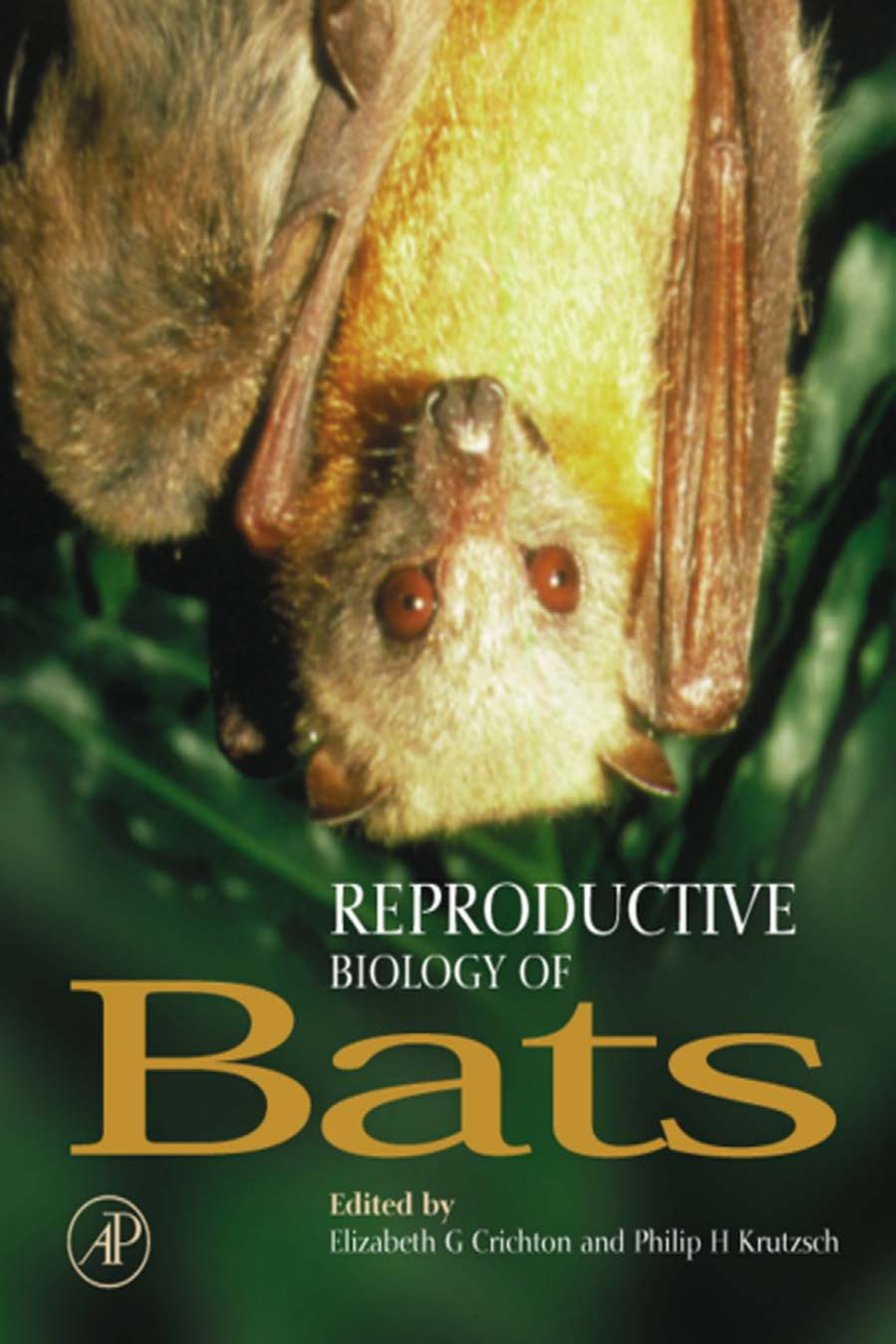

REPRODUCTIVE BIOLOGY OF Bats

Edited by
Elizabeth G Crichton and Philip H Krutzsch

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ACADEMIC PRESS

A Harcourt Science and Technology Company

San Diego San Francisco New York
Boston London Sydney Tokyo

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Academic Press
A Harcourt Science and Technology Company
32 Jamestown Road
London NW1 7BY
http://www.academicpress.com

Academic Press
A Harcourt Science and Technology Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Library of Congress Catalog Card Number: 99-066843

A catalogue record for this book is available from the British Library

ISBN 0-12-195670-9

Typeset by Phoenix Photosetting, Chatham, Kent
Printed in Great Britain at the University Press, Cambridge
00 01 02 03 04 05 9 8 7 6 5 4 3 2 1

Contents

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Preface

Some 20 years ago we discussed with Dr William A. Wimsatt the possibility of bringing together a volume devoted to the reproductive biology of Chiroptera. Our idea, at that time, was to present such a work as Volume 4 in the *Biology of Bats*. Many events interfered with the initiation of such a review, and finally the untimely death of Dr Wimsatt almost permanently set it aside. It is to Bill's vision and memory that we gratefully and lovingly dedicate this volume. He is sorely missed not only for his contributions to this science but for his deep and sincere friendship and support.

The great and ever growing interest in these unique and highly specialized mammals and the many new findings about their reproduction caused us to realize the importance of bringing together the available data concerning the reproductive aspect of the life history of these mammals. Knowledge of reproductive events and their controlling mechanisms may ultimately be central to the management of vulnerable populations. Perhaps the application of assisted reproductive technologies will become critical.

The current knowledge of the reproductive biology of bats is fragmentary, appears in a wide range of scientific literature, and only concerns a small fraction of the 960+ species of Chiroptera. This mammalian order is worldwide in distribution and comprises nearly 25 per cent of the existing living mammal fauna. Volumes that synoptically consider aspects of bat reproduction include: Slaughter and Walton (1970), Kunz (1982, 1984), Hill and Smith (1984) and Altringham (1996). A comprehensive volume reviewing this critically significant area of chiropteran biology, however, is lacking and leaves us without an in-depth compilation of pertinent literature, which is worldwide, in many languages and often difficult to obtain.

Our aim is to summarize (in one volume) the current knowledge of reproductive biology in bats. Perhaps this will provide a stimulus to students and/or established investigators to find answers to some of the many unknowns.

Authors contributing to this volume were selected on their contributions to the subject field that they were to review. Their reviews were to bring forth and summarize data on specific topics. Each chapter was to include and summarize primarily reviews of the published literature and, less often, original work in progress. Contributors were encouraged to freely develop their ideas and presentations which resulted in some, but meaningful, redundancy.

We are forever grateful to a number of individuals who helped make this immense task possible. We wish to thank our spouses Dorothy and Harold for their special support and understanding. We also owe special thanks to David Piper who took the time from his demanding schedule to advise upon and provide computer support, and David Karabinus, a trusted colleague, for his unselfish help in the mechanics of word processing. Secretarial assistance was graciously given by Lori Taylor and Susan Eastman of the Department of Cell Biology and Anatomy, University of Arizona. Robert S. McCuskey, Department head, is thanked for his support in the use of many department facilities and services.

We are especially indebted to Dr. Ted Fleming for his contribution in the preparation of the Subject Index. In addition, we acknowledge the following colleagues who freely provided their professional insight and expertise in anonymously reviewing one or more of the chapter manuscripts: B.B. Benson, R.T.F. Bernard, J. Bradbury, G. McCracken, M. Cukierski, M.B. Fenton, A.W. Gustafson, Mac E. Hadley, P.D. Heideman, P. Hoyer, C. Pow, P.A. Racey, E.H. Studier, E.L. Cockrum and E.D. Widmaier.

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1

Endocrinology of Reproduction in Bats: Central Control

Edythe L.P. Anthony

1.1 INTRODUCTION

One of the most compelling questions addressed by bat physiologists today is how neuroendocrine mechanisms support the diverse array of reproductive patterns that have evolved within the chiropteran order. It is assumed that in bats, as in all mammals studied, gametogenic and steroidogenic activities of the gonads are regulated by hormones of the anterior pituitary gland. Secretion of pituitary hormones is controlled by the hypothalamus, which in turn is influenced by hormonal milieu as well as by neural pathways originating in other areas of the brain.

These complex central mechanisms have been examined most thoroughly in laboratory rodents, in a few primates, in sheep and in certain other livestock species. Research has focused on these animals for several practical reasons, including feasibility of establishing and maintaining breeding populations as well as availability of financial support for work with clearly apparent medical or economic implications. Furthermore, inbred strains have been favored in many

Reproductive Biology of Bats
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physiological studies, as fundamental mechanisms may be more readily discerned when variability among individuals is minimal.

Biologists attempting to investigate central endocrine mechanisms in bats face obvious difficulties on all these fronts. Wild-caught bats, sampled at different phases of their reproductive cycles, have provided the basis for most of our current knowledge. As a result, most studies have been descriptive in nature, documenting apparent changes in hypothalamic and pituitary activity that occur over the course of male and female reproductive cycles. However, efforts to maintain healthy captive bat colonies, in which individuals not only survive over the long term, but also reproduce, are becoming increasingly successful. These advances are creating new research environments in which investigators can conduct studies that are more experimental in design.

This chapter will review information derived from all these sources. Primary emphasis will be placed upon form and function of the hypothalamic-pituitary complex in bats within the broader context of their reproductive physiology. Given the wide variety of reproductive patterns observed, it is important to emphasize that there may also be considerable variation in the underlying neuroendocrine mechanisms. We are far from being able to assess, in any comprehensive way, how conserved or how varied these mechanisms may be. However, it is of great interest to begin to explore the variations on the 'basic mammalian scheme' that may be necessary to achieve such fascinating diversity in reproductive function.

1.2 THE HYPOTHALAMIC-PITUITARY COMPLEX

At one time it was believed that the pituitary gland, acting as an autonomous 'master gland,' served as the primary central regulator of mammalian reproduction. This role was ascribed to the pituitary by virtue of critical influences exerted by luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin on ovarian, testicular and mammary gland function. While these influences are still acknowledged as fundamental to reproductive physiology, it has become clear that the pituitary is not an autonomous regulator. Rather, the pituitary stands at a 'crossroads' where the nervous and endocrine systems converge; thus, it represents only one part of a highly sophisticated neuroendocrine complex. With respect to controlling reproductive activity, this complex is in a position to integrate neural and hormonal input from various sources (including indirect input from sensory systems) and to respond appropriately with endocrine signals. It is, therefore, reasonable to suspect that the hypothalamic-pituitary complex plays a pivotal role in coordinating reproductive activities in response to changing physiological and environmental conditions.

Among mammals, the hypothalamic-pituitary complex exhibits considerable variation with respect to anatomical relationships among its various components. The components of neural origin include the hypothalamus itself, with the median eminence (ME) protruding from its base at the midline. Emanating directly from the ME is the narrow infundibular stem, which in turn expands to form the neural lobe of the pituitary gland. Together, the ME, stem and neural lobe constitute the neurohypophysis. Tissues of non-neural origin comprise the adenohypophysis, those parts of the pituitary gland known as the *pars distalis*, *pars intermedia* and *pars tuberalis*.

Relative orientations of these structures vary widely among mammals (Figure 1.1). In microchiropteran bats (Anthony *et al.*, 1984), the infundibular stem and pituitary neural lobe extend posteriorly from the ME. Because the *pars intermedia* and *pars distalis* develop anterior to the neural lobe, the stem crosses their dorsal surface, creating an extensive region of direct contact between the stem and the *pars distalis*. Similar anatomical relationships have recently been described in megachiropteran bats (O'Brien, 1996); three-dimensional reconstruction of the pituitary gland of *Pteropus scapulatus* (Figure 1.2) clearly reveals that the infundibular stem occupies a sulcus bounded by the *pars distalis* and *pars intermedia*. In contrast, direct contact between the stem and *pars distalis* is minimal in rodents, in which the stem is similarly directed, but the *pars intermedia* and *pars distalis* develop ventral to the neural lobe (Figure 1.1). It is also limited in primates in which the infundibular stem and neural lobe extend inferiorly from the ME and the *pars distalis* is located anterior to the neural lobe.

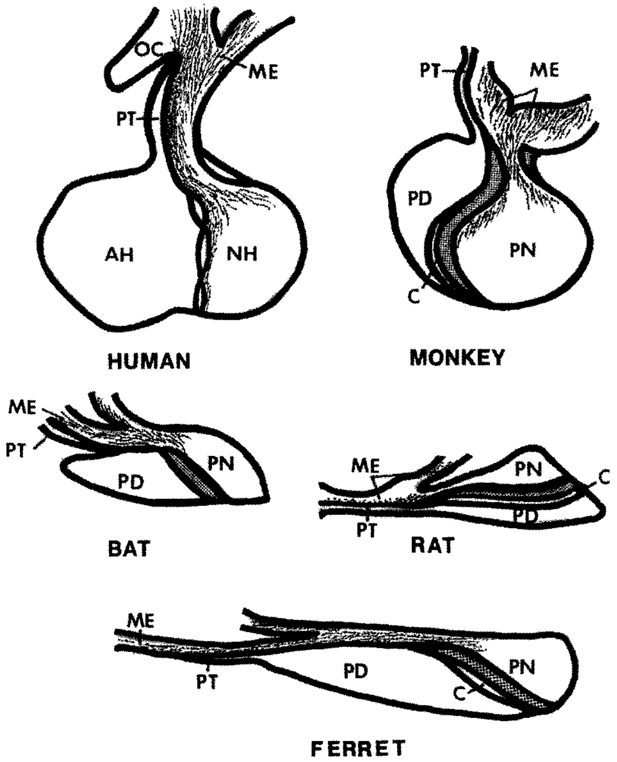

Figure 1.1 A diagram of pituitary morphology and neurohypophysial GnRH projections in five mammals. AH = adenohypophysis; C = hypophysial cleft; ME = median eminence; NH = neurohypophysis; OC = optic chiasm; PD = *pars distalis*; PN = *pars nervosa* (neural lobe); PT = *pars tuberalis*; stippled areas = *pars intermedia*. General outlines adapted from Adams *et al.* (1971) and Hanström (1966). Reprinted from Anthony *et al.* (1984) with permission from Springer-Verlag.

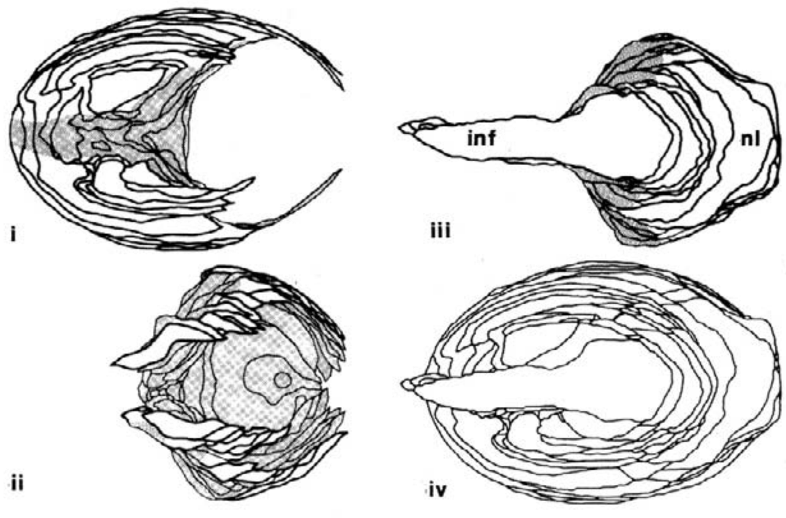

Figure 1.2 Three-dimensional reconstructed image from horizontal sections of pituitary gland of *P. scapulatus*: dorsal view. The faces that are normally hidden inside the gland have been shaded for this illustration. (i) *Pars distalis*. The infundibulum (see iii and iv) lies across the sulcus (shaded) formed by regions of *pars distalis* (rising out of the page) which surround it. This view illustrates how the *pars distalis* extends around the space occupied by the other two regions. (ii) The *pars intermedia* forms the wall of an incomplete spherical shape which cups around the neural lobe (which abuts the inner shaded face) and fits inside the *pars distalis* (which abuts the outer shaded face). Dorsally (rising out of the page), it extends around the infundibulum contributing to the sulcus described by the *pars distalis*. (iii) The neurohypophysis is represented by part of the infundibulum (inf) superficially and the neural lobe (nl) deep to that (disappearing into the page). The shaded face is usually hidden by the overlying *pars intermedia* and the infundibulum fits into the sulcus that is shaded in (i). (iv) The entire gland, constructed by adding the segments from i, ii and iii together and removing any hidden lines. What remains can be constructed from the unshaded regions of the first three illustrations. Figure and legend reprinted from O'Brien (1996) with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

1.3 GnRH AND PORTAL MECHANISMS

In all mammalian species studied to date, gonadotropin releasing hormone (GnRH), also known as luteinizing hormone releasing hormone (LHRH), directs tonic pulsatile secretion of LH and FSH from gonadotropes within the *pars distalis*. This peptide of hypothalamic origin also stimulates the gonadotropin surge that precedes ovulation. GnRH consists of ten amino acids, the form first isolated in mammals having the sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. This molecule has been identified in all mammalian species examined. An additional molecular form with three amino acid substitutions (termed chicken GnRH II) has been identified in some metatherian mammals and in eutherians of the order Insectivora. Chicken GnRH II was not detected in the brain of the only bat species that has been examined (*Miniopterus schreibersii*), although expression of the unsubstituted form has been confirmed (King *et al.*, 1994).

As GnRH is synthesized and secreted in very small amounts, mechanisms responsible for transporting GnRH from sites of secretion to target cells in the *pars distalis* must be highly efficient. In all mammals that have been studied, GnRH is transported in portal vessels which conduct blood flow from capillary beds at sites of releasing hormone secretion to capillary beds within the *pars distalis*. The architecture of these portal vessels varies among mammals, however, depending upon (1) how the *pars distalis* is oriented relative to the neural components of the hypothalamic-pituitary complex, and (2) where specifically within the neurohypophysis releasing hormones are secreted.

Sites of GnRH synthesis and secretion have been studied in several vespertilionid bats using immunocytochemical techniques: *Myotis lucifugus* (King *et al.*, 1984), *M. schreibersii* (Mikami *et al.*, 1988b; Fernandez *et al.*, 1992), *Eptesicus fuscus* (Oelschläger and Northcutt, 1992). In all cases the neuronal cell bodies responsible for synthesis are reported to be widely distributed within the hypothalamus and in surrounding forebrain areas. Processes that extend from these cell bodies toward the medial basal hypothalamus converge at the level of the ME, but few if any, appear to terminate within its external zone. Instead these fibers course via the internal zone, projecting abundantly into the infundibular stem, and many can be traced into the neural lobe of the pituitary gland. In *M. lucifugus*, authenticity of GnRH-immunoreactive material in the neural lobe has been established by high performance liquid chromatography (Anthony *et al.*, 1987b). The principal sites of GnRH secretion in vespertilionid bats thus seem to be within the lower infundibular stem and the neural lobe. This is in sharp contrast to rats, in which the primary site of secretion is the external zone of the ME, with some fibers also terminating in the upper infundibular stem.

Not surprisingly, organization of the portal vasculature differs significantly between bats and rats. The only bat in which the portal vasculature has been examined in detail is *M. lucifugus* (Anthony and Gustafson, 1995). In this study, corrosion casting techniques were used to produce three-dimensional replicas of the microvascular network within the hypothalamic-pituitary complex. Scanning electron microscopic analysis of these replicas revealed a highly anastomotic capillary bed extending from the ME to the proximal neural lobe, ensheathing the infundibular stem in the manner of a ‘mantle plexus’ (Figure 1.3). This continuous capillary network is supplied by at least three small arteries which are probably analogous to the anterior, middle and posterior hypophysial arteries in other mammals, as they associate with the capillary bed at the levels of the ME, stem and neural lobe, respectively. Several straight vessels emerge from portions of this capillary network within the lower infundibular stem and neural lobe (Figure 1.3); these pass directly into the adjacent *pars distalis* where they give rise to arcing parallel arrays of sinusoids. In *M. lucifugus*, the straight vessels comprise a series of short portal conduits, which appear to represent the primary vascular link between the neurohypophysis and the *pars distalis*. A few additional short portal vessels run between the anterior margin of the neural lobe and the posterior margin of the *pars distalis* (Figure 1.4). Long portal vessels, which would originate in the ME, have not yet been observed in bats, whereas in rats they constitute the principal source of portal flow to the *pars distalis*.

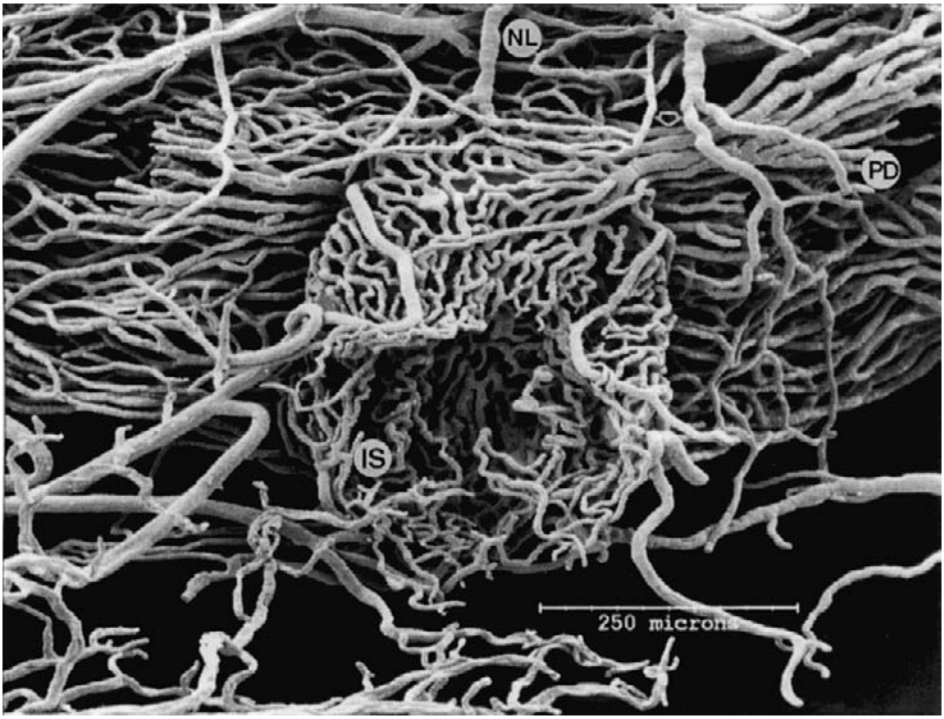

Figure 1.3 A microvascular corrosion cast of pituitary portal vasculature in *M. lucifugus*. The capillaries of the infundibular stem (IS) are organized as a superficial mantle plexus, within which the vessels form a complex anastomosing network. From these capillaries arise a series of short portal vessels (at white arrowhead) that in turn give rise to arcing parallel cascades of sinusoids within the *pars distalis* (PD). The capillary network of the infundibular stem is continuous with that of the neural lobe (NL). Reprinted from Anthony *et al.* (1998) with permission from Elsevier Science.

These observations support the implications of immunocytochemical studies on the GnRH system. In bats, peptide released from neuronal terminals within the lower infundibular stem and neural lobe can gain access to target cells throughout the *pars distalis* via extremely short portal vessels. Paucity of neuronal terminals at the level of the ME is associated with a significantly reduced contribution of long portal vessels to releasing hormone transport.

Direct apposition of the infundibular stem and the dorsal surface of the *pars distalis*, however, suggests that GnRH may additionally gain access to some target cells by non-vascular mechanisms (Anthony *et al.*, 1992). In *M. lucifugus*, the portion of the *pars distalis* immediately surrounding the stem (a region termed the '*zona tuberalis*') is enriched in gonadotropes (Anthony and Gustafson, 1984). Ultrastructural characteristics of the gonadotropes located here, including moderate to extreme dilation of rough endoplasmic reticulum and limited numbers of stored secretory granules, suggest heightened synthetic and secretory activity (Figure 1.5; Anthony *et al.*, 1992). This distinctive functional status (which seems

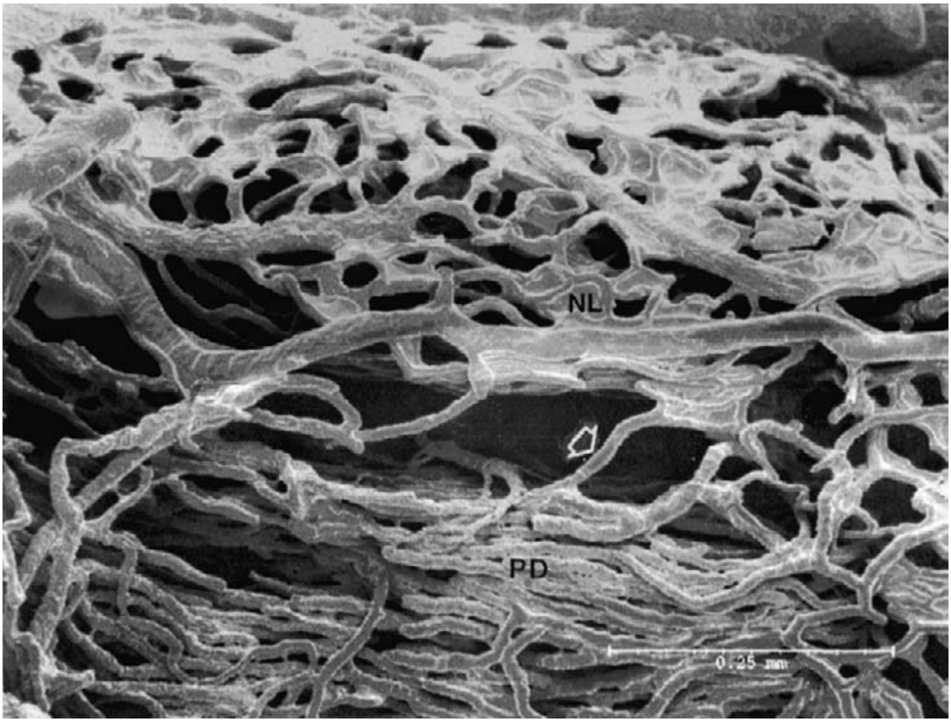

Figure 1.4 In *M. lucifugus*, microvascular corrosion casts further reveal short portal vessels (such as the one indicated by the white arrowhead) running between the anterior margin of the neural lobe (NL) and the posterior margin of the *pars distalis* (PD). These vessels cross the dorsal surface of the relatively avascular *pars intermedia*.

unrelated to endocrine condition) may result from the close proximity of these gonadotropes to GnRH-containing neuronal processes that traverse the infundibular stem; furthermore, they occupy the portion of the *pars distalis* closest to sites of initial entry of the short portal vessels identified by corrosion casting.

When the interface between the tissue of the stem and the *pars distalis* is examined with transmission electron microscopy, profiles of portal vessels are often observed along this boundary. In areas lacking blood vessels, the boundary zone is reduced to a very thin layer of connective tissue containing collagen fibers distributed in scattered groups (Anthony *et al.*, 1992). Because of the delicate nature of this intervening tissue, it is possible that GnRH could diffuse *directly* from some sites of secretion within the stem to a limited neighboring subpopulation of gonadotropes. Clearly, fine structural features of this interface suggest localized facilitation of GnRH delivery to target cells, possibly by both vascular and non-vascular routes. An intriguing question under investigation is whether gonadotropes clustered within this '*zona tuberalis*' area are functionally

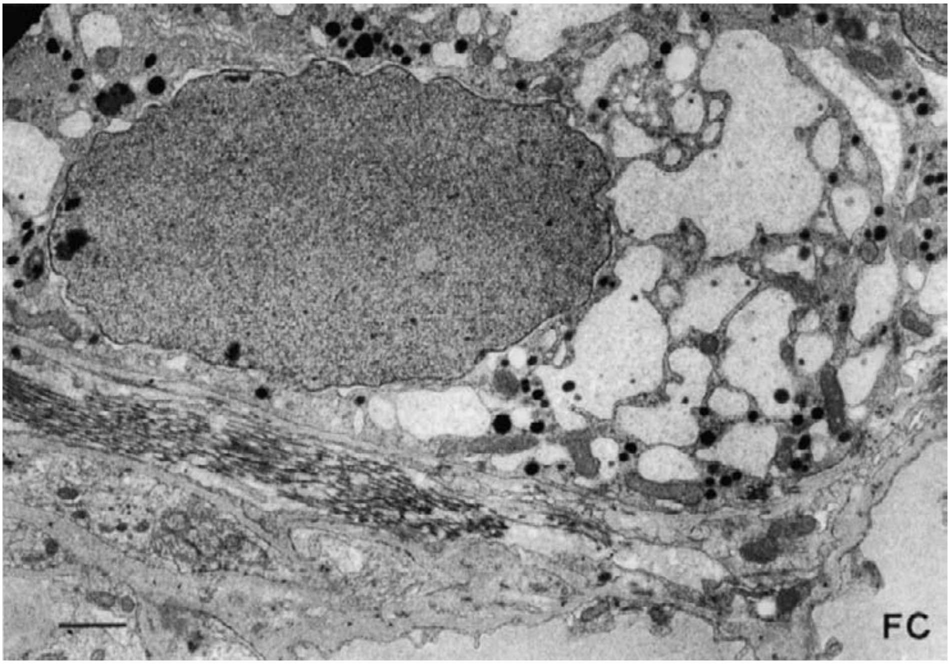

Figure 1.5 An example of a secretory cell of the *pars distalis* located in close proximity to a fenestrated capillary (FC) at the boundary between the lower infundibular stem and the *pars distalis* in *M. lucifugus*. Note the swelling of the rough endoplasmic reticulum and the paucity of stored cytoplasmic secretory granules. Scale bar = 1 µm. Reprinted from Anthony *et al.* (1992) with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

distinct in their contribution to neuroendocrine control of reproduction compared to gonadotropes scattered throughout the rest of the *pars distalis*.

With respect to GnRH transport within the hypothalamic-pituitary complex, bats and rats seem to exemplify two extreme and opposite conditions. In rats, the majority of fibers terminate at the level of the ME, making this species heavily dependent on long portal routes of delivery. Bats, on the other hand, rely heavily (if not exclusively) on short portal and possibly non-vascular routes, as GnRH fibers extend to more distal sites of secretion. Locations of GnRH terminal fields in representatives of other mammalian groups (Figure 1.1) suggest that primates and carnivores fall between these two extremes, with long and short portal vessels contributing more equally to GnRH transport (Anthony *et al.*, 1984).

It is tempting to argue that specific sites of GnRH secretion within the neurohypophysis are physiologically irrelevant as long as mechanisms exist for its efficient delivery to target cells. On the other hand, recent evidence suggests that GnRH secretion can be modulated *at secretion sites* by microenvironmental changes such as extension or retraction of glial processes between GnRH terminals and portal capillaries (King *et al.*, 1995). Paracrine interactions with other peptidergic or catecholaminergic systems may also occur within terminal fields. Realization that peptide hormone secretion may be modulated locally at sites of release introduces an entirely new dimension to investigation of central control of reproductive processes. Glial cell populations vary between the ME and the neural lobe, and these microenvironments also potentially differ with respect to interacting neuronal systems. Therefore, extension of GnRH processes to sites distal to the ME could be an indication that release of this hormone is regulated differently in bats than it is in rodents and perhaps in other mammalian taxa as well.

1.4 GnRH PERIKARYA AND SEASONAL DYNAMICS OF THE GnRH SYSTEM

Similar spatial considerations apply to the distribution of GnRH neuronal cell bodies. These perikarya are distributed diffusely throughout the basal forebrain in all mammals; typically, they are not organized within specific nuclear regions. In rats, the majority are located rostrally, within the preoptic area and anterior hypothalamus. In contrast, in vespertilionid bats, immunocytochemistry has shown that the greatest accumulation of GnRH perikarya is considerably more caudal (Figure 1.6), in the arcuate nuclei and in surrounding regions of the medial basal hypothalamus (King *et al.*, 1984; Mikami *et al.*, 1988b; Fernandez *et al.*, 1992; Oelschläger and Northcutt, 1992).

In *M. lucifugus*, this distribution of perikarya has been verified using primary GnRH antisera with a wide variey of binding characteristics in peroxidase-antiperoxidase (PAP) immunocytochemistry. These antisera include one that binds

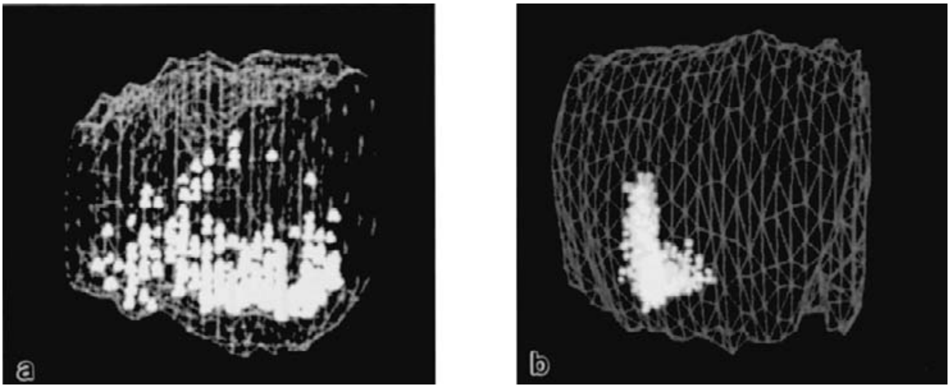

Figure 1.6 Distribution of GnRH-immunoreactive perikarya (white dots) in *M. lucifugus* (a) and laboratory rats (b). Each of these images was reconstructed in three dimensions based on serial coronal sections extending rostrally (at left) from the diagonal band of Broca (where the two hemispheres join) to the anterior margin of the midbrain (at right). Both computer-generated reconstructions are viewed from the lateral aspect, with wire frames representing the outer contours of the brain. Panel (b) reprinted from King and Rubin (1995) with permission from Plenum Publishing Corporation.

only the mature GnRH decapeptide (with its cyclized amino terminal and amidated carboxy terminal) as well as several antisera that can bind both the decapeptide and extended precursor forms (King *et al.*, 1984). Immunocytochemical detection of the fully processed decapeptide within GnRH perikarya in little brown bats suggests that intraneuronal dynamics of post-translational GnRH processing may also differ between bats and rats. At least in this bat species, cleavage of the extended GnRH precursor and modification of the decapeptide terminals apparently occurs to a large extent in cell bodies. In rats, on the other hand, processing appears to occur primarily during axonal transport, as the mature decapeptide is detected (at least with the PAP immunocytochemical technique) only in neuronal processes and terminals (King and Anthony, 1983).

Regarding the distribution of GnRH perikarya within the forebrain, one might argue that the placement of cell bodies bears little functional significance as long as processes extend from them to appropriate sites of secretion. However, depending upon position within the forebrain, individual GnRH neurons undoubtedly establish different kinds of synaptic interaction with other neuronal systems. In all species, the dispersed GnRH 'system' probably represents the aggregate of several functional subpopulations of neurons, each with different afferent influences and each with somewhat different roles to play in supporting tonic gonadotropin secretion and in triggering the preovulatory LH surge.

Painstaking analyses of GnRH neurons in gonadectomized male and female rats and in females at different stages of the estrous cycle have provided striking evidence of functional subpopulations which can be delineated by their position within the larger population (King *et al.*, 1987; Hiatt *et al.*, 1992; King and Rubin, 1995). Studies this extensive have not been conducted in bats, but Anthony *et al.* (1989) examined the frequency and distribution of GnRH perikarya in pre- and post-ovulatory *M. lucifugus* for evidence of discrete functional groups. The results disclosed that the total number of immunocytochemically detectable perikarya was much lower in post-ovulatory ($\bar{X} = 280$; $n = 7$) than in pre-ovulatory ($\bar{X} = 423$; $n = 6$) females. This suggested that during the periovulatory period, some of the neurons had become more extensively depleted of GnRH than others. This reduction in number of detectable cells was equally evident throughout the forebrain, as the rostro-caudal and medio-lateral distributions of immunoreactive cells were similar in both groups of bats. Thus, this study supports the existence of physiologically heterogeneous neurons within the GnRH system of bats, but it does not yet provide a basis for topographical delineation of subgroups.

In this same study, a unique phenomenon was observed, involving the dense clusters of GnRH neurons associated with the arcuate nuclei. Direct contact between GnRH-immunoreactive neuronal elements (verified by electron microscopy) occurs in these regions with surprisingly high frequency. Cell bodies are often contacted by numerous fibers, membranes of neighboring perikarya are apposed along considerable distances, and more complex aggregates of multiple immunoreactive perikarya and small to large caliber fibers are established (Figure 1.7). These GnRH–GnRH contacts are far more extensive than those heretofore described in other mammals. Furthermore, they are more elaborate in active, post-ovulatory bats than in bats collected in hibernation a month prior to ovulation (Anthony *et al.*, 1989).

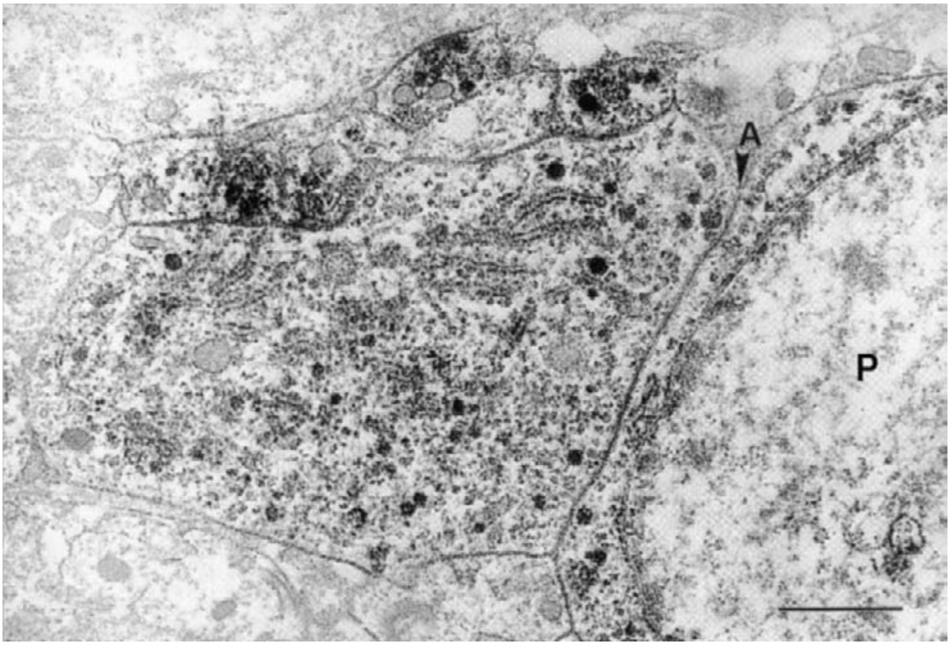

Figure 1.7 At arrowhead (A), a GnRH-immunoreactive perikaryon (P) is contacted by a large caliber GnRH-immunoreactive process. In turn, this process is contacted at top by several small caliber GnRH-immunoreactive fibers. Scale bar = 1 μm. Reprinted from Anthony *et al.* (1989) with permission from the Journals of Reproduction and Fertility, Ltd.

This apparent increase in physical association among GnRH neurons may be related to activation of the system during the spring periovulatory period, and may facilitate synchronous activity of groups of neurons. Supporting this hypothesis is a recent study in rats examining the synchronized firing of multiple GnRH neurons that typifies pulsatile GnRH secretion (Hiruma and Kimura, 1995). The results strongly suggested that GnRH itself, acting locally within the hypothalamus, initiates electrical activity in GnRH neurons, and thus may be responsible for synchronizing secretion from multiple cellular sources.

Other seasonal changes related to reproductive activity observed in wild-caught *M. lucifugus* include a 48% reduction in hypothalamic GnRH content (measured by radioimmunoassay) in post-ovulatory females. GnRH content of the isolated infundibular stem/neural lobe and neural lobe fiber density were also significantly reduced when compared to pre-ovulatory females (Anthony *et al.*, 1989). In *M. schreibersii fuliginosus* studied in Japan, Mikami *et al.* (1988b) reported that GnRH-immunoreactive perikarya were larger in hibernating male and female bats than in active (non-pregnant, pregnant and lactating) females. Fernandez *et al.* (1992), studying *M. schreibersii schreibersii* in Spain, found that perikarya of pregnant and lactating bats were smaller and fewer in number than those in hibernating and periovulatory individuals. (Whether ovulation had already occurred in these bats was not specifically indicated.) These authors were also unable to assign the functionally heterogeneous neurons to specific anatomical subgroups. Interestingly, however, they noted in their light microscopic observations that ‘spiny’ GnRH perikarya were observed only in the arcuate nuclei of periovulatory bats. It is tempting to speculate that the spiny appearance of these cell bodies may reflect direct contacts of other GnRH neuronal processes which cannot be resolved at the light microscopic level, but which were documented at the fine structural level in *M. lucifugus* (Anthony *et al.*, 1989).

1.5 THE *NERVUS TERMINALIS*

A major breakthrough in understanding the ontogeny of the GnRH system derived from the observation made in rodents that during fetal development, GnRH immunoreactivity is detected earliest in the olfactory placode (Schwanzel-Fukuda and Pfaff, 1989). Neurons expressing GnRH proceed to migrate caudally, most of them ultimately becoming dispersed in the basal forebrain. Others remain, even in adult animals, in the *nervus terminalis*, a cranial nerve derived from the olfactory placode. This nerve, lacking a designated number and therefore sometimes referred to as ‘cranial nerve zero,’ innervates the nasal septal mucosa and associates with olfactory, septal and preoptic areas of the brain.

A well-developed terminal nerve has been shown to persist in adult specimens of several microchiropteran and megachiropteran bat species (Oelschläger, 1988; Oelschläger and Northcutt, 1992). In *E. fuscus*, GnRH-immunoreactive neurons have been identified in the portion of the nerve within the cranial vault and also in the meninges surrounding the basal forebrain (Oelschläger and Northcutt, 1992). Superficial cells associated with the medial and basal surfaces of the olfactory bulbs and cells apparently in the meninges were observed also in *M. lucifugus* (King *et al.*, 1984), but at the time were not recognized as components of a terminalis system.

Because the *nervus terminalis* is a critical component of the migration route of GnRH neurons, this nerve contributes in a fundamental way to the establishment of hypothalamic-pituitary neuroendocrine circuits responsible for controlling reproductive function. In addition, because of its association with nasal structures, this nerve may mediate some pheromonally induced aspects of reproductive activity (Oelschläger and Northcutt, 1992). Whether it plays such a role in bats is currently only a matter of speculation, but if it does mediate pheromonal influences in vespertilionids such as *Myotis* and *Eptesicus*, it does so in the absence of a vomeronasal system (Mann, 1961; Cooper and Bhatnagar, 1976).

1.6 PITUITARY GONADOTROPINS

Two previous reviews have addressed pituitary research in Chiroptera (Richardson, 1979 and Anthony, 1987); therefore, the following discussion will focus primarily on more recent contributions that advance our understanding of central control of reproduction. Morphological studies conducted at light and electron microscopic levels continue to extend our knowledge of the pituitary's functional organization in a growing number of microchiropteran groups (e.g. the emballonurids; Badwaik, 1988, 1989) and are setting the stage for detailed neuroendocrine investigation in the Megachiroptera (Bhiwgade *et al.*, 1989; O'Brien, 1996).

Immunocytochemistry has proved to be a useful tool in the transition from traditional descriptive histological approaches into studies designed to explore relationships between individual pituitary hormones and well-known physiological phenomena in bats. A decade ago, *M. lucifugus* (which exhibits delayed ovulation/fertilization) and *Macrotus californicus* (which exhibits delayed development) were the only species in which pituitary gonadotropes had been identified immunocytochemically. Since then, pituitary hormones of the cosmopolitan species *M. schreibersii* (which exhibits delayed implantation) have received considerable attention. Gonadotropes have been identified in a Japanese population of this species using polyclonal antisera raised against rat FSH, rat LH and the beta subunit of ovine LH (Mikami *et al.*, 1988a). In South African *M. schreibersii*, a monoclonal antibody directed against the beta subunit of bovine LH and a polyclonal FSH-beta antiserum have been used successfully in immunocytochemical labeling (Bojarski, 1990; Bernard *et al.*, 1991a). In each of these studies, many of the pituitary gonadotropes were reported to exhibit both LH- and FSH-immunoreactivity, based upon similarities of labeling patterns in adjacent paraffin sections. The implication is that in bats, as in many other mammals, individual gonadotropes typically synthesize, store and secrete both LH and FSH. The possibility that monohormonal gonadotropes may also exist has not been *rigorously* investigated in bats, although studies in *M. schreibersii* (Mikami *et al.*, 1988a) and in other species (Richardson, 1981; Singh and Krishna, 1994) point to a lack of complete concordance of labeling in adjacent sections to support the assertion that some gonadotropes contain only one of the two hormones.

Conclusive evidence concerning the hormonal content of individual gonadotropes is very difficult to obtain; molecular similarities between FSH and LH raise concerns about how reliably either monoclonal or polyclonal antisera can discriminate between the two hormones when used in immunocytochemical protocols. Another technical consideration is that it is very difficult to identify with certainty serial profiles of an individual pituitary cell in paraffin sections. Depending upon section thickness, a cell that is conspicuous in one section may be minimally represented (if at all) in the next. Furthermore, while immunocytochemistry is a highly sensitive technique, minute quantities of a hormone undoubtedly escape detection. Therefore, it is difficult to assert that a cell that demonstrates only LH-immunoreactivity is strictly monohormonal, as it could represent a multihormonal gonadotrope that is depleted of stored FSH at the time of fixation.

In studies focused on laboratory animals, some of these technical problems have been avoided by highly scrupulous use of double-immunogold labeling procedures at the electron microscopic level (Childs *et al.*, 1986). These techniques utilize two different sized gold particles to discriminate subcellular sites of FSH- and LH-immunoreactivity in single ultrathin sections. Yet another approach has been the use of *in situ* hybridization to localize LH-beta messenger RNA in combination with immunocytochemical localization of FSH-beta in whole dispersed pituitary cells (Childs *et al.*, 1987). Adaptation of these types of protocols for use in bats would be highly instructive, particularly with respect to the question of whether gonadotropes function as a homogeneous population or whether they are heterogeneous with respect to LH and/or FSH synthesis, storage and secretion.

The distribution of gonadotropes in *M. schreibersii* is similar to that previously reported in other bat species, the cells being scattered throughout the *pars distalis*, either singly or in small groups (Mikami *et al.*, 1988a; Bojarski, 1990). In specimens obtained in Japan, Mikami *et al.* (1988a) additionally reported an accumulation of gonadotropes in the portion of the *pars distalis* previously described in this chapter (p. 6) as the '*zona tuberalis*,' which also contains clustered gonadotropes in *M. lucifugus*. Extension of this observation to a second vespertilionid species provides additional impetus to investigate whether the gonadotropes located in this distinct anatomical site play a unique role in neuroendocrine control of reproduction.

Seasonal studies of pituitary gonadotropins in *M. schreibersii* have focused primarily on wild-caught females. Using immunocytochemical techniques, Mikami *et al.* (1988a) reported a reduction in size and abundance of gonadotropes during pregnancy and lactation relative to the hibernation period. This seasonal fluctuation is reminiscent of patterns reported for female *M. lucifugus* (Anthony and Gustafson, 1984).

In a study more directly focused on relating changes in gonadotropin secretion to the phenomenon of delayed implantation, which is characteristic of *M. schreibersii*, Bernard *et al.* (1991b) used radioimmunoassay to measure plasma LH levels on a monthly basis throughout the year in South Africa, where these bats do not hibernate, but do exhibit periodic torpor (Figure 1.8). These authors reported relatively low circulating LH levels in the two months prior to significant follicular development (January and February), which rose in March toward a peak in April that coincided with ovulation. Plasma levels then dropped precipitously during the first two months of delayed implantation, beginning to rise again in July, prior to the end of the delay period. After implantation, LH levels continued to increase, attaining a second seasonal peak in November. This occured prior to parturition and coincided with high circulating levels of plasma progesterone. While this study clearly documents relationships between patterns of LH secretion and the changing reproductive status of bats throughout the year, the authors point to a lack of any 'clear indication' that LH directly orchestrates delayed implantation in this species.

In this same population of bats, Bernard *et al.* (1991b) took a fresh morphometric approach to seasonal assessment of LH-immunoreactive gonadotropes. Not only did they calculate relatively standard light-microscopic volume densities (percentage of the volume of the *pars distalis* occupied by gonadotropes), but they additionally developed an index which quantitated results of immunogold labeling at the electron microscopic level. Dubbed 'pituitary LH immunoreactivity,' this interesting index was derived by multiplying the volume density of labeled secretory granules within the cytoplasm of individual gonadotropes by the observed number of gold particles associated with each granule. Fluctuations in this index throughout the year were remarkably consistent with changes in plasma LH levels (Figure 1.8), both being high in April, very low in May, and rising again later in the year. The only discrepancy occurred in the months of January and

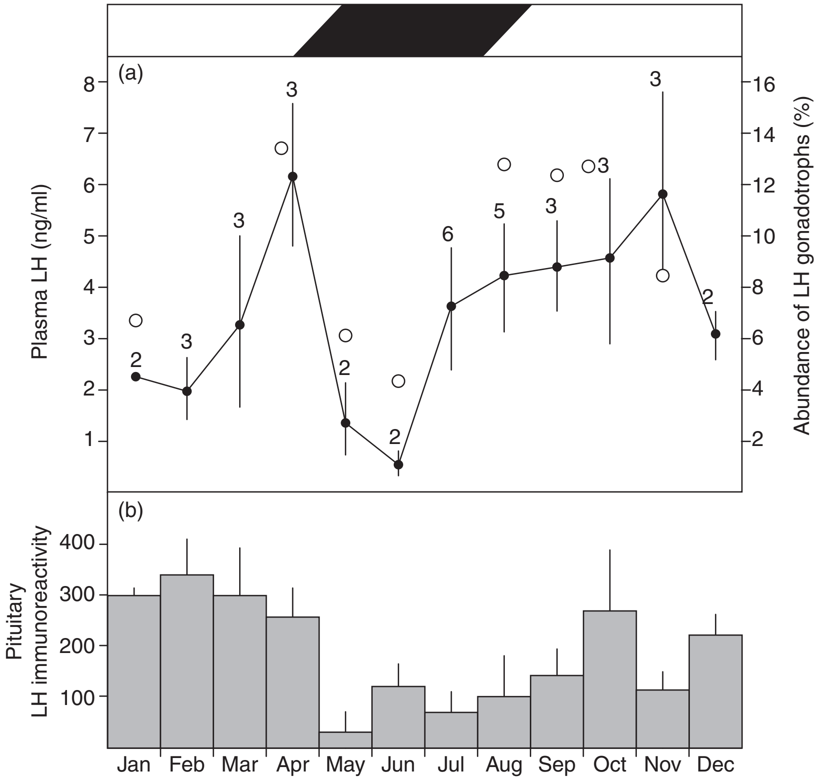

Figure 1.8 Monthly changes in (a) plasma luteinizing hormone (LH) concentration (solid circles) and abundance of LH gonadotrophs (open circles) and (b) pituitary LH-β immunoreactivity in the long-fingered bat. LH concentration is given as mean ± 1 s.d., with sample size above each value. The index of pituitary LH-β immunoreactivity was obtained by multiplying the volume density of secretory granules by the number of gold particles per granule. The period of delayed implantation is indicated by the dark bar at top. Figure and legend reprinted from Bernard *et al.* (1991b) with permission from the Journal of Reproduction and Fertility, Ltd.

February, when circulating LH levels were relatively low, but the immunoreactivity index was high. This discrepancy suggests that during these two months preceding annual follicular development, LH is being stored in the pituitary with only minimal amounts being released. An even stronger correlation appears to exist in this study between volume density measurements and plasma LH levels.

As this study is the first within the realm of bat research to present morphometric analyses of gonadotropes together with data on circulating LH levels, its importance should not be underestimated. The majority of recent seasonal studies of pituitary function in bats have utilized immunocytochemical techniques. Due to the very nature of this approach, data obtained over the course of a reproductive cycle reflect changes in cellular hormone *content*. This is at best indirect evidence upon which to base hypotheses concerning seasonal patterns of hormone *secretion*. However, the relatively good agreement between immunocytochemical parameters and circulating hormone levels reported by Bernard *et al.* (1991b) lends credence to predictions derived from the several immunocytochemical studies conducted before radioimmunoassays for bat pituitary hormones were validated.

Radioimmunoassay (RIA) has also been used to describe seasonal changes in circulating LH levels in *M. lucifugus* (Canney and Butler, 1987). This study revealed low plasma LH levels in late summer, prior to the autumn mating period. These levels remained low in mid-hibernation (December and January) during the period of delayed ovulation, but were significantly elevated in spring, just prior to arousal. In contrast, pituitary LH content, also assessed by RIA, did not change significantly between the mid-winter and spring collection periods. Taken together, these results suggest that LH is stored in pituitary gonadotropes throughout hibernation, as has been inferred from immunocytochemical studies (Anthony and Gustafson, 1984). However, secretion of this hormone is apparently minimal throughout much of the course of delayed ovulation, beginning to rise at an as yet unspecified time point prior to spring arousal.

Interestingly, recent studies of the vespertilionid *Scotophilus heathi* indicate that mechanisms resulting in accumulation of pituitary LH during delayed ovulation can operate independently of an annual hibernation and arousal cycle. In this tropical bat, LH-gonadotropes progressively enlarge and occupy increasing areal fractions of the *pars distalis* beginning at the time of annual ovarian recrudescence and continuing throughout the ovulatory delay (Singh and Krishna, 1996). Following ovulation, LH-gonadotropes appear degranulated and are significantly reduced in size and areal density.

While neuroendocrine mechanisms responsible for forestalling LH secretion during delayed ovulation are far from understood, recent observation of high circulating testosterone and androstenedione in female *S. heathi* during the delay period (Abhilasha and Krishna, 1996) suggests a role for long-loop negative feedback. These androgens, apparently of ovarian interstitial rather than adrenal cortical origin, may inhibit secretion of pituitary gonadotropins. Immunocytochemical studies on the GnRH system of *M. lucifugus* suggest that events at the hypothalamic level parallel those within the pituitary during delayed ovulation, with secretion of stored GnRH being delayed until the periovulatory period (Anthony *et al.*, 1989). Therefore, if feedback is responsible for suppressing the reproductive axis of this bat in winter, it probably intervenes at both hypothalamic and pituitary levels.

Seasonal studies of pituitary function in male bats are generally fewer in number than those in females, but Bernard *et al.* (1991a) included males in their long-term study of *M. schreibersii*, reporting that immunocytochemical indices of gonadotropic function rise significantly during the spermatogenic period, coincident with high plasma testosterone levels. Similar findings were obtained for the rhinolophid bat *Rhinolophus capensis* in South Africa (Bojarski and Bernard, 1992). Combining immunocytochemical results with monthly measurements of plasma LH levels, this study revealed that gonadotropic activity increases during the six-month period of spermatogenesis (October to April), with all indices peaking in March. This pattern suggests that the high levels of testosterone secretion which presumably support later stages of spermatogenesis in this species are supported by heightened gonadotropic function. Gonadotropic activity was notably reduced during the subsequent period of sperm storage (May to September).

1.7 PROLACTIN

Prolactin has received considerable attention from mammalian reproductive biologists not only because of its several widely recognized roles in lactation, but also because of growing evidence linking this pituitary hormone to central control of seasonal reproductive delay phenomena. Studies in mustelid carnivores (reviewed in Curlewis, 1992) conclude that prolactin plays a pivotal role in synchronizing neuroendocrine events of delayed implantation with photoperiodic changes in the environment. This model attributes the delay to a period of inhibited prolactin secretion, during which circulating levels are insufficient to support critical functions of the corpus luteum. In the mustelids this period coincides with short days, and increased pineal melatonin activity is believed to be responsible for suppressing prolactin release, possibly acting by modulating amounts of dopamine (a prolactin-inhibiting factor) reaching the pituitary in portal blood.

Some aspects of this model have been tested in the bat *M. schreibersii*. Bojarski (1993) combined immunocytochemistry and RIA to examine the role of prolactin in the female reproductive cycle of this species in South Africa. Lactotropes were identified at the light and electron microscopic levels, and several morphometric criteria (volume density of lactotropes in the *pars distalis*, percentage of secretory granules within lactotropes bearing gold immunocytochemical label, and the number of gold particles per granule) were evaluated throughout the year. All of these criteria pointed to reduced activity of lactotropes from March through June (during follicular development and the early part of the delayed implantation period), when plasma prolactin levels were consistently low. Lactotropes exhibited signs of increasing activity and plasma prolactin levels rose in July and August as the delay came to an end and implantation occurred. Lactotropes further increased their activity, and circulating prolactin concentrations continued to rise as embryonic development proceeded. These observations are consistent with inhibition of prolactin secretion as day-length shortens from March to June, and further suggest that increasing synthesis and release of this hormone after the winter solstice may activate the corpus luteum and create an endocrine environment that is conducive to implantation.

More definitive evidence for a critical role for prolactin is derived from endocrine manipulation experiments conducted in the same bat species (Bernard and Bojarski, 1994). Pregnant females collected in May were given daily subcutaneous injections of prolactin (0.01 mg), human chorionic gonadotropin (hCG; 1.0 iu), or progesterone (0.4 mg) over a period of 10 days. Control animals were left untreated or were treated with injection vehicle (olive oil or saline) alone. Implantation was prematurely initiated only in the prolactin-treated bats, which also exhibited significantly elevated progesterone levels and ultrastructural changes in the corpus luteum consistent with increased steroidogenic activity. Treatment with hCG, a gonadotropin with LH-like activity, stimulated luteal steroidogenesis, but did not induce implantation. Of the hormones tested, then, only prolactin supplementation was shown to be necessary and sufficient to bring about all the events associated with terminating the delay. However, these results do not exclude pituitary gonadotropins as potential factors in the changing endocrine milieu that promotes implantation.

Prolactin has not been specifically implicated in neuroendocrine mechanisms responsible for delayed ovulation; nonetheless, studies of pituitary lactotropes in bats exhibiting this reproductive pattern have brought to light significant changes in this cell population associated with pregnancy and lactation. Increases in size and/or abundance of prolactin-immunoreactive cells have been observed during pregnancy in *S. heathi* (Singh and Krishna, 1996); similar changes have been reported to persist into lactation in *Myotis myotis* (Jimenez *et al.*, 1987). In pregnant females of the latter species, ultrastructural features of lactotropes, including highly developed rough endoplasmic reticulum and Golgi apparatus, suggest a heightened state of hormone synthesis (Muniz *et al.*, 1991).

Immunocytochemical study of pituitary prolactin in bats took a surprising turn when Ishibashi and Shiino (1989) reported colocalization of prolactin- and growth hormone-immunoreactivity in pituitary cells of female *Pipistrellus abramus*. Although 'mammosomatotropes' containing both hormones have been described in some mammals, other immunocytochemical studies in bats have described lactotropes and somatotropes as two distinct cell types (Richardson, 1981; Mikami *et al.*, 1988a; Bojarski, 1993). Using a two-face immunogold double-labelling procedure at the electron microscopic level, Ishibashi and Shiino (1989) reported that colocalization of prolactin and growth hormone was widespread in cells of the *pars distalis*, with prolactin being found exclusively in mammosomatotropes in winter.

Given the extent of this apparent colocalization, it is unfortunate that immunocytochemical control procedures for method specificity and antiserum crossreactivity were not more throughly documented. Nonetheless, cells described as mammosomatotropes in *P. abramus* hypertrophied during pregnancy, accompanied by an increase in size of cytoplasmic secretory granules. Only during pregnancy and lactation did some pituitary cells exhibit prolactin-immunoreactivity alone. This observation suggests functional plasticity within a population of pituitary cells, allowing them to synthesize varying proportions of prolactin and growth hormone depending on physiological demands. Early in the onset of lactation, secretory granules within the mammosomatotropes appeared to aggregate and coalesce within large cytoplasmic vacuoles, possibly as a means to reduce cellular hormone stores at the end of pregnancy (Ishibashi and Shiino, 1989).

While studies of prolactin in microchiropteran bats have clearly focused on females, male megachiropterans have received some attention in preliminary seasonal investigations. Although sympatric within Australia, little red flying foxes (*Pteropus scapulatus*) and grey-headed flying foxes (*Pteropus poliocephalus*) breed at different times of year, with the former breeding in long days and the latter breeding in short days. Immunocytochemical study of lactotropes in male *P. scapulatus* has revealed increased volume density of these cells during its long-day breeding season (O'Brien *et al.*, 1989). Interestingly, however, plasma levels of prolactin measured by RIA in *P. poliocephalus* were also high in summer, declining as these individuals entered breeding condition in autumn (O'Brien *et al.*, 1990, 1993).

This apparent dichotomy illustrates an important caveat regarding the potential role(s) of prolactin in seasonal reproductive cycles. Increased prolactin secretion in spring and summer is a phenomenon common to many temperate zone mammals, suggesting an early evolutionary origin of this photoperiodic response (Curlewis, 1992). With radiation of mammals and development of diverse seasonal reproductive strategies, neuroendocrine mechanisms driving reproductive cycles have probably been superimposed on this previously established annual cycle of prolactin secretion. Consequently, in different species, prolactin may or may not have assumed an important role in the timing of reproductive events. Furthermore, depending upon species and reproductive strategy, prolactin may exert stimulatory or inhibitory effects on various reproductive processes (Curlewis, 1992).

Studies in flying foxes have also provided evidence that within the hypothalamic-pituitary complex of bats, prolactin secretion is, at least in part, regulated by the inhibitory effects of dopamine. Treatment with the dopamine agonist bromocriptine reduced circulating levels of prolactin in female *P. poliocephalus* (O'Brien *et al.*, 1994). Further, as in other mammals, suckling appears to represent an important stimulus for prolactin release in bats, as in this same study, plasma prolactin levels were suppressed after removal of young from lactating *P. scapulatus*.

1.8 PINEAL MELATONIN AND THE SUPRACHIASMATIC NUCLEUS

Outside the hypothalamic-pituitary complex *per se*, a variety of other central mechanisms may influence reproductive function in bats, especially those known in other groups to mediate endogenous rhythms and to transduce photoperiodic information into endocrine signals. The pineal organ has been the subject of detailed morphological study in a large number of bat species (Bhatnagar *et al.*, 1986, 1990). These studies have confirmed the presence of a pineal in almost all of over 85 species examined, although its size, shape and relationships to other brain structures vary considerably. Much less is known, however, about pineal secretion of melatonin in bats and the role (if any) it may play in their physiology. It is well established in many seasonally breeding mammals that photoperiodically controlled melatonin secretion influences gonadal activity via the hypothalamic-pituitary-gonadal axis (Reiter, 1991), but whether melatonin functions similarly in temperate zone and/or tropical bats remains a matter of speculation.

In vivo and *in vitro* experiments have, however, provided evidence that testicular functions of at least two bat species exhibit sensitivity to melatonin. In *Antrozous pallidus*, Beasley *et al.* (1984) showed that captive males maintained on long-day light conditions, but receiving subcutaneous melatonin implants in mid-summer, experienced an acceleration of changes normally occurring in autumn (e.g. testicular regression, activation of the accessory gland complex, and introduction of sperm into the epididymides).

Singh and Krishna (1995) observed that testicular tissue derived from *S. heathi* and maintained in short-term culture responds to melatonin in a dose-dependent manner, but only if the tissue is obtained during the mid-winter period of testicular 'dormancy.' Tissues obtained from these animals exhibited reduced LH-stimulated testosterone (but not LH-stimulated androstenedione) secretion, whereas tissue obtained from males at three other times of year exhibited no response to melatonin treatment. These results suggest that seasonal changes in tissue responsiveness to melatonin could play a role in the annual reproductive cycle of this species. Caution must be exercised in interpreting results of both these studies, however, because doses of melatonin used *in vivo* and *in vitro* were above the normal physiological range.

A recent study of the phyllostomid *Anoura geoffroyi*, obtained from Trinidad at 10 °N latitude, provides evidence that pineal mechanisms may be physiologically relevant even in near-equatorial bat species. Despite the small size of the gland in this bat, a distinct diurnal rhythm in circulating melatonin levels has been documented in captive males, plasma concentrations reaching nocturnal peaks similar to those seen in temperate zone mammals (Heideman *et al.*, 1996). A unique and intimate relationship of the pineal organ with cerebral blood vessel walls may facilitate entry of melatonin into the blood stream in this species.

Exactly how melatonin may interact with the hypothalamic-pituitary-gonadal axis in bats is unclear, although in *M. lucifugus*, as in many mammals examined, melatonin receptors have been identified in the pituitary *pars tuberalis* as well as in the suprachiasmatic nucleus of the hypothalamus (Bittman, 1993). The role of the *pars tuberalis* within the hypothalamic-pituitary complex remains a mystery, and therefore one can only speculate on implications of melatonin binding at this anatomically privileged site. In *M. lucifugus*, the *pars tuberalis per se* is represented only by two to four layers of cells applied to the ventral surface of the median eminence. It does not ensheathe the more distal infundibular stem (Anthony *et al.*, 1992); however, the '*zona tuberalis*,' previously discussed in this chapter, may be composed of *tuberalis* cells that during development become situated between the lower infundibular stem and the *pars distalis*, ultimately appearing upon routine histological examination to be part of the *pars distalis* itself. If this is the case, it would be very instructive to determine whether cells of the *zona tuberalis* contain melatonin receptors. Interestingly, Williams (1988) reported melatonin binding in a portion of the rat pituitary that may represent a *zona tuberalis*. If in *M. lucifugus* gonadotropes aggregated in this part of the pituitary are melatonin-responsive, this may in part explain differences between them and gonadotropes scattered in other regions of the gland.

The suprachiasmatic nucleus (SCN), another confirmed binding site of melatonin in *M. lucifugus*, is believed to embody the mammalian 'biological clock.' It is also a component of the neural circuitry that relays photoperiodic information from the retina to the pineal. In the rhinolophid bat *Rhinolophus rouxi*, projections from the retina to the SCN have been described as 'extremely weak' (Reimer, 1989). More recent study of three vespertilionid species confirmed retinal input to the SCN, although the entire hypothalamic component represented only 0.5% of total retinal projections (Nemec *et al.*, 1996). These observations suggest that sensory input to the pineal complex may be quite limited.

Several peptides have been identified immunocytochemically in and around the SCN of bats. In *Myotis lucifugus*, neuronal cell bodies and processes containing vasoactive intestinal polypeptide occur in considerable numbers in the SCN (Laemle and Cotter, 1988), whereas cell bodies containing cholecystokinin are more scarce in that location (Cotter and Laemle, 1990). Perikarya containing growth hormone releasing hormone (GHRH) have also been identified within the SCN in this same species (Anthony *et al.*, 1991). With respect to somatostatin, Cotter and Laemle (1987) reported immunoreactive fibers passing through the SCN, whereas Anthony *et al.* (1987a) observed that somatostatin-immunoreactive fibers appear to congregate at the borders of the SCN, with very few of them actually entering the central region occupied by perikarya of GHRH neurons. Vasopressin immunoreactivity has been identified in both neuronal perikarya and processes in the SCN of *Rhinolophus ferrumequinum* (Kumamoto *et al.*, 1992). In contrast to these other peptides, Neuropeptide Y has been described as ‘conspicuously absent’ from the SCN of *Myotis lucifugus* (Laemle and Cotter, 1992). Bewildering in its chemoarchitecture, the SCN undoubtedly represents an area of complex interaction among neural systems that influence diverse physiological rhythms. However, its specific relationships to pineal function and to reproductive function in bats are yet to be investigated.

1.9 SUMMARY AND FUTURE PERSPECTIVES

Despite continuing efforts of investigators all over the world, our knowledge of central control of reproductive function in bats is fragmented and remains heavily biased toward the vespertilionids. Immunocytochemical studies of GnRH neurons have revealed a characteristic distribution of perikarya that differs in potentially significant ways from that seen in other mammalian groups. Differences in GnRH transport routes are also suggested by abundant neuronal projections that extend into the pituitary neural lobe as well as by the architecture of the portal vasculature, which at least in *M. lucifugus* appears to be composed primarily of short portal vessels. Distributions of pituitary gonadotropes and lactotropes have been determined by immunocytochemistry. In combination with information derived from plasma gonadotropin assays, these studies have provided insight into the seasonal dynamics of LH-, FSH- and prolactin-secreting cells in species exhibiting varied reproductive patterns.

Progress is slow yet steady toward the ultimate goal of understanding how central neuroendocrine mechanisms orchestrate the truly fascinating array of reproductive phenomena exhibited by bats. Research has brought to light that delayed ovulation may result from long-loop negative feedback of steroids on the hypothalamic-pituitary complex. However, it is yet to be determined whether seasonal modifications in hypothalamic and/or pituitary sensitivity to steroids contribute to this mechanism. In turn, could pineal mechanisms modulate this sensitivity? Separate lines of investigation have yielded evidence that prolactin rhythms may hold the key to delayed implantation. However, it is not yet known how prolactin secretion is regulated in bats, and how LH might interact with prolactin to fine-tune the endocrine mechanisms synchronizing reproductive cycles with environmental changes. While we have become familiar with GnRH neurons in a few bats, very little is known of the 'higher' neuronal systems that modulate their activity and thus intervene in reproductive physiology from the very top of the hypothalamic-pituitary-gonadal axis. For example, how might stress introduced by captivity alter the central mechnisms which have evolved in free-living populations?

These are all challenging questions, and the seemingly endless variety of reproductive patterns in bats multiply the challenges ahead manyfold. At times the task seems daunting and growth of our knowledge painfully slow. Nevertheless, future generations of curious investigators will continue in the tradition of Bill Wimsatt to deepen as well as broaden our understanding of endocrine mechanisms that support reproductive diversity.

ACKNOWLEDGMENTS

Microvascular corrosion casting studies in *M. lucifugus*, which were conducted by the author in collaboration with Dr Alvar Gustafson and Ms Patricia Overdeep, were supported by the Rhode Island College Faculty Research Fund. We would like to thank Mr Patrick Verdier and Dr Paul McMillan of the Rhode Island Hospital Central Research Facility for their technical assistance with this project.

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2

Endocrine Regulation of Reproduction in Bats: The Role of Circulating Gonadal Hormones

Len Martin and Ric T.F. Bernard

2.1 INTRODUCTION

The Chiroptera have undergone a rich adaptive radiation producing a high degree of diversity in reproductive habits, structures and mechanisms (Krutzsch, 1979; Racey, 1982; Rasweiler, 1993; and elsewhere in this book) and we should expect the endocrine control of reproduction, in at least some bats, to be quite different

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Reproductive Biology of Bats
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from that of other mammals. Indeed, in many bats, there are no obvious changes in vaginal cytology during the breeding season or pregnancy; often the state of the ovary cannot be judged from visual examination; radioimmunoassays of estrogen and progesterone give anomalous results; there is no distinct period of behavioural oestrus to pinpoint time of ovulation; and females may copulate frequently and repeatedly over long periods and well into pregnancy. Such variation from the mammalian norm is perhaps at its most extreme in the hibernating bats (Wimsatt, 1960, 1969) where there may be temporal separation of spermatogenesis, libido and accessory gland function in males, and temporal separation of behavioural oestrus and ovulation in females. In addition, delays in implantation and development of the embryo occur in some bats. Not surprisingly, where data are available, patterns of circulating hormones are often peculiar.

2.2 PERIPHERAL HORMONE LEVELS AND THEIR EVALUATION

2.2.1 Factors Affecting Peripheral Plasma Hormone Levels

The peripheral concentration of a hormone reflects the balance between rates of secretion and metabolic clearance, with the latter influenced by the extent to which the hormone is bound by plasma proteins. Steroids may be bound weakly by serum albumin (non-specific, high-capacity, low-affinity binding) and strongly by proteins such as steroid binding globulin (SBG) and corticosteroid binding globulin (CBG) all of which exhibit limited-capacity, stereospecific, high-affinity binding of specific steroids (Gustafson and Damassa, 1987; Kwiecinski *et al.*, 1987). It is generally accepted that the *effective* hormone concentration is that of the free or weakly-bound hormone in the vicinity of its cellular receptor. Steroid binding globulins have been reported in a number of bats and knowledge of total peripheral hormone concentration may be of limited value.

2.2.2 Direct Assay of Peripheral Plasma Hormone Levels

A range of techniques has been used to assay plasma hormone levels in bats and, consequently, it is often difficult to compare the results from different laboratories. A detailed critique of RIA methodology is beyond the scope of this review but we emphasize that, for results to be scientifically acceptable, it is essential that the assay is validated on the material (e.g. bat plasma) under study; commercially available kits have usually only been validated for human or laboratory animal plasma. Many aspects of assay validation are made difficult in bats because of the limited blood volumes available and limited opportunity for repeated sampling from individuals. Nevertheless Gustafson and Damassa (1984a) have described a method for repeated blood sampling from microbats and McGuckin (1988) and Kennedy (1993) collected blood repeatedly from male and female *Pteropus* spp. through successive breeding seasons and pregnancies.

Given the limits of sensitivity of existing assays, published data for bats indicate that plasma androstenedione (A), testosterone (T), 5α-dihydrotestosterone (DHT) and progesterone should be easily assayable in small blood samples. Oestradiol, usually being in the pg range, will be at the limits of sensitivity and better indicators of ovarian function may be A, T or inhibin.

2.2.3 Assay of Steroid Hormones in Faeces and Urine

Measurement of urinary and fecal steroids is a useful non-invasive means of assessing reproductive status and ovarian cycles (Hodges *et al.*, 1983; Hodges, 1986; Hindle *et al.*, 1992; Atkinson *et al.*, 1995). By their nature, faeces and urine require much more purification than plasma before assay since the bulk of steroids are excreted as conjugated metabolites which require hydrolysis and other sophisticated methodology for adequate quantification. Also there is much unpredictable between-species variation in the spectrum of metabolites excreted. Nevertheless, this is an established methodology which might find further application in bat research.

2.2.4 Other Means of Assessing Gonadal Endocrine Activity

Gonadal endocrine activity can be inferred from the structure, ultrastructure and enzyme histochemistry of endocrine tissues (Racey and Tam, 1974; Jerrett, 1979; Crichton *et al.*, 1990; Abhilasha and Krishna, 1996). Further evidence of changes in gonadal endocrine activity may be gained from examination of target organs such as the reproductive accessory glands, and sebaceous glands in males, and the uterus and vagina in females. However, changes, or often their absence, in the morphology of target organs of bats must be interpreted with caution. For example, the vaginal epithelium, which undergoes cycles of oestrogen-dependent cornification in rodents, is always cornified in *Pteropus* spp., even in pregnancy, and in the Cape horseshoe bat (*Rhinolophus capensis*) vaginal cornification does not appear to be tightly linked to ovarian endocrine activity (Bernard, 1985). Similarly, in male *Myotis lucifugus* (and many other vespertilionids), accessory glands are maintained in a hypertrophied state and appear active during winter, when Leydig cells are small and plasma T low (Gustafson, 1987).

2.3 SOURCE AND SYNTHESIS OF HORMONES IN THE CHIROPTERA

2.3.1 Source and Synthesis of Hormones in Male Bats

The important circulating gonadal hormones in male mammals are the androgens (An) T, A and DHT, oestrogens such as oestradiol, and inhibin. In male bats, the most commonly measured steroid hormones have been the androgens.

Inhibin, oestrogen and progesterone

There are no available data for circulating levels of inhibin and information on estrogens is limited to a report of levels of oestradiol in *Myotis lucifugus* which notes that the source of the hormone is unknown (Damassa and Gustafson, 1984). Wang *et al.* (1992) and Martin *et al.* (1995), found an unusually high peripheral progesterone (10–40 ng/ml) in intact and castrate male Australian flying-foxes

Pteropus poliocephalus, *P. alecto* and *P. scapulatus* and suggested the adrenal cortex as a probable source of this hormone.

Androgens

In mammals 95% of T is synthesized by the Leydig cells and other sources of androgens include the adrenal gland and the Sertoli cells (Bardin *et al.*, 1988; Coffey, 1988). The Sertoli cells do not contribute significantly to the plasma androgen pool (Bardin *et al.*, 1988), however the adrenal should always be considered as a possible source of sex steroids, particularly when peripheral levels are unusual (Parkes and Deanesly, 1965). Although 3β-hydroxysteroid-dehydrogenase (3β-HSD) and 17β-hydroxysteroid-dehydrogenase (17β-HSD) have been localized in the seminiferous tubules and Sertoli cells of *Myotis lucifugus* (Gustafson, 1975) there have been no other studies of the androgenic properties of Sertoli cells of bats. The very limited data for the adrenal gland of Microchiroptera suggest that it is not a significant source of androgens (Racey and Tam, 1974; Racey, 1974). In *Pteropus poliocephalus* adrenal weight is greater in the breeding season than the non-breeding season; however, adrenal T is much lower than testicular T and the adrenal gland contributes little to the breeding season increase in peripheral T (McGuckin and Blackshaw, 1991a). However, T is detectable in castrate *P. poliocephalus* and McGuckin (1988) speculates that this might be of adrenal origin. In the present review, we concentrate on the structure and function of Leydig cells.

The Leydig cells of bats undergo changes in ultrastructure that coincide with spermatogenic activity and, or an increase in plasma T and which are similar to those previously described for other mammals (Kayanja and Mutere, 1978, *Otomops martiensseni*; Ohata, 1979, *Myotis schreibersi* [= *Miniopterus schreibersii*]; Gustafson, 1979, *M. lucifugus*; Loh and Gemmell, 1980, *Myotis adversus*; Bernard, 1986, *Rhinolophus capensis*; Krutzsch and Crichton, 1987, *Mormopterus planiceps*; Krutzsch and Crichton, 1990a, Bernard *et al.*, 1991a, *Miniopterus schreibersii*). In most studies, the coincidence of changes in Leydig cell ultrastructure with increasing plasma T is taken to indicate that the Leydig cells are steroidogenically active. The pattern of ultrastructural change is not consistent throughout the Chiroptera and in *Taphozous georgianus*, where spermatogenesis is seasonal and plasma T varies from 0.4 to 2.1 ng/ml, the fine structure of the Leydig cells does not change seasonally (Jolly and Blackshaw, 1989). The authors interpret this as indicating the imposition of a cold induced winter shutdown of spermatogenesis on a framework of continuous spermatogenesis. By contrast, in *Molossus fortis*, males are probably spermatogenically active throughout the year but the size of the Leydig cells varies, reaching a maximum at the time of the first oestrus of the annual, bimodal female cycle (Krutzsch and Crichton, 1990b).

Enzyme histochemical studies of the Leydig cells of the seasonally breeding bats *Pipistrellus pipistrellus* and *Myotis lucifugus* have shown the presence of 3β-HSD throughout the year, and 17β-HSD (which was not detected in *P. pipistrellus*) in the Leydig cells of *M. lucifugus* during spermatogenesis only (Racey and Tam, 1974, Gustafson, 1979 for review). The most direct evidence of the role of the Leydig cells in steroidogenesis comes from work on *Pteropus poliocephalus* where plasma T in castrates is an order of magnitude lower than in intact males (McGuckin, 1988). Treatment of reproductively non-active *P. poliocephalus* with human chorionic gonadotropin (hCG) induced an immediate linear increase in T which reached ~16 ng/ml 90 minutes later. Treatment with synthetic gonadotropin releasing hormone (GnRH) had no significant effect on plasma T (McGuckin, 1988).

Steroid binding globulins

In *Myotis lucifugus*, production of SBG is regulated by photoperiod acting via the thyroid, since post-arousal increases in levels of SBG can be induced by increased photoperiod and blocked by thyroidectomy, but not castration (Gustafson and Damassa, 1987).

Commentary: The mass of indirect evidence and the far smaller number of experimental studies all suggest that the Leydig cells are the principal source of peripheral androgens. Changes in Leydig cell size and ultrastructure are reliable indicators of steroidogenic activity but there are anomalous species such as *Taphozous georgianus* and *Molossus fortis* where Leydig cell ultrastructure does not reflect plasma T. A single study provides evidence that the Sertoli cells have the enzymes necessary for steroidogenesis, and several authors have speculated that the adrenal gland may produce some oestrogens, progesterone and T during the non-breeding season.

2.3.2 Source and Synthesis of Hormones in Female Bats

In female mammals, the major circulating ovarian hormones are oestradiol, androgens such as A and T, progestins such as progesterone, and inhibin. The two principal sources of steroid hormones are the ovary (interstitial tissue, thecal cells, granulosa and luteal cells) and placenta (see Gore-Langton and Armstrong, 1994 for review). Inhibin may be derived from luteal cells or the granulosa (Burger *et al.*, 1995).

While the steroidogenic activity of the mammalian ovary has been and remains an active field of research, very few studies have examined ovarian steroidogenesis in bats. This is in spite of the fact that the structure of the ovary of several species poses interesting questions about ovarian steroidogenesis.

Microchiroptera

Androgens and oestrogens. In a typical mammal the interstitial and thecal cells stain positively for 3β-HSD and are involved in the *de novo* production of androgens. The follicular cells (granulosa) stain positively for 17β-HSD, aromatase cytochrome P450, cytochrome $P450_{scc}$ and 3β-HSD and metabolize the androgens to produce oestrogens. The granulosa may also be involved in the *de novo* production of progesterone which can then serve as a substrate for the biosynthesis of further oestrogens (Gore-Langton and Armstrong, 1994).

The interstitial and thecal cells of *Tadarida brasiliensis* and *Scotophilus heathi* possess the enzymes necessary to produce androgens (Jerrett, 1979 and Abhilasha and Krishna, 1996 respectively). However, in most members of the genus *Tadarida*, the left ovary is essentially an interstitial organ with no large Graafian follicles or corpora lutea and the fate of the androgens is unknown.

In *Antrozous pallidus* the interstitial and thecal cells stain positively for both 17β-HSD and 3β-HSD (Oxberry, 1979) suggesting that they may be capable of *de novo* production of oestrogens. The ovaries of *Macrotus californicus* lack interstitial tissue but a proportion of the multilaminar follicles have a prominent theca interna (Crichton and Krutzsch, 1985). The granulosa and theca interna stain positively for both 17β-HSD and 3β-HSD suggesting that they may be capable of *de novo* production of oestrogens (Crichton *et al.*, 1990). This has previously been reported for atretic follicles and the authors note that many of the positive staining follicles in *M. californicus* may be atretic. Follicular atresia is often associated with luteinization of the thecal and granulosa cells, producing accessory corpora lutea. This phenomenon is particularly developed in some vespertilionids (Guthrie and Jeffers, 1938; Mossman and Duke, 1973; van der Merwe, 1979) and the role of these additional endocrine structures warrants study.

In *Miniopterus schreibersii*, plasma oestradiol peaks in mid-gestation, suggesting that, at least during pregnancy, this hormone may be of placental origin (Crichton *et al.*, 1989).

Progesterone. The two major sources for progesterone in bats are the corpus luteum and the placenta with the relative importance of these two organs differing both temporally within a species and between species. Additional sources of progesterone are the ovarian interstitial tissue and the adrenal gland. The most complete data are available for *Myotis lucifugus* and *Miniopterus schreibersii* and clearly illustrate these points. In both species the placenta takes over progesterone production from the corpus luteum in the final third of pregnancy (Buchanan and Younglai, 1988 and van Aarde *et al.*, 1994 respectively). Less complete data, indicating that the placenta takes over progesterone production from the corpus luteum are available for many other species. For example, the corpus luteum of a range of species undergoes luteolysis in late pregnancy (e.g. Kayanja and Mutere, 1975, *Otomops martiensseni*; Kitchener and Halse, 1978, *Chalinolobus gouldi* and *Eptesicus regulus*; Kitchener and Coster, 1981, *Chalinolobus morio*; Gopalakrishna, 1969, Gopalakrishna *et al.*, 1986, *Rousettus leschenaulti*; Towers and Martin, 1995, *Pteropus poliocephalus*). Furthermore, there are a number of species in which the corpus luteum is only present for the first half of pregnancy. This appears to have a taxonomic basis and some members of the Families Nycteridae (Matthews, 1941, *Nycteris luteola*; Bernard, 1982, *N. thebaica*), Rhinolophidae (including the Hipposideridae) (Matthews, 1941, *Triaenops afer*; Bernard and Meester, 1982, *Hipposideros caffer*; Gopalakrishna and Badwaik, 1988, *H. fulvus, Rhinolophus rouxi, R. microphyllum*; Gopalakrishna and Badwaik, 1989, *Hipposideros lankadiva*) and Megadermatidae (Gopalakrishna and Badwaik, 1988, *Megaderma lyra*) have a corpus luteum that is extrovert and which is lost in early or mid-pregnancy. In all these species, the probable source of progesterone after luteolysis is the placenta, but this needs to be established.

Many other species of Chiroptera have a corpus luteum that persists through pregnancy to just before, or after parturition (e.g. *Mormopterus planiceps*; Crichton and Krutzsch, 1987; *Antrozous pallidus*; Oxberry, 1979; *Tadarida brasiliensis*; Jerrett, 1979 and *Macrotus californicus*; Crichton *et al.*, 1990). In *M. californicus* the corpus luteum and multilaminar follicles stain strongly for 3β-HSD and weakly for 17β-HSD during gestation suggesting that both synthesize progesterone and oestrogen (Crichton *et al.*, 1990).

In several species, plasma progesterone levels in non-pregnant females are high (~10 ng/ml; Oxberry, 1979; Krutzsch unpublished; Crichton *et al.*, 1990; Jerrett, 1979; Buchanan and Younglai, 1988; van Aarde *et al.*, 1994). In *Tadarida brasiliensis* and *Myotis lucifugus* the interstitial tissue, which has substantial steroidogenic potential, is possibly the source of this progesterone (Jerrett, 1979 and Guraya and Greenwald, 1964; Guraya, 1967 respectively). In *Antrozous pallidus* enzyme histochemical studies suggest that the ovarian interstitial tissue is not the source of the progesterone (Oxberry, 1979). In *Macrotus californicus*, where there is no interstitial tissue, the granulosa does not stain for 3β- or 17β-HSD at this time and the progesterone in non-pregnant females is not of ovarian origin (Crichton *et al.*, 1990). By contrast, in non-pregnant *Miniopterus schreibersii*, total adrenal progesterone content and adrenal progesterone levels are much higher than ovarian (adrenal, 10 ng and 25 ng/mg; ovary, 0.8 ng and 3 ng/mg), thus, adrenal secretion may well be responsible for the very high plasma progesterone levels in non-pregnant and post-partum animals (van Aarde *et al.*, 1994).

Ovarian steroidogenic cells contain a characteristic suite of organelles (lipid droplets, smooth endoplasmic reticulum and mitochondria) that are typical of tissues involved in steroid biosynthesis (Hall, 1984; Hoyer *et al.*, 1988). The data for Chiroptera (Kayanja and Mutere, 1975; Kimura and Uchida, 1983; Uchida *et al.*, 1984; Crichton and Krutzsch, 1985, 1987; Crichton *et al.*, 1989; Bernard *et al.*, 1991b) indicate that the changes in luteal ultrastructure that occur with changes in steroidogenic activity are similar to those reported for Leydig cells of bats.

Two studies examining the possible occurrence of sub-classes of luteal cells in bats have indicated that there may be two size classes in *Macrotus californicus* and *Miniopterus schreibersii* (Crichton *et al.*, 1989, 1990), but the results are equivocal and require further study.

Megachiroptera

Androgens and oestrogens. Plasma T is detectable in intact and ovariectomized *Pteropus poliocephalus* during the breeding season and is probably of adrenal origin (Towers and Martin, 1985; Kennedy, 1987; Towers, 1988).

In *Pteropus* spp. plasma oestradiol increases through pregnancy, is highly correlated with placental weight ($r = 0.75$, $P < 0.001$), and is probably of placental origin (Towers and Martin, 1985; Towers, 1988). It is, however, likely that the developing preovulatory follicles produce oestradiol and the presence of oestradiol in the plasma of ovariectomized, non-pregnant animals indicates that there is a non-ovarian source of this hormone (Towers, 1988).

Progesterone. The data for Australian *Pteropus* reflect a common pattern in which plasma progesterone does not vary substantially during follicular development or after formation of the corpus luteum or ovariectomy. Plasma progesterone levels increase from mid-pregnancy to reach 200–800 ng/ml close to term. The size of the corpus luteum decreases during pregnancy, and is negatively correlated with plasma progesterone ($r = -0.69$, $P < 0.001$). In late pregnancy, plasma progesterone is positively correlated with placental weight (r = 0.79, $P < 0.001$) and placentae contain 4–8 μg progesterone/g, indicating that the placenta is the major source of progesterone over this period (Towers and Martin, 1985, 1995).

In intact non-pregnant *P. poliocephalus* without corpora lutea, plasma progesterone varies between 2 and 30 ng/ml and is 1–85 ng/ml in ovariectomized bats. The authors suggested, but did not substantiate, that the adrenal may be a significant source of this progesterone.

Inhibin. In intact female *Pteropus* the predominant source of inhibin appears to be the ovary, with relatively little secretion before ovulation, and that presumably from antral follicles (Kennedy, 1993). A large increase in plasma inhibin occurs after formation of the corpus luteum and presumably reflects secretion by luteal cells. Inhibin levels decline during pregnancy, coinciding with the decline in function of the corpus luteum described by Towers and Martin (1995). The placenta does not appear to be a major source of inhibin. However, there is an extra-ovarian source, possibly the adrenal, with secretion exacerbated by the abnormal endocrine balance resulting from ovariectomy (Kennedy, 1993).

2.4 PERIPHERAL HORMONE CONCENTRATIONS, BINDING PROTEINS AND HORMONE ACTION IN MALE BATS

Peripheral hormone concentrations vary with the pattern of reproduction shown by the species and, in this section, species are grouped firstly by pattern of reproduction and secondly, by family (or subfamily). Detailed data are presented for a representative species and, where appropriate, brief summaries are presented for other species.

2.4.1 Microchiroptera (See Table 2.1)

Sperm storage and prolonged libido

Vespertilioninae. The most comprehensive data set is for the hibernating bat *Myotis lucifugus* from north temperate latitudes (~40°N) in the USA (Figures 2.1 and 2.2a). Following arousal from hibernation in March and April, there are coordinated increases in testis size, spermatogenesis and Leydig cell function, and increases in peripheral T and SBG. Plasma T reaches a peak of ~59 ng/ml, which coincides with the peak in spermatogenesis and which is much higher than in many other mammals (Gustafson and Shemesh, 1976). However, there is little increase in accessory gland size until after peak spermatogenesis, when plasma T and SBG levels start to fall (Figure 2.1). The mass of the accessory glands and libido are maintained throughout hibernation, in the face of baseline plasma T and SBG levels (Figure 2.1). Gustafson and Damassa (1984, 1987) argue that, during spermatogenesis, high SBG levels produce a high peripheral T (by reducing clearance rate of T) but block growth of the accessory glands by reducing the free T available to androgen receptors on the accessory glands. At the start of winter, the decreases in peripheral T and SBG levels are phase-shifted so that the free T increases and stimulates the accessory glands. Their evidence comes from an

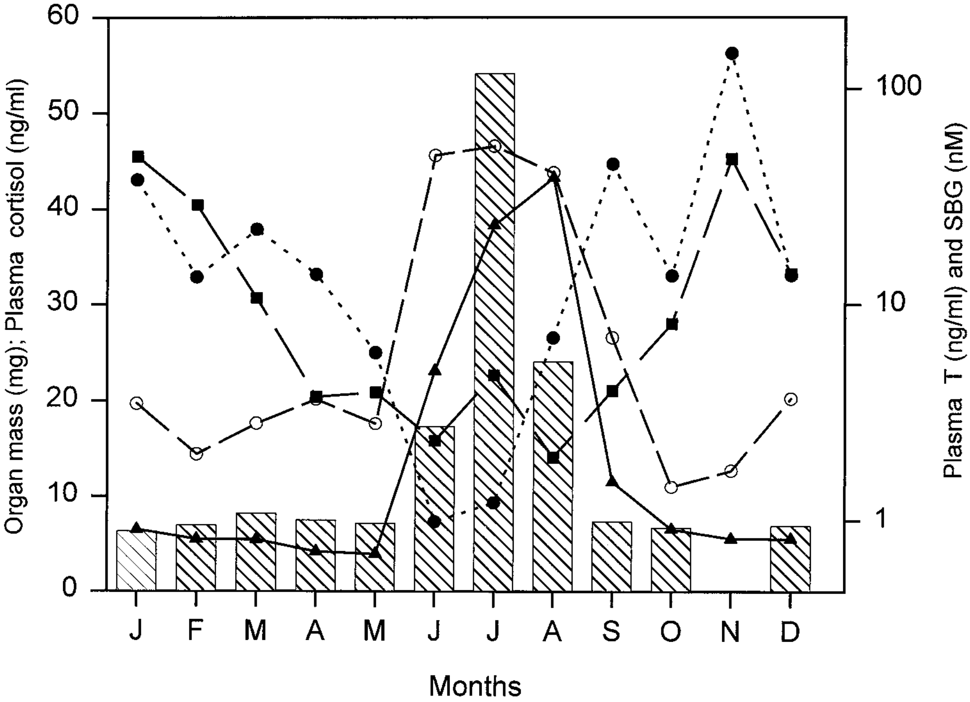

Figure 2.1 Monthly changes in reproductive organs and hormone levels in male *Myotis lucifugus* (Vespertilionidae). This figure is based on data from Gustafson and co-workers, redrawn and scaled to facilitate comparison. □ = testis mass (mg), ● = mass of the accessory gland/3 (mg), ○ = plasma SBG/4 (nM), ▲ = plasma T (ng/ml) from Gustafson and Damassa (1984c, 1987), and ■ = plasma cortisol/25 (ng/ml) from Gustafson and Belt (1981). Note that the left ordinate scale is arithmetic and the right logarithmic.

experiment in which males were removed from hibernation, castrated and thyroidectomized (TRX; low SBG) or sham-thyroidectomized (sham-TRX; high SBG; Gustafson *et al.*, 1985). After exposure to long photoperiod [16L:8D] the bats were implanted with pellets containing 0, 0.03, 0.3 or 1.5 mg T, and maintained for two weeks, being bled at the end of each week. Mean plasma T concentrations show dose-dependent increases in both sham-TRX and TRX groups. However, at every dose level, peripheral T levels are significantly higher in the former [high-SBG group], indicating that SBG diminishes the clearance rate of T. Dose-dependent increases in the mass of the accessory glands occur in both sham-TRX and TRX groups. However, to attain similar gland masses between the groups or to attain the maximal response, a ten- to twenty-fold greater plasma T concentration is required in the high-SBG males than the low-SBG. Since TRX does not reduce the metabolic response of the accessory glands to androgens, it was concluded that inhibition of androgen action is due to high levels of SBG. Plasma SBG and T are high in neonatal *M. lucifugus* from 2–12 weeks of age (Gustafson and Damassa, 1984b). Fluctuations in plasma T and SBG levels are synchronized, but over the period of high plasma T there is no stimulation of the accessory glands and it is suggested that this could be due to elevated SBG levels (Gustafson and Damassa, 1984b).

Circulating cortisol levels are extremely high in *M. lucifugus* (Gustafson and Belt, 1981) and show a circannual cycle which closely parallels that of the accessory glands (Figure 2.1). Glucocorticoids and thyroid hormones can affect the endocrine responses of many tissues and such modulation of target-tissue responsiveness should be kept in mind in each new species investigated.

Plasma oestradiol is undetectable or low in male *M. lucifugus* at a time when SBG and T are elevated (<10 pg/ml, compared with 80 pg/ml in a plasma pool from late pregnant females; Damassa and Gustafson, 1984).

The asynchrony between elevated plasma T and activity of the accessory glands that characterizes *M. lucifugus* has been reported for *Nyctalus noctula* (~50°N, England; Racey, 1974; Figure 2.2b), *Pipistrellus pipistrellus* (~50°N, England; Racey and Tam, 1974; Figure 2.2c), *Vespadelus vulturnus* (South-eastern Australia; Tidemann, 1993; Figure 2.2d) and *Nyctophilus geoffroyi* (South-western Australia; Hosken *et al.*, 1998, Figure 2.2e). Seasonal changes in plasma T in *N. noctula* and *V. vulturnus* are very similar to those described for *M. lucifugus*. The results for *P. pipistrellus* are anomalous in that plasma T, which was measured three times during the year, is high at all times. However, the large gaps in the data make interpretation very difficult. The morphology of the Leydig cells of *P. pipistrellus* does not change during the year, but testicular T is an order of magnitude lower at the end of hibernation (February, 12.7 ng/pair) than at the start of hibernation (August, 125.3 ng/pair) suggesting that activity of the Leydig cells may be reduced during winter (Gustafson, 1979).

Rhinolophidae. In the Cape Horseshoe bat (*Rhinolophus capensis*; 33°S, South Africa; Bernard, 1985, 1986; Figure 2.3a), spermatogenesis starts in spring (October) and ceases after early winter (May) although sperm remain in the caudae epididymides through winter. Copulation and ovulation occur in August–September, at the end of winter hibernation. Peripheral T, which is low, peaks during late spermatogenesis in March at 6 ng/ml and declines through autumn to 0.2−1.2 ng/ml in winter. Accessory glands enlarge in early winter, as T declines, are maintained and active during the period of low T and start to atrophy as T increases in spring (September–November).

Molossidae. In *Mormopterus planiceps* from southeast Australia (36°S; Krutzsch and Crichton, 1987; Figure 2.3b), the annual spermatogenic cycle is prolonged, starting in spring (September/October) and ceasing in autumn (May). Spermiogenesis occurs from February to May and epididymal sperm persist to September/October. The accessory glands reach a maximum size in May and thereafter decrease in size through to mid-summer (December). This species does not appear to hibernate, although its metabolism is greatly reduced during winter. Mating probably occurs from late summer (May) through winter and births occur in December. Peripheral A and T range from 0.2−5 ng/ml, increasing over autumn and winter to a peak in August/September, as the spermatogenic cycle begins, and declining to a mid-summer minimum. There is a small March peak in plasma T which coincides with maximum testis size and a sharp increase in the size of the accessory glands. Both testes and accessory glands regress from May to midsummer,

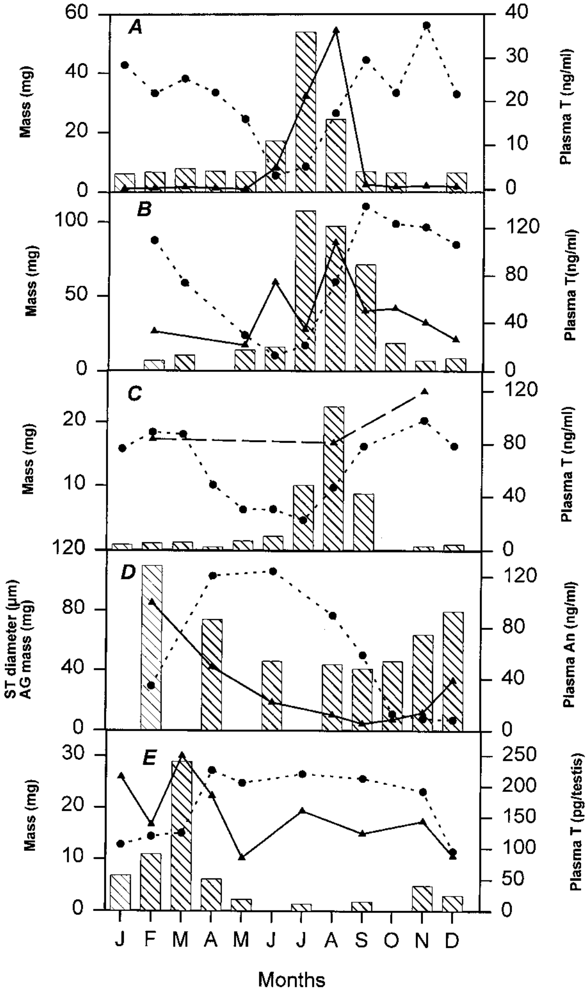

Figure 2.2 Seasonal changes in size of the testes (□) and accessory glands (●) and plasma androgen levels (▲) in male vespertilionids redrawn from published data and scaled to facilitate comparison of different circannual patterns. Where possible, organ weights are given; where not available, we have used testis length or volume, or seminiferous tubule (ST) diameter for gonad size and accessory gland (AG) volume, length, or epithelial height. (A) *Myotis lucifugus* [Northern hemisphere]. Data and sources as for Figure 2.1. (B) *Nyctalus noctula* [Northern hemisphere]. Redrawn from Racey (1974) and scaled as follows. Testis mass/5 and accessory gland mass/3 (mg). Plasma androgen is T (ng/ml). (C) *Pipistrellus pipistrellus* [Northern hemisphere]. Redrawn from Racey and Tam (1974) and scaled as follows. Testis mass/10 and accessory gland mass/3 (mg). Plasma androgen is T (ng/ml). (D) *Vespadelus (Eptesicus) vulturnus* [Southern hemisphere]. Redrawn from Tidemann (1993) and scaled as follows. ST diameter (µm) and accessory gland mass/2 (mg). Plasma androgen (ng/ml; determined by RIA with an antibody showing 32% cross-reaction to DHT). (E) *Nyctophilus geoffroyi* [Southern hemisphere]. Redrawn from Hosken *et al.* (1998) and scaled as follows. Testis mass (mg) and prostate epithelial height x1.5 (µm). 'Plasma androgen' is T (pg/testis, determined by RIA of ether extracts of formalin-fixed testes which had been stored in 70% aqueous ethanol).

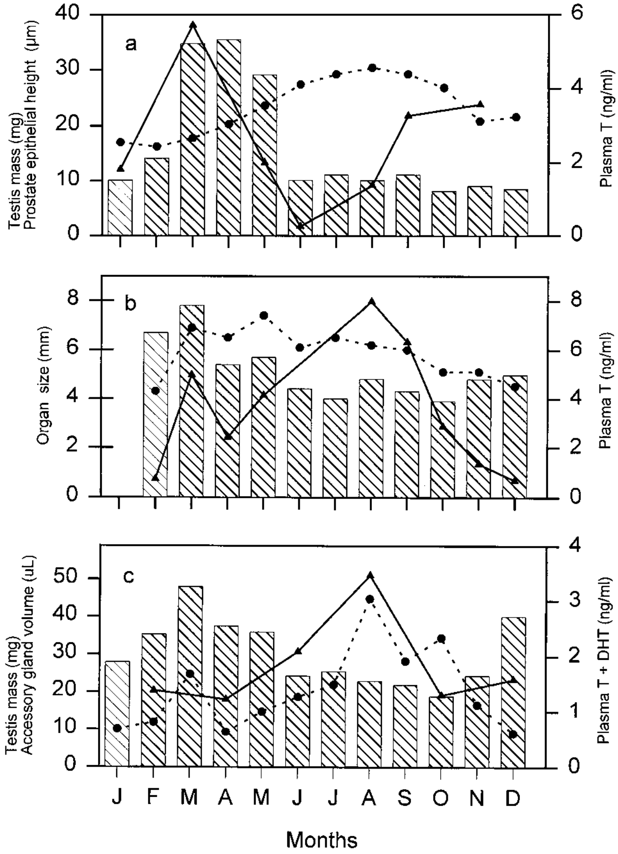

Figure 2.3 Seasonal changes in size of the testes (□) and accessory glands (●) and plasma androgen levels (▲) of non-vespertilionid bats which store sperm in the cauda epididymidis. (a) *Rhinolophus capensis* (Rhinolophidae) [Southern hemisphere]. Redrawn from Bernard (1986) and scaled as follows. Testis mass (mg) and prostate epithelial height × 1.5 (µm). Plasma androgen is T (ng/ml determined by RIA with check that no DHT was detectable). (b) *Mormopterus planiceps* (Molossidae) [Southern hemisphere]. Redrawn from Krutzsch and Crichton (1987) and scaled as follows. Testis length and length of Cowpers gland ×2 (mm). Plasma androgen is T (ng/ml, determined by separate RIAs after chromatographic separation). (c) *Taphozous georgianus* (Emballonuridae) [Southern hemisphere]. Redrawn from Jolly and Blackshaw (1989) and Jolly (1990) and scaled as follows. Testis mass (mg) and seminal vesicle volume ×3 (µl). Plasma androgen is T+DHT (ng/ml; RIA after chromatographic separation).

despite rising concentrations of androgens. Thus there is only loose linkage between peripheral androgen concentrations and testicular and accessory gland function.

Emballonuridae. The reproductive cycle of *Taphozous georgianus* in Queensland, Australia (~24°S; Figure 2.3c) is surprisingly similar to that of *Mormopterus planiceps*. Peak spermatogenic activity occurs in summer and autumn (January to May), declines over winter and ceases in spring (Jolly and Blackshaw, 1989). However, spermatozoa are present in the caudae epididymides throughout the year. The accessory glands enlarge in August/September, when mating occurs, and are regressed at other times of the year (November–July; Jolly and Blackshaw, 1989). Peripheral A and DHT levels are high compared with T and follow the same pattern of annual change. Peripheral androgen levels peak in late winter (August), coincident with increased volume of the accessory glands, and about one month before mating. Interestingly, in this species, as in *M. planiceps*, the seminiferous tubules regress as androgen levels peak in winter. Thus spermatogenesis and activity of the accessory glands are dissociated.

Commentary: With the exception of *Mormopterus planiceps* and *Taphozous georgianus*, the preceding examples are characterized by an asynchrony between elevated plasma T and activity of the reproductive accessory glands. Plasma T increases with increasing spermatogenic activity, but, as plasma T declines, so the accessory glands increase in size. The only exception is *Pipistrellus pipistrellus* where the Leydig cells appear to remain active and plasma T is elevated throughout winter. The data for *Myotis lucifugus* suggest that SBGs, by effectively decreasing the amount of free hormone, are responsible for the asynchrony in the development of the testes and accessory glands. Although *M. planiceps* and *T. georgianus* store sperm through winter, the hormonal control of reproduction is quite different from the other members of the group. Spermatogenesis and activity of the accessory glands is synchronized but not obviously linked to plasma androgen concentrations. Clearly, in these examples, SBGs may play an important role and it is very difficult to interpret the data without information on peripheral SBG levels. It is thus essential that all future studies that aim to contribute to our understanding of the endocrine control of sperm storage and prolonged libido measure both androgens and the SBGs.

Leydig cell activity

Early studies (Courrier, 1927; Racey, 1974; Racey and Tam, 1974; Krutzsch, 1961, 1975; Gustafson, 1979) suggested that species in which sperm storage and prolonged libido occur during winter possess one of two quite different patterns of Leydig cell activity. In the *Pipistrellus* Pattern, Leydig cells are active during winter, while in the *Myotis* Pattern they are inactive. Since, in both cases, spermatogenesis occurs in summer and the accessory glands are active during winter, these early results suggested an astonishing asynchrony between activity of the Leydig cells and activity of the accessory glands in the *Myotis* Pattern. Gustafson (1979) carefully analysed the available information and concluded that the *Pipistrellus* and *Myotis* Patterns were probably more similar than first appeared, and that the apparent asynchrony between exocrine and endocrine components of the testis of bats that hibernate may not be real. Based on the information presented in the preceding species accounts, it appears that the general trend is for steroidogenic activity of the Leydig cells and high plasma T to coincide with spermatogenesis in summer, while in winter, when accessory glands are active (or maintained in a hypertrophied state) and libido maintained, the Leydig cells are inactive and plasma T is low. This is confirmed in a range of other species for which data are less complete (Kayanja and Mutere, 1978, *Otomops martiensseni*; Krutzsch and Crichton, 1990b, *Molossus fortis*; Krutzsch *et al.*, 1992, *Rhinolophus megaphyllus*). Thus, as data accumulate, the existence of the *Myotis*

Pattern is confirmed but there is little support for the occurrence of the *Pipistrellus* Pattern. Of the three species that have high plasma T during winter, one is *P. pipistrellus* (after which the pattern is named) while in *Mormopterus planiceps* the Leydig cells are inactive in winter and in *Taphozous georgianus* there are no seasonal changes in the ultrastructure of the Leydig cells. It should however be noted that the *Pipistrellus* Pattern was originally described from high latitudes (above 50°N) and the information presented above is for species from lower latitudes; thus the occurrence of the *Pipistrellus* Pattern remains untested.

Synchronized male reproductive activity, no sperm storage

A variety of species show what might be regarded as typical mammalian reproduction.

Phyllostomidae. In the non-hibernating *Macrotus californicus* in Arizona (~35°N; Krutzsch *et al.*, 1976; Figure 2.4a), activity of the accessory glands and seminiferous tubules are synchronized. Plasma T peaks in July and August at ~2.7 ng/ml, coincident with spermatogenesis and development of the accessory glands. Plasma T declines progressively from August to ~0.5 ng/ml in mid-winter (January), then increases to 2.0 ng/ml in March, well before the start of spermatogenesis in June, and without any apparent stimulation of accessory glands. Leydig cell activity mirrors that of plasma T.

Miniopterinae. In *Miniopterus schreibersii* (e.g. 33°S, South Africa; Bernard *et al.*, 1991a; Figure 2.4b) spermatogenesis occurs in late summer (February to April) and coincides with activity of the Leydig cells and accessory glands. Plasma T is greatest (15–20 ng/ml) between March and May, coinciding with peak testis weight and activity of the Leydig cells and accessory glands. After spermatogenesis, plasma T falls to 2–3 ng/ml in winter. A second peak in T (~10 ng/ml) occurs in spring (October), some four months before the next spermatogenic cycle. This peak is not associated with any apparent increase in testis size, accessory gland function or Leydig cell activity and is another example of a dissociation between peripheral T and accessory gland response.

Krutzsch and Crichton (1990a) found low peripheral androgen levels in Australian *M. schreibersii* throughout the reproductive cycle (Figure 2.4c; A, 0.1–0.6 ng/ml; T, 0.1–0.2 ng/ml). Testis and accessory gland weights cycle in parallel and peak in late autumn (April/May), yet peripheral A and T are minimal in May. Peripheral T shows virtually no other seasonal change, while A is maximal in November at the start of the annual testicular cycle.

Commentary: The control of spermatogenesis and accessory gland activity in these species appears normal. There is however some evidence for both species that at certain times of the annual cycle, the accessory glands do not respond to increasing plasma T and this again highlights the need to measure both androgens and SBGs.

Steroid binding globulins

Kwiecinski *et al.* (unpublished data referred to in Gustafson and Damassa, 1987) detected a SBG in the vespertilionids *Myotis keenii, Eptesicus fuscus* and

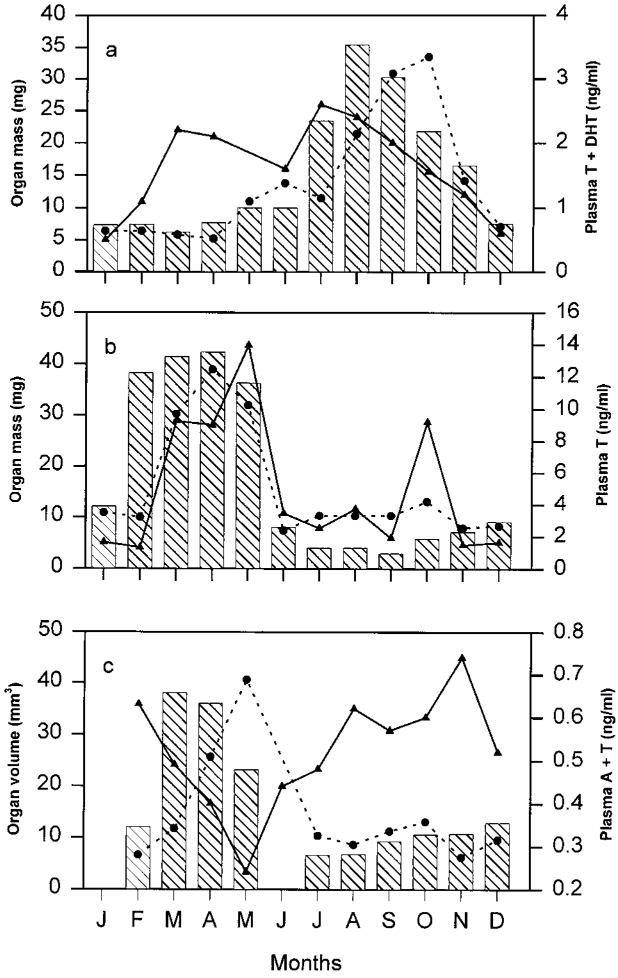

Figure 2.4 Seasonal changes in size of the testes (□) and accessory glands (●) and plasma androgen levels (▲) in male Microchiroptera with synchronized reproductive activity. (a) *Macrotus waterhousii* (Phyllostomidae) [Northern hemisphere]. Redrawn from Krutzsch *et al.* (1976) and scaled as follows. Testis mass (mg) and mass of the prostate gland and seminal vesicle ×2 (mg). Plasma androgen is T+DHT (ng/ml). (b) *Miniopterus schreibersii* (Vespertilionidae) in South Africa. Redrawn from Bernard *et al.* (1991a) and showing monthly changes in mass of the testes and accessory glands (mg) and plasma androgen (T, ng/ml, RIA with antibody showing 5.1% cross-reactivity to DHT). (c) *M. schreibersii* in SE Australia. Redrawn from Krutzsch and Crichton (1990a) and scaled as follows. Testis volume (mm^3) and volume of the Cowpers gland ×7 (mm^3). Plasma androgen is A+T (ng/ml, determined by specific RIAs after chromatographic separation).

Pipistrellus subflavus, and the phyllostomids, *Artibeus jamaicensis* and *Brachiphylla cavernarum*, but not the desmodontid, *Desmodus rotundus murinus*. This SBG bound T, DHT and oestradiol with high affinity. Kwiecinski *et al.* (1987) also detected a CBG which bound androgens to varying degrees in all of the species examined, except *D. rotundus*. Given the wide variation in peripheral T among male bats and the asynchrony between Leydig cell activity and activity of the accessory glands, a broad survey of plasma SBGs in bats is long overdue.

2.4.2 Megachiroptera (see Table 2.1)

The reproductive cycles of all male Megachiroptera are similar and characterized by synchronized activity of the Leydig cells, spermatogenesis and activity of the reproductive accessory glands.

There are no published data on peripheral oestrogens in male Megachiroptera; however, peripheral progesterone in intact and castrate male Australian flying-foxes *Pteropus poliocephalus, P. alecto* and *P. scapulatus* is high (10–40 ng/ml; Wang *et al.*, 1992 and Martin *et al.*, 1995; Figure 2.5a).

Androgens

The most extensive studies of androgen concentrations are those of McGuckin (1988) and McGuckin and Blackshaw (1987, 1991a, b) who investigated seasonally breeding *Pteropus* spp. in Southeast Queensland, Australia. In *P. poliocephalus* shot in the wild (Figure 2.5b), testis size increases from November to peak in March, early in the autumn mating season, then decreases to July and remains minimal until November. Plasma T is basal at ~2 ng/ml in winter and early summer (June–December), increases sharply from January to peak at 46.3 ng/ml in March, and falls progressively thereafter to minimal values in winter (June; Figure 2.5b). Peripheral A follows essentially the same pattern, but is significantly lower than T during the breeding season (March peak = 16 ng/ml; non-breeding season ~4 ng/ml). Seminal vesicle weight increases sharply after the rise in peripheral T to peak at the height of the mating season, then falls rapidly to minimal values in June (Figure 2.5b).

Similar seasonal changes occur in peripheral T and A in captive adult *P. poliocephalus* maintained in open cages in Southeast Queensland. However, peak T is approximately half that of wild-shot bats, and occurs in April at the height of the mating season, while testicular recrudescence appears earlier than in the wild.

Pteropus scapulatus is nomadic, about half the body mass of *P. poliocephalus*, and breeds approximately six months later than *P. poliocephalus*. Mating occurs in spring (November–December) and young are born in autumn/early winter (May–June). In a small series of *P. scapulatus* from the wild, McGuckin (1988) recorded testis weights of ~1.8 g in February and June, and 6.9 g in October. Plasma T is high during spermatogenesis and immediately after mating (October, 2.6 ng/ml; January, 2.4 ng/ml) and lower in February (1.2 ng/ml), April (0.5 ng/ml) and June (0.8 ng/ml).

In captive *P. scapulatus*, testis size peaks in early summer (October) (McGuckin and Blackshaw, 1991b). Peripheral T is maximal in captives in October–November (~3 ng/ml), but other peaks of 2–3 ng/ml occur in February–March and May–June. In samples collected over 12 months from four captive castrate *P. scapulatus* (McGuckin, 1988), annual mean T ranges from 0.4–0.7 ng/ml for individuals. Thus peripheral T appears to be lower in *P. scapulatus* and the annual cycle less well defined than in *P. poliocephalus*. There may also be a proportionately larger adrenal contribution.

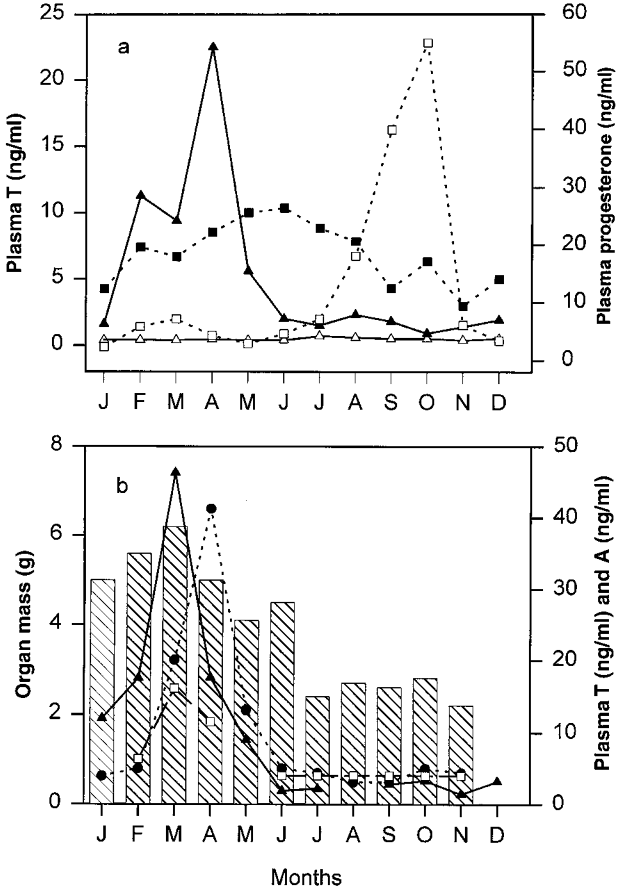

Figure 2.5 Seasonal changes in reproductive organs and plasma androgens and progesterone in male *P. poliocephalus* (Pteropodidae) [Southern hemisphere]. (a) Monthly changes in plasma progesterone (■) and T (▲, ng/ml; determined by RIA of ether extracts of pools of plasma from 3–5 captive bats, with each point the mean of two separate pools; redrawn from Martin *et al.*, 1995). Data for female bats, progesterone (□) and T(△), are included to allow comparisons. (b) Monthly changes in testis mass (□, g), mass of the seminal vesicle ×2 (●, g) and plasma A and T (respectively □ and ▲, ng/ml). Redrawn from McGuckin (1988) and McGuckin and Blackshaw (1987, 1991b).

Peripheral T is low in the central African fruit bat *Epomops franqueti*; 0.3 ng/ml in wild-caught adults with scrotal testes, compared with ~0.1 ng/ml in wild-caught adults and juveniles with abdominal testes (Ifuta *et al.*, 1988).

Steroid binding globulins

Kwiecinski *et al.* (unpublished data referred to in Gustafson and Damassa, 1987) failed to detect an SBG, but did detect a CBG in *Pteropus giganteus* and *Rousettus aegyptiacus*. The CBG binds androgens to varying degrees, and in

Pteropus and *Rousettus* shows relatively high affinity for DHT (Kwiecinski *et al.*, 1987). Wang *et al.* (1992) and Martin *et al.* (1995) also failed to detect an androgen binding SBG in three Australian species of *Pteropus*, but demonstrated a CBG which binds cortisol, corticosterone and progesterone with high affinity, but not oestradiol, T or DHT.

Commentary: Other than the anomalous high plasma progesterone levels and the extremely high T levels seen in the mating season in the absence of any high-affinity androgen binding protein, there appears to be nothing profoundly unusual about the endocrine-reproductive profile of male *P. poliocephalus*. The situation in *P. scapulatus* is less clear and needs further study. Questions remain about actual T, whether its annual pattern is different and possible adrenal contributions. The substantial difference in peak plasma T levels during spermatogenesis in *P. poliocephalus* and *P. scapulatus* (Table 2.1) is intriguing and cannot be attributed to differing methodologies since the results were obtained by the same researchers.

Table 2.1 Summary of plasma T or An in male Chiroptera during spermatogenic inactivity, spermatogenesis and, where applicable, winter sperm storage. This table is designed to reveal trends and readers are directed to the text and the original papers for exact values. NA, not applicable.

SPECIES (source)	Plasma [T] or [An] in ng/ml		
	Basal level during spermatogenic inactivity	Peak during spermatogenesis	Winter sperm storage
Microchiroptera			
	SPERM STORAGE AND PROLONGED LIBIDO DURING WINTER		
M. lucifugus[1]	<0.1	59	<0.1
N. noctula[2]	30	100	30
P. pipistrellus[3]	90	81	120
V. vulturnus[4]	[An]20	100	10–20
N. geoffroyi[5]	80 pg/testis	254 pg/testis	~100 pg/testis
M. planiceps[6]	<1	4	8
R. capensis[7]	2–3	6	1
T. georgianus[8]	1	1	3.5
	SYNCHRONIZED MALE REPRODUCTIVE ACTIVITY; NO SPERM STORAGE		
M. schreibersii[9]	3	15	NA
M. schreibersii[10]	0.1	0.2	NA
M. californicus[11]	0.5	2.7	NA
Megachiroptera			
P. poliocephalus[12]	2.0	46	NA
P. scapulatus[13]	<1	2.6	NA
E. franqueti[14]	0.1	0.3	NA

Data are from the following references: 1, Gustafson and Shemesh, 1976; 2, Racey, 1974; 3, Racey and Tam, 1974; 4, Tidemann, 1993; 5, Hosken *et al.*, 1998; 6, Krutzsch and Crichton, 1987; 7, Bernard, 1985; 8, Jolly and Blackshaw, 1989; 9, Bernard *et al.*, 1991a; 10, Krutzsch and Crichton, 1990a; 11, Krutzsch *et al.*, 1976; 12, McGuckin and Blackshaw, 1987; 13, McGuckin, 1988; 14, Ifuta *et al.*, 1988.

2.5 PERIPHERAL HORMONE CONCENTRATIONS, BINDING PROTEINS AND HORMONE ACTION IN FEMALE BATS

2.5.1 Microchiroptera

Sperm storage and delayed ovulation

Vespertilioninae (Tables 2.2, 2.3). The most comprehensive data are for *Myotis lucifugus* in which mating occurs in autumn and sperm are stored throughout winter in the female reproductive tract. At the same time the ovaries contain a single large Graafian follicle (Buchanan and Younglai, 1988). Ovulation and fertilization occur in spring and births in mid-summer.

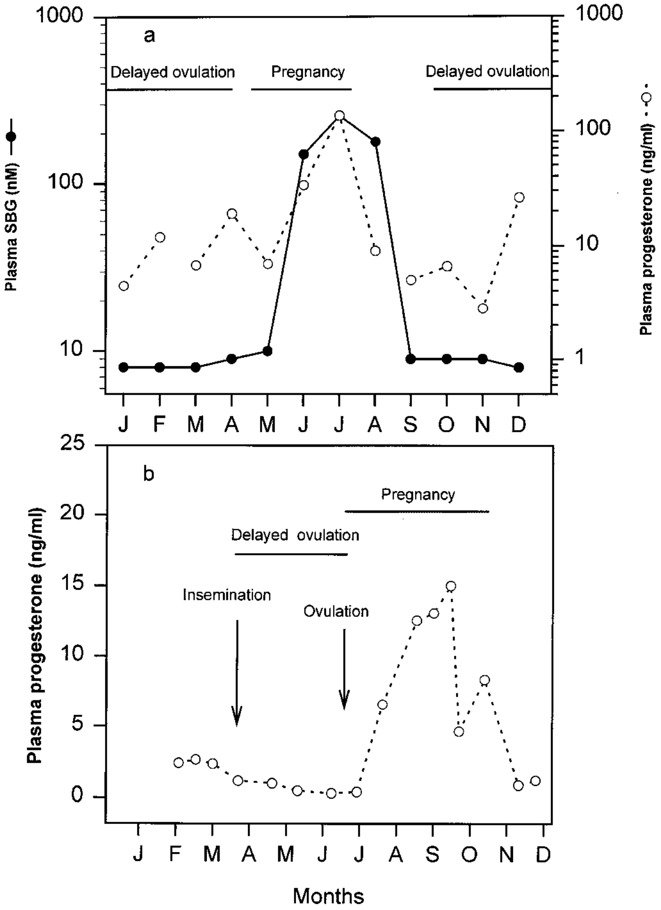

Figure 2.6 Monthly changes in gonadal hormones of female vespertilionids in which ovulation is delayed. (a) Plasma progesterone (○) and steroid-binding globulin (●) during delayed ovulation and pregnancy in *Myotis lucifugus* (Vespertilionidae) [Northern hemisphere]. The results for SBG (nM) are redrawn from Kwiecinski *et al.* (1991). The results for progesterone (ng/ml) are redrawn from Buchanan and Younglai (1986, 1988). (b) Progesterone concentrations in female *Chalinolobus gouldii* (Vespertilionidae) [Southern hemisphere]. Redrawn from Hosken *et al.* (1996).

1. Progesterone: Plasma progesterone is high and variable in *M. lucifugus* (Buchanan and Younglai, 1986, 1988; Figure 2.6a). In nulliparous, anovular bats, plasma progesterone is 2–11 ng/ml, and is ~19 ng/ml around the time of ovulation. In hibernating sexually mature and immature, unovulated bats, peripheral progesterone fluctuates significantly over time, from a basal 5 ng/ml to 12–26 ng/ml. There are no significant differences between bats of differing reproductive status, *viz.* nulliparous with small uteri, nulliparous with swollen uteri, or parous. At implantation, plasma progesterone is ~7 ng/ml and rises exponentially in late pregnancy to 81–206 ng/ml at term, with no evidence of a prepartum fall. In lactating females plasma progesterone is 6–16 ng/ml and does not decline further until the end of lactation.

   Currie *et al.* (1988) measured plasma progesterone in pregnant *M. lucifugus* and related levels to foetal crown–pump length. Plasma progesterone increases exponentially with increasing crown–rump length, doubling for every 2 mm of foetal growth, to reach ~30 ng/ml at a crown–rump length of 8 mm. Between crown–rump lengths of 8 and 16 mm, plasma progesterone doubles again and then remains steady at ~65 ng/ml until term. The highest value (175 ng/ml) was associated with a near-term foetus (crown–rump length, 22 mm).
2. Oestrogen: Buchanan and Younglai (1988) were unable to measure plasma estrogen in *M. lucifugus* but Damassa and Gustafson (1984) recorded levels of 80 pg/ml in pooled plasma from females in late pregnancy.
3. Steroid binding globulins: An SBG, which binds both oestradiol and DHT, occurs in female *M. lucifugus* at baseline levels during hibernation, ovulation and early pregnancy (September–May; Kwiecinski *et al.*, 1991; Figure 2.6a). During the second half of pregnancy, SBG levels increase 15–30 fold and remain elevated through lactation, anoestrus and pro-oestrus. This seasonal pattern in plasma SBG is similar to that seen in male *M. lucifugus*.

Plasma progesterone levels (Table 2.2) show similar trends in *Antrozous pallidus* (Oxberry, 1979) and *Chalinolobus gouldii* (Hosken *et al.*, 1996; Figure 2.6b), being much lower during delayed ovulation than in late pregnancy.

In *Antrozous pallidus*, plasma oestrogen peaks at oestrus (August; ~55 pg/ml; Table 2.3) and then falls to baseline levels during hibernation and delayed ovulation (~20 pg/ml). There is a small peak in oestrogen at ovulation and levels increase as pregnancy progresses. A maximum plasma oestrogen level is reached at mid-gestation (~90 pg/ml) and thereafter oestrogen concentration falls to ~25 pg/ml immediately after birth (Oxberry, 1979).

The reproductive cycle of *Scotophilus heathi* in India (~26°N) is characterized by arrested ovarian activity between December and mid-February (Abhilasha and Krishna, 1996). Copulation occurs between mid-January and mid-February and sperm are stored by the female for a short period until ovulation occurs. During delayed ovulation plasma progesterone (~2 ng/ml) and oestradiol (~50 pg/ml) are low. The androgens A (~80 ng/ml) and T (~5 ng/ml) are high during early delayed ovulation (December) and lower in January. Plasma A and T remain low during enlargement of antral follicles (February), at which time oestradiol rises. Interestingly, copulatory activity starts in January, well before the increase in level of oestradiol. The authors argue that intra-ovarian actions of androgens, originating from the interstitial tissue, might inhibit development and maturation of ovarian follicles, and so be responsible for delayed follicular development. However, peripheral androgen levels are so much higher than in most female (or male) mammals, that they could well affect gonadotropin secretion by systemic actions on the hypothalamic-pituitary axis.

Commentary: In all the above cases, plasma progesterone is much lower during delayed ovulation than in late pregnancy; nevertheless, by non-chiropteran standards, it is high. Progesterone is a well-known inhibitor of pituitary gonadotropin secretion, and cannot be excluded from having a role in delayed ovulation in these bats. In female *M. lucifugus*, plasma SBG is at a minimum over the period of delayed ovulation (Figure 2.6a). Thus circulating androgens (see Chapter 7 for preliminary data) might be expected to have a free range of action at this time.

An experiment cited by Wimsatt (1969; and see review by Racey, 1982) is pertinent here. Ovulation is delayed throughout hibernation in *Myotis lucifugus*. If animals are aroused prematurely in the first half of hibernation there is no ovulation and the follicle degenerates. Moreover, ACTH, GH and T_4 have no effect, whereas LH stimulates immediate ovulation. Arousal during the second half of hibernation results in immediate ovulation following abrupt release of LH from the pituitary. Thus the primary deficit in early hibernation appears to lie in the failure of the aroused pituitary to secrete LH. Wimsatt referred to an endogenous rhythm in the hypothalamus, and one can envisage how in early hibernation the hypothalamic-pituitary timer has not reached maturity. In the light of Abhilasha and Krishna's (1996) results one could postulate that the early-hibernation failure to release LH stems from a chronic suppression of the pituitary by high androgen levels. Androgens have not been measured in any of the other species in which ovulation is delayed. However, in *Antrozous pallidus* (Oxberry, 1979), oestradiol levels during delayed ovulation are of the same order of magnitude as in *Scotophilus heathi*. Since oestradiol may be produced from androgen precursors, it is possible that peripheral androgens are high in female *A. pallidus* and may play a role in delay.

Molossidae (Tables 2.2, 2.3). In *Mormopterus planiceps*, mating occurs during winter, fertilization occurs in late winter (August) and early spring and births occur in summer (December and January). The reproductive cycle is unusual in that pro-oestrus is protracted, sperm are stored and embryonic stages up to implantation span several months in spring (August and September; Crichton and Krutzsch, 1987). Plasma progesterone concentration is elevated from the time of fertilization and establishment of the corpus luteum (August) and reaches a peak of ~38 ng/ml in late pregnancy (November and December; Table 2.3). For the rest of the year, plasma progesterone is low (~1 ng/ml). Plasma oestrone and oestradiol are lower than progesterone. Plasma oestradiol is elevated during the prolonged pro-oestrus (late summer; February to May; ~1 ng/ml), low during winter (~0.2 ng/ml), and reaches peak values during pregnancy (~2.0 ng/ml in November and December; Crichton and Krutzsch, 1987).

Table 2.2 Summary of peripheral progesterone levels in female Microchiroptera at various stages of the reproductive cycle. This table is designed to summarize trends and readers are directed to the text and the original papers for exact values. (ND, not detectable; DD, delayed development; DOv, delayed ovulation). The second column (copulation/implantation) includes measures of plasma progesterone at the time of copulation or shortly thereafter. NA, not applicable.

	Plasma progesterone (ng/ml) at various stages of the female reproductive cycle				
SPECIES (source)	Inactive base line	Copulation implantation	Winter DD or DOv	Pregnancy peak	Lactation
M. lucifugus[1,2]	2–10	7	5–10	100–200	<20
A. pallidus[3]	5	20	5	50	<10
C. gouldii[4]	2	no data	<1	15	ND–<10
S. heathii[5]	5	no data	2	no data	no data
T. brasiliensis[6]	10	20	NA	100	20–30
M. planiceps[7]	1	no data	1	38	no data
M. californicus[8,9]	ND–<20	20	7–22	30–108	<10

Data are from the following references: 1, Buchanan and Younglai, 1988; 2, Currie *et al.*, 1988; 3, Oxberry, 1979; 4, Hosken *et al.*, 1996; 5, Abhilasha and Krishna, 1996; 6, Jerrett, 1979; 7, Crichton and Krutzsch, 1987; 8, Burns and Easley, 1977; 9, Crichton *et al.*, 1990.

Table 2.3 Summary of plasma oestrogen levels in female Microchiroptera at various stages of the reproductive cycle. This table is designed to summarize trends and readers are directed to the text and the original papers for exact values. Base line values are those recorded prior to follicular development. NA, not applicable.

	Plasma estrogen (pg/ml) at various stages of the female reproductive cycle				
SPECIES (source)	Base line	Oestrus/ copulation	Winter/delay	Pregnancy peak	Lactation
A. pallidus[1]	10	60	20	90	25
S. heathi[2]	no data	300	50	no data	no data
M. schreibersii[3]	200	200	200	1500	2
M. californicus[4]	no data	20	30	80	no data
M. planiceps[5]	400	900	NA	2000	900

Data are from the following references: 1, Oxberry, 1979; 2, Abhilasha and Krishna, 1996; 3, Crichton *et al.*, 1989; 4, Burns and Wallace, 1975; 5, Crichton and Krutzsch, 1987.

Commentary: During the prolonged proestrus and delayed ovulation peripheral oestradiol levels are high. It is thus possible that, as described for *Scotophilus heathi*, androgens from the interstitial tissue play a role in delaying ovulation.

Delayed implantation

Miniopterinae (Table 2.4). The female reproductive cycle of members of the genus *Miniopterus* from subtropical and temperate latitudes is characterized by a period of delayed implantation that coincides with the winter/cool dry season of that latitude. Copulation, ovulation and fertilization occur prior to winter, implantation occurs at the end of winter and births in early to mid-summer. In Australia and Japan delayed implantation is followed by a period of delayed embryonic

Table 2.4 Summary of the available data for plasma progesterone levels for *Miniopterus schreibersii* from Japan (column 1), Australia (column 2) and South Africa (columns 3–5) at various stages of pregnancy. All samples for the post-fertilization stage were collected within one month of fertilization but the stage of development varied from a tubal morula to bilaminar blastocyst. NA, not applicable.

	Plasma progesterone (ng/ml)				
Stage of pregnancy	Ref. no 1	2	3	4	5
Non-pregnant	7.0±0.1	0.5±0.1	1.4±0.3	1.5±1.0	3.4±0.4
Post fertilization	11.1±1.8*	2.0±0.5	12.9±3.5*	6.2±3.5	6.2±0.4*
Delayed implantation	ND	2.6±1.3	18.5±3.2	1.7±0.6*	3.2±0.3*
Delayed embryonic dev.	4.7±0.6*	3.5±0.7	NA	NA	NA
Late foetal dev.	11.8±1.5*	22.6±4.6	127.7±9.4*	64.8±4.7*	35.6±1.4*

Data are from the following references: column 1, Kimura *et al.*, 1987; 2, Crichton *et al.*, 1989; 3, van der Merwe and van Aarde, 1989; 4, Bernard *et al.*, 1991b; 5, van Aarde *et al.*, 1994.

* Indicates that the mean value is significantly different from the preceding value as presented in the original paper.

development that does not occur in Europe or Africa (Kimura and Uchida, 1983; Crichton *et al.*, 1989; Bernard *et al.*, 1996 for review).

1. Progesterone: A number of authors have measured plasma progesterone throughout pregnancy in *Miniopterus schreibersii*. However, comparison of these data is difficult because they are based on populations of *M. schreibersii* with different patterns of reproduction, and in which the timing and duration of gestation differ slightly. Some trends are, however, evident if plasma progesterone is compared for similar stages of gestation (Table 2.4). Progesterone is detectable in non-pregnant females (~0.5–7.0 ng/ml) and levels are elevated (~2–12 ng/ml), sometimes significantly, in the month following fertilization. The variation in plasma progesterone levels in the month following fertilization probably reflects variation in the developmental stage of the embryo in the females that were sampled. For example, two females with a single morula in the left oviduct had plasma progesterone of 3.6 and 4.9 ng/ml while a third female, caught at the same time, but with a unilaminar blastocyst in the right uterine horn, had a progesterone level of 10.2 ng/ml (Bernard *et al.*, 1991b). During delayed implantation plasma progesterone is typically low (1.7–3.2 ng/ml) and not significantly different from that of non-pregnant females. The apparent exception is the high plasma progesterone (18.5 ng/ml) recorded for *M. schreibersii* by van der Merwe and van Aarde (1989). However, this value should be interpreted with care because a similarly elevated level was not recorded by the same authors in a later study, using the same assay (van Aarde *et al.*, 1994). Post-implantation progesterone level remains low in populations in which there is a period of delayed development. In all populations progesterone increases during foetal development in conjunction with development of the placenta, reaching peaks about one month before parturition of ~25–125 ng/ml (Crichton *et al.*, 1989; van der Merwe and van Aarde, 1989).

2. Oestrogen: In *M. schreibersii*, plasma oestradiol in the first half of delayed implantation (200–340 pg/ml) is not significantly different from that prior to

conception (~250 pg/ml; Crichton *et al.*, 1989). However, prior to implantation oestradiol declines to its lowest value (~130 pg/ml) and then increases, post-implantation, to reach ~290 pg/ml during delayed embryonic development and a peak of ~1500 pg/ml in mid-gestation. Plasma oestrone is highest at the time of ovulation, declines during the following months until formation of the placental discs and thereafter climbs, before decreasing prior to parturition (Crichton *et al.*, 1989).

Commentary: The low levels of plasma progesterone during delayed implantation and delayed development suggest that a fully functional corpus luteum and elevated plasma progesterone levels may be required for implantation. The elevated plasma oestradiol during delayed implantation led Crichton *et al.* (1989) to suggest that it may delay development by suppressing a luteotrophin that is required for optimal luteal function but this suggestion remains untested. More recently it has been shown that neither exogenous progesterone, nor exogenous hCG (which activates the corpus luteum and elevates endogenous progesterone) will terminate delayed implantation in *M. schreibersii* (Bernard and Bojarski, 1994). A similar situation occurs in some of the mustelid carnivores where progesterone alone will not terminate delayed implantation (Berria *et al.*, 1989).

Delayed embryonic development

Phyllostomidae (Tables 2.2, 2.3). In *Macrotus californicus* copulation and fertilization occur in autumn (October). Post-implantation embryonic development is retarded during winter (October–March), normal embryonic development resumes in spring (March) and births occur in mid-summer (June; Bradshaw, 1962; Crichton *et al.*, 1990).

1. Progesterone: Plasma progesterone is low during delayed embryonic development (7 ng/ml; Burns and Easley, 1977; ~12 ng/ml; Crichton *et al.*, 1990) and rises significantly at the resumption of normal embryonic development (32 ng/ml; Burns and Easley, 1977; 108 ng/ml; Crichton *et al.*, 1990; Table 2.2). Burns and Easley (1977) detected a peak in plasma progesterone at the time of placentation, but this was not recorded by Crichton *et al.* (1990). The changing plasma progesterone is mirrored by changes in the ultrastructure of the luteal cells and intensity of staining for 3β-HSD and 17β-HSD which indicate that the corpus luteum is not fully active during delayed embryonic development (Crichton *et al.*, 1990). Abortion follows ovariectomy at any stage of pregnancy (Burns, 1981), indicating that the corpus luteum is required throughout, but progesterone was not measured post-ovariectomy.
2. Oestrogen: Plasma concentrations of oestrone and oestradiol are relatively high at implantation and shortly thereafter (~18 pg/ml and 60 pg/ml respectively) and much lower during delayed embryonic development (~12 pg/ml and 35 pg/ml respectively; Burns and Wallace, 1975). After the resumption of normal embryonic development, oestrone and oestradiol increase to ~25 pg/ml and 75 pg/ml respectively.

Commentary: Based on the coincidence of low plasma steroid concentrations with the period of delayed development, Burns and Easley (1977) and Burns and

Wallace (1975) have proposed that delayed embryonic development is a consequence of hormonal inadequacy. This represents an ideal starting point for further experiments to determine which hormones are responsible for retarding embryonic development and how their levels are controlled.

No reproductive delay

Molossidae (Table 2.2). The reproductive cycle of *Tadarida brasiliensis* from North America (~35°N) is characterized by synchronized spermatogenesis and follicular development in spring (Jerrett, 1979). Copulation, ovulation and fertilization occur in early summer (March) with birth in early to mid-summer (late June and early July).

1. Progesterone: Baseline plasma progesterone levels during dioestrus, in the absence of a corpus luteum, are high (~10 ng/ml; Table 2.2). Plasma progesterone increases with ovulation and establishment of the corpus luteum and rises to a peak (106 ng/ml) in late pregnancy. At parturition, the corpus luteum regresses and plasma progesterone drops to baseline (Jerrett, 1979).

Commentary: The non-ovulatory left ovary of *T. brasiliensis* is filled with interstitial cells which stain positively for 3β-HSD (Jerrett, 1979) and is the probable source of progesterone in non-pregnant animals.

2.5.2 Megachiroptera (Table 2.5)

The reproductive cycles of female Megachiroptera are not complicated by the reproductive delays which occur in some of the Microchiroptera and this section is not subdivided as previously. However, information is available for a wider range of hormones than is the case for the Microchiroptera, and the section is subdivided by hormone.

Table 2.5 Summary of peripheral levels of progesterone (P) and oestradiol (E_2) in female Megachiroptera at various stages of the reproductive cycle. This table is designed to summarize trends and readers are directed to the text and the original papers for exact values. ND, not detectable.

Species	Plasma P and E_2 (ng or pg/ml) at various stages of the female reproductive cycle			
	Base line	Copulation implantation	Pregnancy peak	Lactation
E. franqueti (E_2)[1]	6 pg/ml	no data	20 pg/ml	no data
P. hypomelanus (E_2)[2]	no data	1456 pg/ml	no data	no data
P. hypomelanus (P)[2]	no data	1343 pg/ml	3200 pg/ml	no data
P. poliocephalus (E_2)[3,4,5]	10 pg/ml	26 pg/ml	700 pg/ml	10 pg/ml
P. poliocephalus (P)[3,6,7]	20 ng/ml	20 ng/ml	800 ng/ml	20 ng/ml
P. scapulatus (E_2)[4]	ND	15 pg/ml	181 pg/ml	<20 pg/ml
P. scapulatus (P)[6]	4 ng/ml	4 ng/ml	195 ng/ml	4 ng/ml

Data are from the following references: 1, Ifuta *et al.*, 1988; 2, Atkinson *et al.*, 1995; 3, Towers and Martin, 1985; 4, Towers, 1988; 5, Kennedy, 1987; 6, Towers and Martin, 1995; 7, Kennedy, 1993.

Inhibin

To date, inhibin appears to be the only effective marker of changing ovarian function in Australian *Pteropus*. In captive adult female *Pteropus poliocephalus* (Queensland, Australia; Figure 2.7a), plasma inhibin levels are low or not detectable prior to the start of the breeding season in March, although occasional animals show sporadic transient spikes of 5–12 pM (Kennedy, 1993). Inhibin concentrations increase abruptly to a peak in April, when most females conceive, and well before peripheral progesterone rises significantly. During the ensuing pregnancy, inhibin levels fall progressively while progesterone continues to rise (Figure 2.7a). A similar pattern occurs in *P. alecto* where inhibin is low or undetectable in late pregnancy.

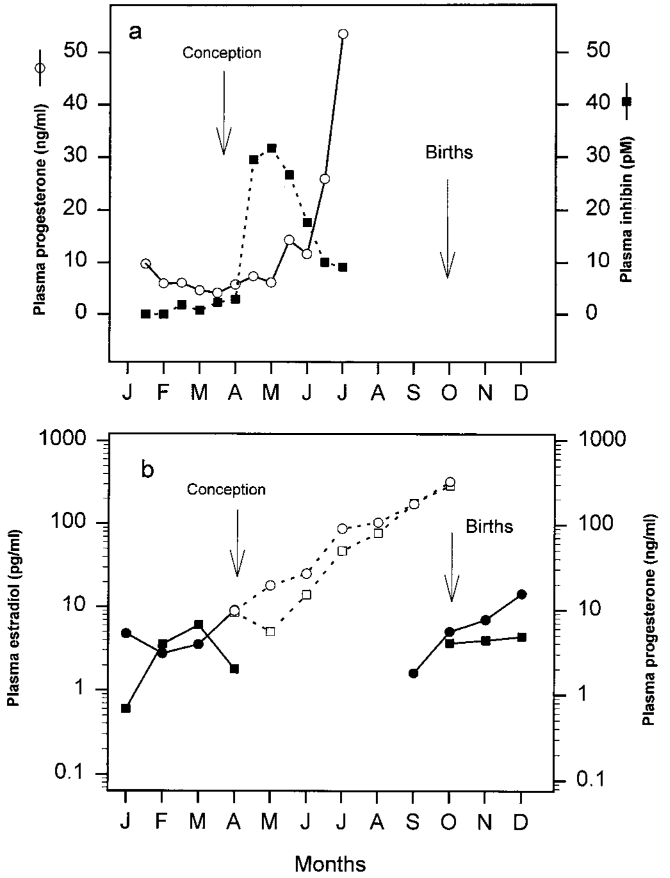

Figure 2.7 Monthly changes in reproductive hormones in female Australian flying foxes (Pteropodidae) [Eastern Australia]. (a) Plasma progesterone (○) and inhibin (■) in seasonally breeding captive adult female *Pteropus*. Redrawn from Kennedy (1993). (b) Plasma oestradiol (□) and progesterone (○) in adult pregnant (open symbols) and non-pregnant (closed symbols) female *P. poliocephalus* shot in the wild. Redrawn from Towers (1988) and Towers and Martin (1995).

Kennedy (1993) examined levels of inhibin and progesterone in 16 adult female *Pteropus* spp. for four weeks before, and one week after, bilateral ovariohysterectomy in late April. At operation, 11 bats had ovarian luteal tissue, and the remainder had follicles at various stages of development. Bats with luteal tissue had significantly higher levels of inhibin than those without (means of 41 pM and 10 pM, respectively), whereas there was no difference in plasma progesterone (both means of 8 ng/ml). Post-ovariectomy, inhibin fell significantly (mean of 2 pM), whereas progesterone did not (mean of 5 ng/ml).

In a large series of measurements taken during pregnancy and before and during the mating season in intact and ovariectomized *P. poliocephalus* and *P. alecto*, Kennedy (1993) found no significant correlation between inhibin and progesterone. Ovariectomized bats typically continued to show a high plasma progesterone (see below), whereas inhibin was usually undetectable. Nevertheless, several ovariectomized bats occasionally showed detectable levels of inhibin and one consistently returned values of >10 pM.

Oestrogens (Table 2.5)

The most comprehensive data are for the Australian flying fox, *Pteropus poliocephalus*. In this species, mating occurs in March and April and pregnancy from April/May to October/November. In non-pregnant, early mating season bats, with ovaries containing antral but no preovulatory follicles, oestradiol concentrations range from undetectable to 16 pg/ml (Figure 2.7b; Towers and Martin, 1985; Towers, 1988). There is no correlation between plasma oestradiol and follicular development and none of the uteri show signs of oestrogen stimulation. It is now established that, in Australian *Pteropus*, the uterus does not exhibit oestrogen stimulation until a preovulatory follicle develops, and then only in the ipsilateral horn (Martin, *et al.*, 1995).

In pregnancy, plasma oestradiol rises exponentially, but does not differ significantly from levels of oestradiol in non-pregnant bats until about three months post-conception (July; Figure 2.7b). In late pregnancy, oestradiol ranges from 100–700 pg/ml, and falls to baseline levels post-partum.

Kennedy (1987), using similar methods to Towers (1988), measured oestradiol in sequential plasma samples from 16 captive adult *P. poliocephalus* (13 of which had given birth in the previous season) and from two bats that had been ovariectomized (OVX) shortly before the period of study. Samples were taken monthly in December, February and March, then weekly during the peak mating season (23 March to 30 April). Over the latter period, all females (including the OVX) mated repeatedly and 14 of the intact bats subsequently gave birth at the usual time (October–November). Pre-mating season (December–March) mean monthly oestradiol levels were 3–7 pg/ml in both intact and OVX bats. During April mean oestradiol was 6–9 pg/ml in intact bats and 4–7 pg/ml in the OVX. Before April, no sample had an oestradiol level >10 pg/ml, whereas in April, 9 bats had one or more samples with a peak oestradiol concentration of 10–26 pg/ml. Similarly high plasma oestradiol levels have been reported for the same species (Towers, 1988). Conception in *P. poliocephalus* occurs in April, and it is tempting to speculate that each oestradiol peak might reflect maturation of an oestrogen-secreting preovulatory follicle. We quote Kennedy's data at length to emphasize that, in mating-season *P. poliocephalus*, oestradiol is low, at the limits of assayability and, on average, little different from that in OVX bats. Such low levels are consistent with observations that uterine growth does not precede preovulatory follicle maturation and is then restricted to the ipsilateral horn (Martin *et al.*, 1995).

In *P. scapulatus*, which mates in mid-summer (November–December) and gives birth in early to mid-winter (May–June), seasonal changes in oestradiol are similar to those in *P. poliocephalus* (Towers, 1988). In contrast to the results for *P. poliocephalus*, oestradiol is undetectable in plasma from adult *P. scapulatus*, seven days post-OVX in June (Towers, 1988).

In the central African fruit bat, *Epomops franqueti*, levels of oestradiol were ~20 pg/ml in wild-caught adult pregnant bats compared to 6–7 pg/ml in wild-caught juveniles and non-pregnant adults (Ifuta *et al.*, 1988).

Atkinson *et al.* (1995) measured oestradiol in faeces and plasma from captive *P. hypomelanus* [tropical, East Asia, housed in Florida]. They reported that reproduction was non-seasonal and polyoestrous, with a cycle length of 31.1 days, but no discrete period of behavioural oestrus. Sperm were present in vaginal flushings throughout the cycle and pregnancy. At oestrus, oestradiol (2296 pg/g faeces and 1456 pg/ml plasma) is substantially higher than that reported for Microchiroptera or for mammals generally.

Commentary: Although the actual levels of oestradiol in the various studies differ markedly (Table 2.5), there are common trends. Oestradiol is relatively low during follicular development, but may increase episodically with follicle maturation and through pregnancy. In *P. poliocephalus*, where oestradiol levels in intact bats are similar to those of OVX bats, oestradiol is clearly not of ovarian origin and the source may be the adrenal gland. Overall, oestradiol does not appear to be a useful marker of ovarian function in these species.

Progesterone (Table 2.5)

In *Pteropus poliocephalus* plasma progesterone rises and falls at the onset of the mating season and there appears to be a broad peak at the time of conception (Towers and Martin, 1985, 1995; Kennedy, 1993; Figures 2.7a, b; 2.8a). However, because of substantial variation between individual bats, these changes are not statistically significant. Progesterone levels do not increase when the corpus luteum forms but a significant increase occurs from mid-pregnancy to reach 200–800 ng/ml close to term. There is no evidence that progesterone levels fall before parturition, but there is an abrupt fall post-partum.

Data from non-pregnant, late-pregnant and OVX *P. scapulatus* and *P. alecto* indicate that plasma progesterone follows similar patterns to that of *P. poliocephalus* (Figure 2.8a).

Commentary: The data for Australian *Pteropus* reflect a common pattern in which plasma progesterone does not alter substantially during follicular development or after formation of the corpus luteum or ovariectomy (Table 2.5). Thus progesterone is not a useful marker of ovarian function in these species. Plasma progesterone increases from mid-pregnancy to reach a maximum in late pregnancy.

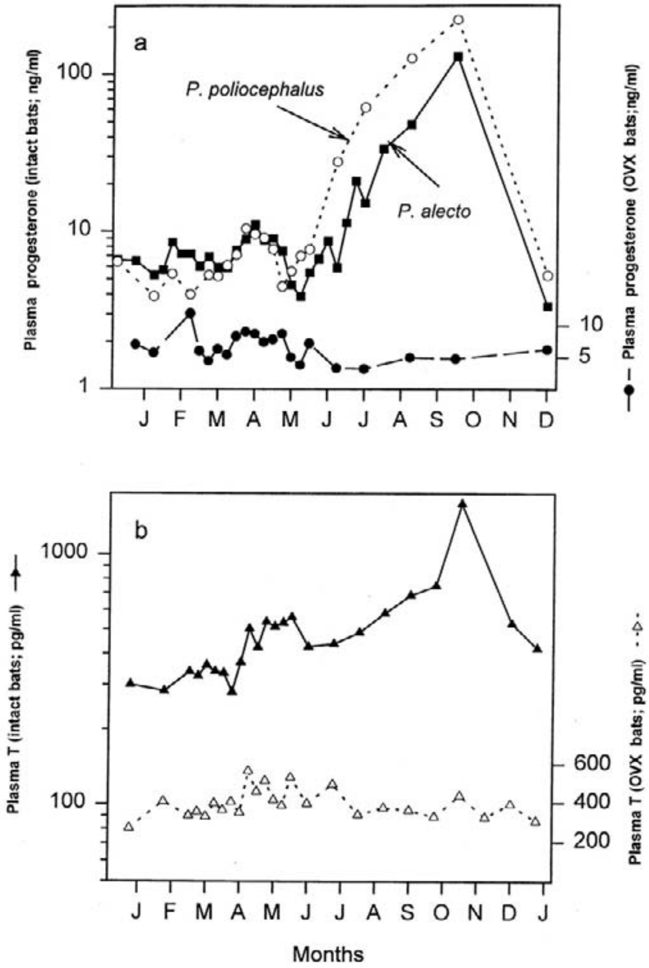

Figure 2.8 Plasma progesterone and testosterone in seasonally breeding captive adult female *Pteropus* (Pteropodidae) [Southern hemisphere]. (a) Monthly changes in plasma progesterone (ng/ml; RIA of hexane extracts of serial plasma samples) in intact (○) and ovariectomized (●) *P. poliocephalus* and adult, intact *P. alecto* (■). Redrawn from Kennedy (1993). (b) Monthly changes in plasma T (pg/ml) from four intact (▲) and one ovariectomized (△) adult *P. poliocephalus*. Redrawn from Kennedy (1993).

Androgens

In *P. poliocephalus* and *P. alecto*, A ranges from 1–10 ng/ml in non-pregnant bats, but shows no systematic change over the breeding season or increase in pregnancy (Kennedy, 1993). Plasma T ranges from 200–600 pg/ml in non-pregnant bats, increasing systematically in late pregnancy to 800–1600 pg/ml (Figure 2.8b). Plasma T is elevated in intact females during the mating season, but comparable increases occur in OVX bats and individual profiles of intact bats show no meaningful patterns.

2.6 UNILATERAL ENDOMETRIAL GROWTH (UEG) IN *PTEROPUS* SPP.; IMPLICATIONS FOR SYSTEMIC HORMONE LEVELS

In the genus *Pteropus* and in *Rousettus aegyptiacus* endometrial growth is restricted to the uterus ipsilateral to the ovary with the preovulatory follicle or corpus luteum (Marshall, 1949, 1953; Bernard, 1988; Martin *et al.*, 1995). The ovaries of many megachiropterans, including *Rousettus* and *Pteropus* spp. possess an unusual morphology and vascular complex (Bernard, 1988; Pow, 1992; Pow and Martin, 1994, 1995; Towers and Martin, 1995). The distal half of the ovarian artery is highly coiled and completely enclosed in a venous sinus derived from the uterine and ovarian veins. Just before entering the ovary, the ovarian artery branches to form a uterine artery which runs to the ipsilateral uterine horn. Such a rete might allow estrogens and progesterone to pass from the ovarian vein into the ovarian artery and then via the uterine artery to the ipsilateral uterine horn. In this way the unilateral endometrial growth in *Pteropus* and *Rousettus aegyptiacus* may be explained.

Pow and Martin (1991) demonstrated the efficacy of the route in *Pteropus* by injecting [^{3}H]-estradiol into one ovary and measuring uterine levels one hour later. Although recovery of [^{3}H]-estradiol was low, it was preferentially transferred to the ipsilateral uterine horn. A similar pathway, albeit in the reverse direction, exists in sheep where endometrial luteolytic factors pass from uterine vein to ovarian artery and influence the ipsilateral ovary only (Ginther, 1974; Mapletoft *et al.*, 1975). Preferential transfer of progesterone from the corpus luteum to the ipsilateral endometrium may explain why peripheral progesterone levels do not increase in early pregnancy in *Pteropus* spp. If progesterone underwent endometrial metabolism, as in rodents (Clark, 1974), luteal secretion would have a minimal effect on peripheral progesterone levels. Unilateral endometrial growth associated with a preovulatory follicle presumably involves oestradiol, which usually does not undergo uterine metabolism and which, by binding to target-organ oestrogen receptors with high affinity, is usually taken up preferentially from the circulation by target organs like the uterus. Martin *et al.* (1995) questioned whether preferential local transport alone was sufficient to explain the stimulation of unilateral endometrial growth by oestradiol. Their suggestion that saturation of contralateral oestrogen receptors is prevented by a high-affinity oestradiol binding in plasma was not substantiated (Wang *et al.*, 1992). Metabolism of oestradiol by *Pteropus* endometrium needs to be examined.

Australian *Pteropus* spp. appear to be spontaneous ovulators and such mammals usually require ovarian oestradiol to induce the LH surge essential for ovulation. If the single ovary containing the developing follicle does not communicate with the contralateral uterus, we must question how it communicates with the hypothalamus. If ovarian oestradiol *were* the messenger, the apparent paradox might be explained in terms of differing oestrogen receptor populations in the hypothalamus and contralateral uterine horn giving different levels of sensitivity to oestrogens. Alternatively, ovarian T or A could act as messenger, with aromatase present in the hypothalamus but not the contralateral uterine horn.

2.7 GENERAL DISCUSSION

Since the last major review of reproduction in bats (Racey, 1982), data on the circulating gonadal hormones of many more species have become available yet our knowledge of the sources, nature, peripheral metabolism and plasma concentrations of reproductive hormones and the properties of their receptors, and how these are regulated, remains poor. For example, despite the anomalous levels of progesterone found in many bats, there have been few attempts to chemically identify the immunoactive material, or to determine, by removal of the appropriate organ, if it is of ovarian or adrenal origin. There is substantial evidence in some bat species to support the adrenal gland as an extra-gonadal source of steroid hormones. For example, Wang *et al.* (1992) and McGuckin (1988) invoked adrenal synthesis to explain the high levels of progesterone and T in intact and castrate male *Pteropus*. We are not aware of reports of high plasma progesterone levels in other male mammals, but there is a substantial literature on adrenal progesterone secretion in females (Resko, 1969; Fajer *et al.*, 1971; Shakh and Shakh, 1975; Plotka *et al.*, 1983; Asher *et al.*, 1989) even to levels which influence sexual receptivity (Feder and Ruff, 1969).

High baseline progesterone could result from high-affinity protein binding. All Microchiroptera studied by Kwiecinski *et al.* (1987) have a CBG-like plasma-protein, but there are no data to indicate if this binds progesterone as strongly as the CBG-like protein of *Pteropus* (Martin *et al.*, 1995). Overall therefore, we have little idea of how progesterone actually functions in any species of Chiroptera.

The most commonly measured hormones in bats have been T in males, and oestradiol and progesterone in females; this, in spite of the fact that there is an ever-increasing list of intra-gonadal hormones and an ever-increasing awareness of how gonadal hormones regulate gametogenesis by intra-gonadal paracrine and autocrine actions (Hillier, 1994; Burger and de Kretser, 1989). To use T as an example, modulation can occur systemically via changing level of a specific binding protein like SBG. This is seen in *M. lucifugus*, where thyroid hormone modulates the action of T by altering SBG levels and so the free-T reaching specific target-cell receptors (Damassa *et al.*, 1985, 1995; Kwiecinski *et al.*, 1986). Non-gonadal hormones might modulate the action of T by altering 5α-reductase or aromatase levels differentially in different target-tissues. Thus thyroid or adrenocortical hormones (which rise and fall seasonally in *M. lucifugus*) might regulate aromatase in central neuronal tissues, and 5α-reductase in the accessory glands but not gonadal spermatogenic tissues. The action of T could be reduced in a target-tissue by specifically increasing enzymes which metabolically inactivate T; this is believed to be one mechanism whereby progesterone inhibits the action of oestradiol in the human uterus (Tseng and Gurpide, 1975).

Sex-steroids are known to regulate the synthesis of their own, and each other's receptors in target-cells. Receptor levels might also be up- or down-regulated in specific tissues by thyroid hormones, adrenocorticosteroids or pituitary hormones such as growth hormone. Receptors may be absent at certain developmental stages (e.g. neonates) or from particular tissues such as the vaginal epithelium of *Pteropus* (Pow and Martin, 1988; Pow, 1992). It is also clear that hormone action can be blocked or modulated by altering the intracellular expression of the hormone-receptor complex, i.e. via changes in intracellular regulators such as transcription factors controlled by other hormones. We suggest that future efforts should continue to use properly validated RIAs to measure peripheral hormone levels, but applied to a wider choice of gonadal hormones, and with better characterization of what hormones are actually circulating. We would expect these assays to be complemented with assays of thyroid and adrenocortical hormones, and steroid-binding proteins, and with immunocytochemistry and *in situ* polymerase-chain-reaction cytochemistry to elucidate expression of hormone receptors and hormone-metabolizing enzymes in target-tissues.

When Wimsatt (1960, 1969) speculated on the peculiarities of reproduction in hibernating bats, sensitive hormone RIAs were in their infancy, details of steroid hormone action were poorly known, the significance of target-organ metabolism of T was not appreciated, and immunocytochemical methods for detecting cellular hormone receptors and metabolizing enzymes were unknown. Despite advances in technology and understanding, it remains true that we are no closer to understanding the role of gonadal hormones in the control of the reproductive asynchronies and delays that characterize many of the Microchiroptera. The available data provide an ideal starting point for studies of the endocrine control of reproductive delays. For example, SBGs may play a significant role in the asynchronous activity of Leydig cells, seminiferous tubules and accessory glands in many vespertilionid and rhinolophid bats. High concentrations of androgens, possibly in conjunction with base-line SBGs in winter may play a key role in delaying ovulation in some vespertilionids. The termination of delayed implantation in *Miniopterus schreibersii* is temporally linked to activation of the corpus luteum, but is not stimulated by progesterone. Delayed development in *Macrotus californicus* is associated with low plasma progesterone, oestrone and oestradiol. Future studies which aim to contribute to the understanding of the control of reproductive delays should measure the full range of hormones and assess the abundance of receptors and enzymes involved in metabolism of the hormone. Ultimately, hormone manipulation experiments with captive bats will yield the most robust data.

We suggest that future research should address basic questions in bat reproductive biology such as the control of delayed ovulation, sperm storage and delayed implantation and the separation of processes such as oestrus and ovulation, and Leydig cell and accessory gland activity, that are normally coincident. Further studies of plasma T, oestradiol and progesterone in previously unstudied species will provide additional base-line information but will not answer the important fundamental questions.

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3

Peripheral Endocrines in Bat Reproduction

Gary G. Kwiecinski and David A. Damassa

3.1 INTRODUCTION

Bats represent a highly successful order of existing mammals, second only to rodents in the numbers of individuals and diversity of species. Yet most bat species exhibit only one or two reproductive cycles per year, typically producing a single young. This is in marked contrast to rodents which generally breed repeatedly in a given year and have multiple young per litter. Despite the limited reproductive potential of bats, these mammals have achieved extensive radiation in relation to their food sources and, like muroid rodents, populate most regions of the earth. In the sub-arctic and temperate zones, bats are primarily insectivorous and migrate to warmer climates or hibernate during the winters to escape harsh environmental conditions and lack of food. Reproductive potential is maximized in these species by the adoption of diverse strategies. For example, females of some species exhibit embryonic diapause (delayed development or implantation) or delayed

Reproductive Biology of Bats
0-12-195670-9

fertilization in response to inhospitable periods (Oxberry, 1979). In certain vespertilionid and rhinolophid bats, males undergo spermatogenesis in one season and mate in another, storing their sperm in the reproductive tract (Gustafson, 1979). Strategies such as these enable bats to separate the different components of their reproductive cycle, timing energetically expensive parts with maximum food availability. Such asynchronous (disassociative) reproduction depends on monitoring environmental changes and selectively initiating appropriate reproductive responses. The linking of internal conditions for optimal reproductive effort with environmental changes likely involves the central nervous system and a variety of reproductive and peripheral endocrine regulatory factors.

Our knowledge of the endocrine and neuroendocrine control of reproduction in bats is relatively limited when compared to that amassed for laboratory rodents and certain domestic species (e.g. Bronson and Heideman, 1994). In bats, current information on the reproductive hormones of the hypothalamus, pituitary, gonads and pineal are reviewed elsewhere in this volume (see Heideman; Anthony; Martin and Bernard; Krutzsch; Rasweiler and Badwaik). However, many endocrine factors that are classically considered non-reproductive or peripheral to the hypothalamic–pituitary–gonadal axis have been implicated in the control of reproductive processes. These include hormones of the thyroid, adrenal and pancreas, as well as specific extracellular binding proteins for these hormones and for the sex steroids. The following sections review our current knowledge about some of these non-reproductive endocrine factors in bats and assess their possible interactions in modulating reproductive timing. Particular attention is focused on the role of sex hormone-binding globulin (SHBG) in asynchronous reproductive cycles.

3.2 PERIPHERAL ENDOCRINES IN BATS

The reproductive state of an individual at any particular time reflects an integration and coordination of both external and internal signals and not just a simple response to a single environmental factor. Transduction of these signals into organismal responses is primarily effected by the nervous system and its interactions with the endocrine system. Some well-known environmental factors influencing reproductive timing include food availability, ambient temperature and photoperiod. Although nearly 25% of all mammals are bats, we know very little about how environmental and internal factors interact to regulate annual patterns of reproduction in Chiroptera.

In most mammals studied, the hypothalamic–pituitary axis controls the secretion of LH, FSH and hence the gonadal steroids that drive gamete production and activate many reproductive processes. Hormones such as thyroxine, melatonin, prolactin, insulin and the glucocorticoids are also known to modulate reproductive function by acting at various sites, including the brain, pituitary, gonads and steroid target tissues (Knobil and Neill, 1994). Many hormones, particularly the sex steroids, are bound to specific binding proteins. These extracellular hormone-binding proteins are present in the circulation and have been shown to reduce the availability and the clearance of their specific ligands.

However, recent evidence suggests that many of these proteins also act directly on cells to activate intracellular signalling pathways. As such, binding proteins may also serve as endocrine factors in the control of reproduction (Damassa and Cates, 1995).

Study of the endocrine regulation of reproduction in bats has been technically challenging. Bats are among the smallest mammals, making extensive repetitive blood sampling impractical. Most bats tend to breed poorly in captivity, reflecting our lack of knowledge in manipulating dietary, physical, social and emotional needs of these animals in ways that allow reproduction. Thus, detailed endocrine profiles or information about the pulsatile or circadian patterns of hormone secretion are not readily obtained. In the following sections we will review what is currently known about peripheral endocrine factors in bats and their relationship to seasonal changes in the timing and expression of reproductive functions.

3.2.1 Thyroid gland

The thyroid gland has long been recognized as an important modulator of reproductive function. Both hyper- and hypo-thyroidism are associated with reproductive dysfunction and infertility. Direct effects of thyroid hormones on cellular metabolism and hormone responsiveness have been demonstrated in the gonads and the sex accessory organs. In addition, thyroid hormones appear to play a key role in the expression of events that underlie seasonal reproductive cycles. In the European starling, thyroidectomy prevents the seasonal decline in testicular activity and sustains the breeding season indefinitely (Woitkewitsch, 1940). Similarly, in ewes, thyroid hormones have been shown to exert a permissive effect on the reduction of hypothalamic gonadotropin releasing hormone secretion and the consequent transition from the breeding season to anestrum (Karsch *et al.*, 1995). This action of thyroid hormone [thyroxine (T_4) and triodothyronine (T_3)] is apparently confined to a window of time in the seasonal cycle and changes in plasma T_4 at other times during the subjective year have little effect on reproductive function. Thus, studies in several species indicate that thyroid hormones, acting directly or by metabolic effects, can influence reproductive processes.

Identification and assay of T_4, T_3 and TBG in blood

Measurements of plasma T_4 levels in bats (Table 3.1) were first made in female *Macrotus waterhousii* (= *M. californicus*; Burns *et al.*, 1972), a non-hibernating species. Assays were performed on pooled samples using a specially designed assay kit. Depending on reproductive status, total mean plasma T_4 concentrations in these bats ranged from 26 to 64 nM. These values are much lower than those of euthyroid women (117–168 nM) but within the range of serum T_4 concentrations measured in other eutherian mammals (Hulbert and Augee, 1982).

A general radioimmunoassay technique was subsequently validated for the measurement of plasma T_4 concentrations in *Myotis lucifugus* (Kwiecinski *et al.*, 1986). The technique was modified from Alexander and Jennings (1974) for use with small volumes of plasma and employs Sephadex adsorption chromatography

Table 3.1 Serum/plasma thryoxine concentrations measured by radioimmunoassay in Chiroptera. Values are means for samples collected at various reproductive stages.

			T_4		
	Sex	Stage	(nM)	(ng/ml)	Ref
Suborder: Megachiroptera					
Family: Pteropodidae					
Epomops franqueti					
	F	Immature	3.9	3.0	1
	F	Adult non-pregnant	3.5	2.7	1
	F	Pregnant	3.9	3.0	1
	M	Immature	4.0	3.1	1
	M	Adult	5.1	4.0	1
	M	Adult (no epaulets)	3.7	2.9	1
Suborder: Microchiroptera					
Family: Phyllostomidae					
Macrotus waterhousii					
	F	Immature	43.8	34.0	2
	F	Adult non-pregnant	42.5	33.0	2
	F	Pregnant (early)	25.7	20.0	2
	F	Pregnant (late)	63.1	49.0	2
	F	Lactation	64.4	50.0	2
Family: Vespertilionidae					
Myotis lucifugus					
	F	Immature (Aug)	4.2	3.3	5
	F	Adult non-pregnant (Jun)	2.0	1.6	5
	F	Pregnant (early May)	5.2	4.0	3
	F	Pregnant (late Jun)	1.3	1.0	3
	F	Lactation (Jul)	2.0	1.6	3
	M	Immature (Aug)	11.8	9.1	5
	M	Adult (May)	29.6	23.0	4

References:
1. Ifuta *et al.*, 1988
2. Burns *et al.*, 1972
3. Kwiecinski *et al.*, 1991
4. Damassa *et al.*, 1995
5. Unpublished observations; measurements made as described in 3 and 4.

for sample purification. This method was adopted after several unsuccessful attempts were made to obtain valid measurements of *M. lucifugus* plasma T_4 using commercial kits available at the time.

With this assay, mean plasma T_4 concentrations in pregnant and non-pregnant *M. lucifugus* ranged from 1 to 30 nM – even lower than the values obtained for *M. waterhousii*. Measurements of plasma concentrations of T_4 and T_3 were also made year round in *Epomops franqueti* using commercial assay kits (Ifuta *et al.*, 1988). The validation of these assays was not presented, but mean plasma T_4 levels were similar to those reported for active *M. lucifugus*. Concentrations of plasma T_3 in *E. franqueti*, as in other species, were markedly lower than those of T_4 and ranged from 0.3 to 1.0 nM. Interestingly, concentrations of T_4 (Table 3.1) in adult males of *E. franqueti* and *M. lucifugus* tend to be higher than concentrations in adult females.

In vertebrates, circulating thyroid hormones are bound to plasma proteins such as thyroxine-binding globulin (TBG), albumin, and transthyretin [thyroxine-binding pre-albumin (TBPA)] with only a small fraction (<1%) existing in the unbound or free state. TBG is the major plasma T_4-binding protein in many mammals (it binds 75–80% of human thyroid hormones; Burrow, 1991) and has the highest affinity for T_4.

The binding of T_4 to plasma proteins in bats has only been examined in *M. lucifugus* (Kwiecinski *et al.*, 1991; Damassa *et al.*, 1995). In both males and females, iodinated T_4 was bound by TBG and by albumin. Binding to TBG was specific and saturable. In active males and females, levels of TBG binding were approximately 60% of total T_4 binding in plasma, a value comparable to those seen in other vertebrate species (Damassa *et al.*, 1995; Kwiecinski *et al.*, 1991).

Seasonal and pregnancy-associated changes in circulating thyroid hormones

The annual cycle of plasma T_4 and TBG have been studied in *M. lucifugus* males (Damassa *et al.*, 1995) and females (Kwiecinski *et al.*, 1991). As shown in Figure 3.1, males exhibit a well-defined seasonal cycle of plasma T_4 concentrations. Seasonally low levels were measured in the fall and during early hibernation (September–December). At mid-hibernation (January–February) plasma T_4 concentrations began to increase reaching peak levels in May at the time of normal arousal. Throughout the summer (June–August), plasma T_4 remained elevated and

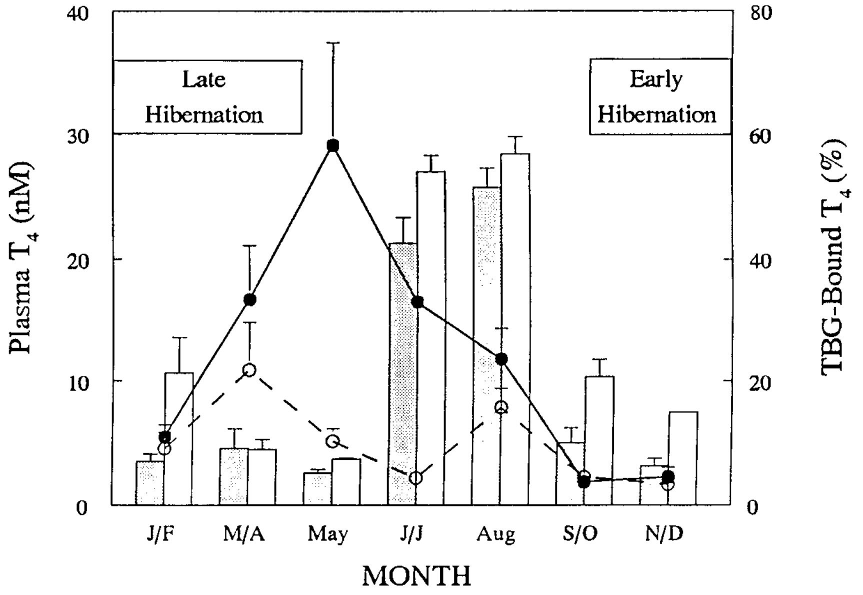

Figure 3.1 Annual cycles of plasma thyroxine (T_4; lines) in adult males (solid circles) and females (open circles) and the % binding of T_4 to thyroxine-binding globulin (TBG; bars) in adult males (solid bars) and females (open bars). Data are means ± SE. Normal periods of hibernation are indicated by open horizontal bars.

then declined to basal levels in the fall prior to the onset of hibernation. TBG activity was elevated only in the summer and remained at basal levels at all other times. Seasonal changes in non-TBG bound T_4 paralleled those of T_4. Interestingly, TBG activity increased in active male bats prematurely aroused from hibernation regardless of laboratory photoperiod or season of arousal. This suggests that the control of TBG may be related to food availability and/or temperature and is not influenced by photoperiod.

In females, overall plasma T_4 concentrations were lower than those observed in males and there was a distinct biphasic seasonal pattern (Figure 3.1). As in males, lowest levels were measured prior to the mid-point of the hibernation period. After mid-hibernation (January–February), plasma T_4 levels increased significantly and peaked in April at the time of normal arousal for females. Circulating T_4 levels declined in May and June but then increased showing a second minor peak in August before declining in the fall.

Reports of pregnancy-associated changes in plasma T_4 have yielded conflicting findings. Increased circulating T_4 levels during late pregnancy, similar to that seen in humans, were observed in *M. waterhousii* (Burns *et al.*, 1972). In contrast, *M. lucifugus* females showed lowest circulating T_4 during the last half of pregnancy (June) with somewhat higher levels during early pregnancy (May). Non-pregnant *M. lucifugus* sampled at the same time as pregnant females showed similar changes in plasma T_4 concentrations. This indicates that measured T_4 changes are most likely related to seasonal cycles than to pregnancy in this species. In *E. franqueti*, plasma T_4 levels did not change during pregnancy but plasma T_3 was significantly increased. Thus, in bats that have been studied, increases in plasma thyroid hormone concentrations are not consistently associated with the physiological state of pregnancy.

The percent binding of T_4 to TBG in *M. lucifugus* showed similar seasonal changes in males and females. Levels decreased in May but increased dramatically in June. The percent binding of T_4 to TBG remained high during lactation and the post-lactational periods (July–August). An additional form of TBG-like activity, seen only in lactating females, had a slightly lower mobility than the TBG of males or non-lactating females (Kwiecinski *et al.*, 1991). This second TBG may be analogous to a TBG variant that has been identified in post-partum human serum (Strel'chyonok *et al.*, 1984). The relationship of this TBG variant to parturition or lactation remains to be determined. It is unknown whether bats possess a TBPA since the low-resolution agarose gels used in studies to date may not have separated TBPA from albumin. In summary, it appears that the changes in circulating T_4 and TBG-bound T_4 that occur during pregnancy in bats may not be the result of pregnancy, but instead reflect seasonal changes that are coincident with pregnancy.

Seasonal changes in thyroid gland morphology

Thyroid glands of mammals are composed of two different kinds of parenchymal cells: parafollicular cells (C-cells or calcitonin-secreting cells) and follicular epithelial cells (responsible for T_4 production). The thyroid gland has been studied histologically in many seasonally breeding microchiropteran bats (for review, see

Kwiecinski *et al.*, 1991; Damassa *et al.*, 1995). In general, these morphological studies indicate that the thyroid increases activity late in the winter or early spring, is active throughout the summer, and regresses by autumn. Such an activity cycle is commonly found in most seasonally breeding mammals.

C-cells in bats, as in other mammals, are scattered throughout the follicular epithelium (but are not in contact with the colloid) and these cells are located throughout the stroma in interfollicular positions. The C-cells of *M. lucifugus* constitute approximately 34% of the total parenchymal cell population of the adult thyroid gland (Nunez and Gershon, 1980). In contrast, the C-cells in humans, mice and rats, constitute 1–5% of total thyroid parenchymal cells. In *M. lucifugus*, C-cells have an activity cycle that parallels follicular epithelial cell activity (Kwiecinski *et al.*, 1987b). Immunocytochemical and biochemical studies have shown that C-cells in bats produce a variety of hormones including: calcitonin, calcitonin gene-related peptide (CGRP), serotonin and somatostatin (Haymovits *et al.*, 1976; Nunez *et al.*, 1988). Many of these hormones appear to undergo seasonal fluctuations but their relationship to reproduction in bats is unknown.

The follicular cells of the thyroid lie immediately adjacent to the follicular lumen and are responsible for the production and secretion of T_4. Seasonal changes in follicular morphology have been most extensively studied in *M. lucifugus* (for review, see Kwiecinski *et al.*, 1987b) and show that during the second half of hibernation (January–March) the follicular epithelium was uniformly low cuboidal to squamous. In March, the thyroid follicles become uniformly small as the colloid is depleted but the follicular epithelium increases in height (indicative of increased synthetic activity). Throughout the summer, most follicles remain relatively small to medium in size with a cuboidal epithelium and minimal colloid. By late summer, thyroids began to show a zonation. Small follicles with cuboidal epithelium persist in the central parts of the gland while peripherally larger follicles with attenuated, squamous epithelial cells predominate.

The characteristic seasonal changes in the morphology of the thyroid follicular cells in bats do not always reflect changes in plasma T_4 concentrations. For example, in the non-hibernating *Macrotus waterhousii*, changes in thyroid follicle dimensions did not correlate with plasma thyroxine values (Burns *et al.*, 1972). Despite its quiescent morphological appearance, the thyroid appears to retain some synthetic capacity during hibernation. Radioiodine uptake studies in *Antrozous pallidus* and *Tadarida (brasiliensis) mexicana* revealed that bats maintained at 4 °C accumulate iodinated compounds in the thyroid and produce iodinated amino acids (Sadler and Tyler, 1960a, b). Although the synthetic activity of the thyroid during hibernation is lower than during active periods, reduced hormone metabolism could translate small changes in synthetic activity into significant changes in plasma T_4 levels. In *Myotis*, the gradual increase in circulating T_4 activity during the second half of hibernation occurs before detectable increases in follicular cell height and when the gland appears morphologically inactive. These findings suggest that the thyroid in bats may play an important physiological role in modulating reproductive function in hibernating bats.

In summary, bats appear to maintain thyroid gland activity throughout the year. The peak seasonal levels of T_4 in *M. lucifugus* occur in the spring and correspond with arousal from hibernation and the onset of reproductive activity. Similar seasonal increases in plasma T_4 levels have been observed in other hibernating species although this is not a universal response (see Damassa *et al.*, 1995). In fact, non-hibernating species typically show peak levels of circulating T_4 at the end of the breeding season (Karsch *et al.*, 1995). Thus, the effects of thyroid hormone on reproductive function in bats are not well understood. In bats, as in birds and other mammals, the effects of T_4 on seasonal reproduction are likely to be permissive. The actions of T_4 are not consistent throughout the subjective year but appear to be influenced by internal seasonal timing mechanisms (Damassa *et al.*, 1995; Karsch *et al.*, 1995). Further work is needed to delineate these mechanisms in hibernating and non-hibernating bats.

3.2.2 Adrenal Gland

A primary function of the adrenal gland is to protect the organism against acute and chronic stress. In acute stress, catecholemines of the medulla mobilize glucose and fatty acids for energy and prepare the heart, lungs and muscles for action. Glucocorticoids protect against overreactions of the body's responses to stress and, in the more chronic stress of food and fluid deprivation, stimulate gluconeogenesis to maintain the supply of glucose. Stress as well as hyper- and hypofunction of the adrenal gland is known to suppress reproduction in mammals, and in women is associated with anovulation and amenorrhea (e.g. Rivier *et al.*, 1986; Burrow, 1991). Measurements of adrenal function in bats to date have been made in a limited number of species but results indicate that there are both seasonal and stress-related changes in adrenocortical activity.

Identification and assay of glucocorticoids and corticosteroid binding globulin (CBG)

Assays of plasma glucocorticoid concentrations in bats (Table 3.2) have typically utilized clinical assay kits. Problems of assay interference and cross-reactivity are less likely with glucocorticoids when compared to the gonadal or thyroid hormones because of the high concentrations of glucocorticoids in blood. This is particularly true for the Megachiroptera. Mean plasma cortisol levels measured in captive *Pteropus hypomelanus, P. vampyrus* and *P. pumulus* were extremely high, ranging from 1.09 to 3.50 μM, depending on species and degree of adrenocortical activation (Widmaier and Kunz, 1993; Widmaier *et al.*, 1994). In these species, cortisol appears to be the principal adrenal glucocorticoid since cortisol concentrations were between two and six times higher than those of corticosterone.

Measurements of plasma cortisol concentrations in Microchiroptera have produced more variable results. In one study, cortisol levels in male *M. lucifugus* (Gustafson and Belt, 1981) measured 15 minutes following arousal from torpor, were similar to those measured in Megachiroptera (Table 3.2). In contrast, Widmaier *et al.*, (1994) found much lower levels of circulating cortisol in wild-caught, predominantly female, *M. lucifugus* sampled in late July, and low circulating cortisol levels were also found in captive *Artibeus jamaicensis* (Table 3.2). For both of these species, plasma cortisol concentrations exceeded those of corticosterone except during the daily nadir in plasma cortisol levels in *M.*

Table 3.2 Basal plasma cortisol and corticosterone concentrations measured by radioimmunoassay in Chiroptera. Values are means for samples collected at various reproductive stages. NP = not present.

	Cortisol		Corticosterone		
	(μM)	(ng/ml)	(μM)	(ng/ml)	Ref.
Suborder: Megachiroptera					
Family: Pteropodidae					
Pteropus vampyrus	1.6	596	0.29	102	1
Pteropus hypomelanus	1.3	1269	1.70	590	1
		483	0.35	121	2
Rousettus aegyptiacus	–	–	0.10	36	1
Pteropus pumilus	1.1	394	0.16	55	2
Suborder: Microchiroptera					
Family: Phyllostomidae					
Carollia perspicillata	–	–	0.10	34	2
Artibeus jamaicensis	0.16	58	0.08	26	2
Family: Vespertilionidae					
Myotis lucifugus[a]					
(morning)	0.04	14	0.24	85	2
(mid-day)	0.14	49	0.15	52	2
(evening)	0.47	170	NP	NP	2
Myotis lucifugus[b]					
Early Hib. (Jan)	3.1	1114	–	–	3
Late Hib. (Apr)	1.4	500	–	–	3
Active (Aug)	1.0	350	–	–	3

References:
1. Widmaier and Kunz, 1993
2. Widmaier *et al.*, 1994
3. Gustafson and Belt, 1981

[a] Samples were collected within 2–3 minutes of handling. Sampled animals consisted of both males and females at various reproductive stages although the majority were post-lactational females.

[b] Samples were collected approximately 15 minutes after arousal from torpor. All animals sampled were adult males.

lucifugus (Widmaier *et al.*, 1994). Differences in the plasma cortisol levels obtained for *M. lucifugus* likely relate to seasonal and stress-related factors (see below).

The levels of plasma glucocorticoids in Megachiroptera are more than five-fold higher than those of humans and comparable levels are found only in a few other species such as the New World monkeys (Cassorla *et al.*, 1982). A specific circulating corticosteroid binding globulin (CBG) with a high affinity for glucocorticoids has been identified in virtually all vertebrate species (Westphal, 1986). In representatives of both the Megachiroptera and Microchiroptera, a CBG was identified and exhibited electrophoretic mobilities distinct from both sex hormone-binding globulin (SHBG) and albumin (Kwiecinski *et al.*, 1987a). Interestingly, no CBG activity was detectible in the common vampire bat, *Desmodus rotundus*. In several species of *Pteropus*, testosterone and progesterone also bound to plasma CBG. This could account, in part, for the unusually high circulating levels of progesterone and corticosterone found in both sexes (Wang *et al.*, 1992). The high levels of circulating glucocorticoids in captive-breeding pteropodids and other bats (Widmaier and Kunz, 1993) represent an interesting model for the study of glucocorticoids, CBG and their role in the timing and modulation of reproductive activity in mammals.

Daily and seasonal changes in circulating adrenal glucocorticoids

A pronounced diurnal rhythm of plasma cortisol and corticosterone has been characterized in *M. lucifugus* (Widmaier *et al.*, 1994). Cortisol levels in the evening, just before the onset of activity, were more than 10-fold higher than those measured in the morning and are consistent with findings in other mammals. Peak corticosterone levels occurred in the morning, when cortisol levels were at their nadir. The function of the reciprocating peaks in these two glucocorticoids is presently undefined.

Plasma cortisol levels in *M. lucifugus* also show a distinct seasonal cycle (Gustafson and Belt, 1981). Cortisol levels were lowest during the months of activity (1.3 μM) and highest during hibernation (2.6 μM). Contrary to the concept of poly-glandular involution during hibernation in mammals, this study indicates that in bats the adrenal gland is not completely involuted but plays a substantial role in the animal's metabolism during hibernation (Gustafson and Belt, 1981). The cortisol levels measured in this study are the highest reported for any of the mammalian hibernators. Although there has not been an ultrastructural investigation of the adrenal cortex in *M. lucifugus*, morphological studies in other hibernating bats indicate that winter is a period of high adrenocortical activity (see below). Adrenal glucocorticoids are produced largely by the adrenal zonae fasciculata and reticularis. These zones of the adrenal cortex are also responsible for production of the androgens, androstenedione and dehydroepiandrosterone (DHEA). Although detailed analysis of steroid production by the adrenal cortex has not been conducted in bats, histological studies have provided evidence for the seasonal activation of these two regions.

Seasonal changes in adrenal morphology

Cells of adrenal zonae fasciculata and reticularis in *Miniopterus schreibersii* were found to be more developed cytologically during hibernation than during the summer active period (Planel *et al.*, 1961). Similar results were also obtained from histochemical and electron microscopic studies (Romita and Montesano, 1972b) of the adrenal cortex of two other microchiropteran species, *Vesperugo savi* (= *Pipistrellus savi*) and *Vesperugo piccolo* (synonym not found). These observations correlate well with the high levels of circulating glucocorticoids found in hibernating *M. lucifugus*. Although it is well recognized that adrenal glucocorticoids can affect reproductive function, the possibility also exists that changes in adrenal androgen production or secretion can also influence reproduction. In the pteropid, *Rousettus aegyptiacus*, increases in adrenal size were measured at times of reproductive activity in both sexes (Baranga, 1980, 1984). In the vespertilionid bat, *Scotophilus heathi*, high levels of circulating androstenedione were associated with the initial round of spermatogenesis, but data on glucocorticoid production are not available in this species (Krishna and Singh, 1997). Interestingly, in *M. lucifugus* and *M. septentrionalis*, very high levels of androstenedione were measured at the time of spermatogenesis (~1000 ng/ml) and throughout the period of winter hibernation

(200–400 ng/ml: see Chapter 7) coincident with the period of maximal adrenocortical activation. Whether androstenedione is of testicular or adrenal origin and how it influences reproduction in bats showing various seasonal reproductive adaptations remains to be investigated.

Adrenal response to stress/arousal

Prior to the development of radioimmuno- and immunometric-assays, measurements of ascorbic acid depletion in various endocrine organs were used to assess the degree of stimulation. Such studies were performed on adrenals of *M. lucifugus* by Krutzsch and Hess (1961). In these studies, captivity *per se* did not change the adrenal ascorbic acid content in either hibernating or active bats. Among captive animals, administration of adrenocorticotrophic hormone (ACTH) to hibernating and epinephrine to active animals decreased adrenal ascorbic acid content. However, the most dramatic depletion occurred in non-captive hibernating bats stressed by arousal, a finding well correlated with blood corticosterone levels in hibernating hamsters, after similar treatment (Elton *et al.*, 1959). Measurements of plasma cortisol levels in captive bats collected during late hibernation (April) showed no significant changes 15 minutes after arousal from hibernation (Gustafson and Belt, 1981) when compared to those for which arousal was prevented. For *M. lucifugus*, April is a transition time between deepest hibernation and greater metabolic activity. Plasma cortisol levels at this time resemble levels observed during the months of activity rather than levels observed during hibernation, even though these bats were found in hibernation at this time. The lack of a stress response in plasma cortisol levels could be attributed to previous arousals from hibernation or to the sluggish nature of the cortisol response to arousal stress.

Further studies of the timing of stress-induced adrenal activation in Megachiroptera were carried out by Widmaier and Kunz (1993). When individual *Pteropus hypomelanus* were subjected to restraint stress, cortisol levels rose 800% by two hours (Widmaier and Kunz, 1993). In a subsequent study (Widmaier *et al.*, 1994), two-fold increases in plasma cortisol levels were measured in *P. pumilus* after one hour of restraint. A one-hour restraint was also shown to produce increases in plasma ACTH levels in *P. hypomelanus* but these were not significant due to high variability. Even the stress associated with fifteen minutes of routine handling (weighing, measuring, etc.) elicited a significant rise in plasma glucocorticoids in *P. hypomelanus*.

In summary, plasma glucocorticoid levels increase in bats in response to standard stressors such as handling or restraint despite very high basal steroid levels in the plasma. In addition, arousal from hibernation also appears to constitute a significant stress and may result in prolonged elevations in plasma glucocorticoids (adrenal activity). Considering the muscle wasting (catabolic) effects of glucocorticoids, this finding would be consistent with observations in *Eptesicus fuscus* that significant gluconeogenesis from tissue protein occurs during spontaneous arousals from hibernation (Yacoe, 1983). Since stress and glucocorticoids are known to suppress reproductive function in mammals, the relationship between reproductive function and the high levels of glucocorticoids reported in bats represents an interesting model for further studies of the physiological role of the adrenal on reproductive timing and modulation in bats.

3.2.3 Endocrine Pancreas

The pancreas plays a key role in the provision of nutrients to all body cells during times of feeding and fasting. The endocrine hormone-producing cells are organized into 'islets' (1–2% pancreatic mass) dispersed among the pancreatic acinar (exocrine) cells. A number of hormones have been localized to the pancreatic islets of mammals by a variety of means, but in bats, studies have focused on insulin and glucagon. Insulin secretion is primarily stimulated by hyperglycemia, and insulin controls cellular storage and metabolism of ingested metabolic fuels. The major physiological stimulus for glucagon secretion is hypoglycemia, and it produces a rise in blood glucose by stimulating hepatic glycogenolysis and gluconeogenesis. Recently, numerous studies have demonstrated that insulin represents an important factor in reproduction in that it regulates both ovarian secretion and peripheral metabolism of androgens (Pasquali *et al.*, 1995). In bats, there is a paucity of information on pancreatic function in general, and no studies have focused on the pancreas and reproductive physiology.

An immunocytochemical study of the pancreatic islets in the vampire bat (*Desmodus rotundus*) identified numerous insulin-positive (beta) cells and moderate numbers of glucagon-immunoreactive (alpha) cells. Although pancreatic polypeptide is widely distributed in mammals (Gepts *et al.*, 1978), no immunoreactivity was observed in *D. rotundus* pancreas or gastrointestinal tract (Yamada *et al.*, 1984). Alpha, beta and delta (somatostatin-secreting) cells were identified in the pancreatic islets of two species of hibernating bats, *Vesperugo savi* (*Pipistrellus savi*) and *Rhinolophus ferrumequinum*. The alpha and beta cells underwent significant seasonal changes, with a morphology consistent with high levels of activity in the spring and summer, and little evidence of activity during hibernation (Romita and Montesano, 1972a). Such morphological studies indicate a change in cellular activity but do not necessarily reflect changes in serum hormone levels.

Plasma levels of glucose in *Pteropus vampyrus, P. hypomelanus, and Rousettus aegyptiacus*, non-hibernating species, were within normal mammalian range (80–100 mg/dl) for both sexes (Widmaier and Kunz, 1993). Glucose was at a minimum before food presentation and maximum after food presentation. In *M. lucifugus*, blood glucose levels during hibernation in April were very low (28 mg/dl) with an insulin level of 21 μU/ml (Bauman, 1990), whereas unfed bats following arousal in April had glucose concentrations of 155 mg/dl and an insulin level of 69 μU/ml. This is consistent with results from other species that abstain completely from food during hibernation (South and House, 1967). Measurements of pancreatic insulin content in *M. lucifugus* (Bauman, 1990) demonstrated slight increases between summer (June) and fall (October) with further increases in the early spring (late April). In contrast, pancreatic glucagon content increased progressively from summer through winter without major increases in the spring. Elevated pancreatic glucagon during hibernation raises the possibility that it assures carbohydrate homeostasis during prolonged fasting. The progressive elevation of pancreatic insulin during hibernation may provide a storage pool of insulin required for immediate release upon arousal.

The preceding sections reviewed peripheral endocrine factors considered 'non-reproductive' that may have effects on the timing and modulation of reproductive expression in mammals. In bats, thyroid, adrenal and pancreatic hormones undergo seasonal changes and likely play a role in the control of reproduction. Another factor that can modulate and directly affect reproduction is sex hormone-binding globulin. This protein not only binds sex steroids but may also provide a link between other hormones and modulation of reproductive timing. The following section reviews current understanding of the control and actions of SHBG in bats.

3.2.4 Leptin

The discovery in 1994 of leptin, the 16-kD cytokine-like hormone product of the *ob* (obese) gene in mice (Zhang *et al.*, 1994), has led to a surge in research activity in a variety of mammals regarding the role of leptin in energy balance. Leptin is produced in several organs, including white and brown adipose tissue, the placenta and fetal tissues. As a hormone, it influences food intake, maintenance of energy expenditure (insulin secretion, lipolysis and sugar transport) and plays roles in reproduction and immune functions (Frübeck *et al.*, 1998; Trayhurn *et al.*, 1999). The production of leptin is modulated by insulin, glucocorticoids, fasting, and β-adrenoceptor agonists (Trayhurn *et al.*, 1999). The leptin receptor gene is widely expressed and the leptin system now appears to be highly complex. The hormone is believed to be involved in a range of physiological processes transcending the initial lipostatic theory. In bats, the relationship of leptin to reproduction has been examined in *M. lucifugus* (Widmaier *et al.*, 1997) and *E. fuscus* (Kunz *et al.*, 1999). In pregnant *M. lucifugus* and *E. fuscus*, leptin levels rose during pregnancy and declined following parturition. During lactation, reduced leptin was significantly correlated with body weight in *E. fuscus*, but not in *M. lucifugus*. Leptin was also found to be significantly reduced in acutely fasted (16 hours) animals. These studies support the suggestion that leptin may play a role in reproduction and regulation of energy balance in bats.

3.3 SEX HORMONE-BINDING GLOBULIN (SHBG)

High-affinity binding proteins for hormones are present in the plasma of most vertebrate species (Westphal, 1986). In eutherian mammals, sex hormone-binding globulin (SHBG) is the protein typically responsible for the high-affinity extracellular binding of androgens and oestrogens. Structural studies of SHBG have been conducted in only a few species but suggest that SHBG is a dimeric glycoprotein with a relative molecular weight of approximately 100,000 D and a single steroid binding site (Joseph, 1994; Damassa and Cates, 1995). The liver is considered to be the principal source of circulating SHBG; however, the testis, placenta, brain, prostate and selected other tissues also express SHBG or SHBG-like proteins (Joseph, 1994; Damassa and Cates, 1995). Recent evidence suggests that these proteins modulate steroid action within specific target tissues. The ability to activate certain steroid-dependent target tissues (e.g. testes) while maintaining others in an inactive state (e.g. sex accessory glands) is the hallmark of the asynchronous reproductive cycles observed in many bat species. In the following section, we will review what is known about SHBG in bats and the relationship of this protein to asynchronous reproductive cycles.

3.3.1 Characterization of Sex Hormone-Binding Globulin (SHBG) in Bats

Among mammals, SHBG typically binds various androgens showing the highest affinity for dihydrotestosterone (DHT). Association constants for DHT-binding at 4°C range from 0.1 to 5 nM^{-1} or approximately an order of magnitude lower than that of the intracellular androgen receptor (AR) (Renoir *et al.*, 1980). However, unlike the AR, SHBG does not bind the synthetic androgen R1881 and shows no sequence homology with the steroid receptor family of proteins. The molecular sequences of SHBG have been determined for human, rabbit, rat, mouse and Djungarian hamster (see Cates *et al.*, 1995). Within a given species, testicular androgen-binding protein (ABP) shares a common sequence with SHBG, differing only in the extent of glycosylation. Among species, the sequence identity of SHBG/ABP is typically more than 70%. Nevertheless, antisera generated against SHBG usually only cross-reacts with SHBG from the same or closely related species (Renoir *et al.*, 1980). Attempts to characterize SHBG in bats using various antisera raised against human, rat or Djungarian hamster SHBG have not been successful. Therefore, bat SHBG has been identified by its affinity for sex steroids and by physicochemical properties.

Sex hormone-binding globulin was consistently found in representatives of two distinct families of bats, the phyllostomids and the vespertilionids (Table 3.3). However, no SHBG activity was detected in random serum samples from adult *Desmodus rotundus* or from several species of pteropodids. Studies of SHBG activity in the serum of rhinolophids or in members of other families of Chiroptera have not been conducted. Species differences in blood SHBG activity have also been observed in other mammalian orders. The golden hamster, like many other rodent species, lacks measurable plasma SHBG activity. In contrast, SHBG expression in the Djungarian hamster increases dramatically at birth and remains high until puberty (Gustafson *et al.*, 1989; Cates *et al.*, 1995). In *M. lucifugus*, SHBG levels were low in near-term foetuses, increased more than 30-fold by one week of age, fell dramatically by four weeks of age (onset of puberty and first spermatogenesis), and returned to basal values by 12 weeks, prior to the onset of hibernation (Gustafson and Damassa, 1984). These species-associated differences in the expression of SHBG may reflect unappreciated divergence in the strategies developed for reproductive control.

As mentioned previously, SHBG universally binds androgens with high affinity. In common with primates, but unlike most other mammals, bat SHBG also shows relatively high affinity binding of the oestrogen, oestradiol (Table 3.3). Thus, in bats, SHBG has the potential for modulating the actions of both androgens and oestrogens. Little is known about the specific actions of SHBG in bats but studies

Table 3.3 Steroid-binding proteins in adult bats. Electrophoretic mobilities under native conditions (Rf) of sex hormone-binding globulin (SHBG) and corticosteroid-binding globulin (CBG). Affinity constants (K_A) for the binding of dihydrotestosterone (DHT) to SHBG were determined at 4°C using steady-state gel electrophoresis. The relative binding affinities (RBA) of testosterone (T) and oestradiol (E_2) to SHBG are expressed as a percentage of DHT-binding.

	Rf		SHBG			
	SHBG	CBG	K_A–DHT		RBA(%)	
			nM^{-1}	T	E_2	Ref.
Suborder: Megachiroptera						
Family: Pteropodidae						
Pteropus giganteus	NP	0.51	NP	NP	NP	1
Pteropus scapulatus	NP	–	NP	NP	NP	2
Pteropus poliocephalus	NP	–	NP	NP	NP	2
Pteropus alecto	NP	–	NP	NP	NP	2
Rousettus aegyptiacus	NP	0.51	NP	NP	NP	1
Suborder: Microchiroptera						
Family: Desmodontidae						
Desmodus rotundus	NP	NP	NP	NP	NP	1
Family: Phyllostomidae						
Brachyphylla cavernarum	0.19	0.42	0.50	36	77	1
Artibeus jamaicensis	0.25	0.47	0.60	28	83	1
Family: Vespertilionidae						
Myotis lucifugus	0.25	0.53	1.02	43	77	3
Myotis keenii	0.26	0.42	–	–	–	1
Eptesicus fuscus	0.27	0.44	–	–	–	1

NP = Not present
– = Not determined
References:
1. Kwiecinski *et al.*, 1987a
2. Wang *et al.*, 1992
3. Gustafson and Damassa, 1985

in other mammals indicate that this protein likely influences steroid hormone action by several distinct mechanisms.

3.3.2 Mechanisms of Action of Sex Hormone-Binding Globulin (SHBG)

The effects of SHBG on steroid target tissues have been traditionally explained by a 'steroid-trapping' model (Figure 3.2). This model states that steroids bound to SHBG are 'trapped' and, therefore, unable to gain access to steroid-sensitive cells (Westphal, 1986). As a corollary, SHBG can also trap steroid metabolites and thus influence cellular steroid metabolism. Studies using mammalian cells and tissues in culture have typically supported the predictions of this model. For example, in androgen-sensitive human prostate cells, addition of SHBG to the media shifts the dose-response curve such that higher concentrations of testosterone are required to produce equivalent biological responses (see Damassa *et al.*, 1991). Based on such studies, the biologically active fraction of a given steroid is generally considered to be that fraction which is not bound to SHBG. Although commonly accepted, this

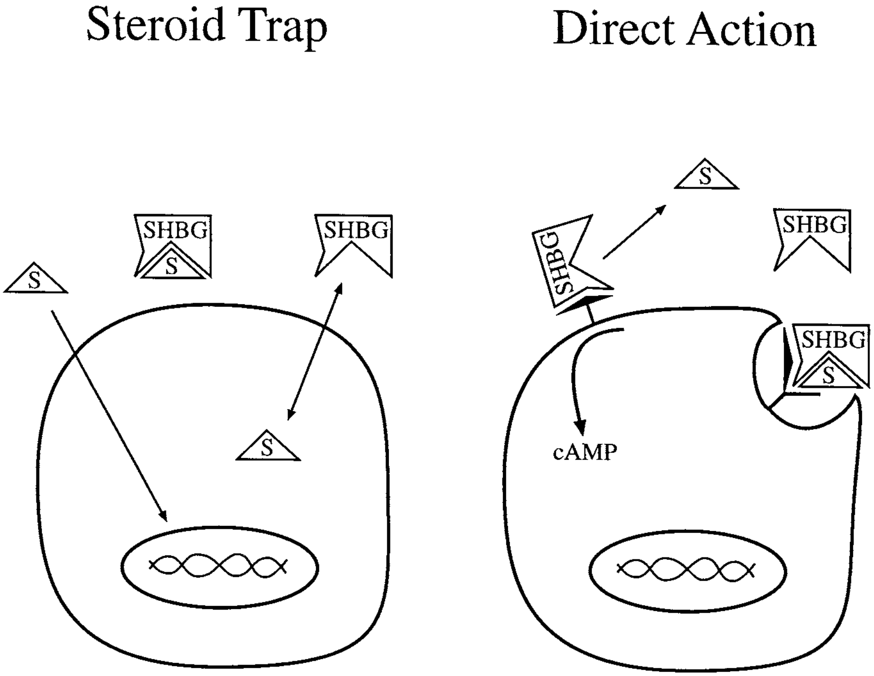

Figure 3.2 Mechanisms of action of sex hormone-binding globulin (SHBG). The demonstrated actions of SHBG have been summarized by two distinct models. The classical Steroid-Trapping model views SHBG as an extracellular sink for steroids. By binding steroids, SHBG can prevent steroid signals from entering the cell. As a corollary, SHBG can also facilitate the removal of specific steroid metabolites and alter steroid metabolism within the cell. A second model, supported by recent studies, proposes direct actions of SHBG on cellular function. According to the Direct-Action model, SHBG specifically binds to SHBG receptors on steroid-sensitive cells. Depending on the cell, SHBG can be internalized and thus provide targeting of steroid to specific cellular compartments. Alternatively, receptor-bound SHBG can interact with steroids and activate second-messenger mediated intracellular signalling pathways.

model now appears to be overly simplistic. Sex steroid levels in the blood are typically regulated by feedback control systems involving the hypothalamus, pituitary and the gonads. The trapping of sex steroids in blood can transiently buffer or modulate hormone delivery to target tissues and affect steroid clearance. However, steroid trapping also reduces the feedback effects of steroids on the hypothalamic-pituitary axis. A marked increase in circulating SHBG could therefore reduce the steroid feedback signal and consequently lead to increased steroid production and a return of free steroid concentrations to the normal range. Thus, simple ligand trapping is likely to have long-term effects on steroid availability only within the normal range of functional feedback systems (Damassa and Gustafson, 1988).

More recent evidence now supports several novel mechanisms of SHBG action. Extracellular SHBG can bind to specific membrane-associated receptors and the process of receptor-mediated endocytosis has been demonstrated in several different cell types (Strel'chyonok *et al.*, 1984; Gerard, 1995). Thus, SHBG could be functioning as a specific carrier of steroids to target cells. Although unbound steroids typically diffuse freely into cells, internalized SHBG-bound steroids could activate different intracellular signalling mechanisms or be targeted to specific cellular compartments (Siiteri *et al.*, 1982). In fact, SHBG may itself be part of a steroid signal transduction system. The binding of SHBG or the SHBG-steroid complex to specific SHBG receptors on the cytoplasmic membrane has been shown to activate cellular cAMP-mediated signalling pathways (Nakhla and Rosner, 1996; Fortunati *et al.*, 1996). In addition, the uterus, prostate, brain, and several other steroid-responsive organs produce SHBG or SHBG-like proteins (Joseph, 1994). Thus, the possibility exists that local SHBG production, independent of circulating SHBG concentrations, could serve as an autocrine regulator of steroid sensitivity. The extent to which these SHBG signalling pathways modulate specific biological responses to sex steroids *in vivo* remains to be determined.

In summary, recent findings suggest that the actions of SHBG are likely to be more extensive than those predicted by the 'steroid-trapping' models. The identification of SHBG receptors on certain steroid target cells as well as the demonstration of local production of SHBG raises the possibility that SHBG can differentially modulate steroid action at target tissues. This would provide a mechanism for circulating androgens and oestrogens to selectively activate certain reproductive organs and not others. Defining the precise actions of SHBG in the control of reproduction in bats will depend on the eventual availability of purified and/or recombinant bat SHBG and the development of appropriate *in vitro* systems to study SHBG action. Nevertheless, studies reviewed below on the control of SHBG secretion and its relationship to seasonal reproductive processes in *M. lucifugus* provide insight into the possible roles of SHBG in species exhibiting asynchronous reproductive cycles.

3.3.3 SHBG and Seasonal Reproductive Processes

Seasonal changes in plasma SHBG activity have been studied in wild-caught adult *M. lucifugus* from northern New England (Gustafson and Damassa, 1985; Kwiecinski *et al.*, 1991). In both males and females, plasma SHBG concentrations are at low basal levels during the winter and early spring (Figure 3.3). In early June, SHBG levels increase dramatically and are elevated throughout July and the first half of August. The increase in plasma SHBG activity in males occurs before seasonal increases in plasma T concentrations. During active spermatogenesis (June–August) plasma T levels increase to extremely high levels. Throughout this period the steroid-binding capacity of plasma SHBG exceeds serum T concentrations by more than two-fold (Gustafson and Damassa, 1985, 1987). SHBG and T levels decrease prior to hibernation and remain at low basal levels throughout the winter. In juveniles, total plasma T levels were also elevated during the postnatal period and declined in parallel with SHBG reaching a nadir prior to hibernation (Gustafson and Damassa, 1984). Interestingly, the androgen-dependent accessory sex glands do not reach maximal levels of stimulation until late in August when SHBG levels begin to decline and circulating T levels transiently

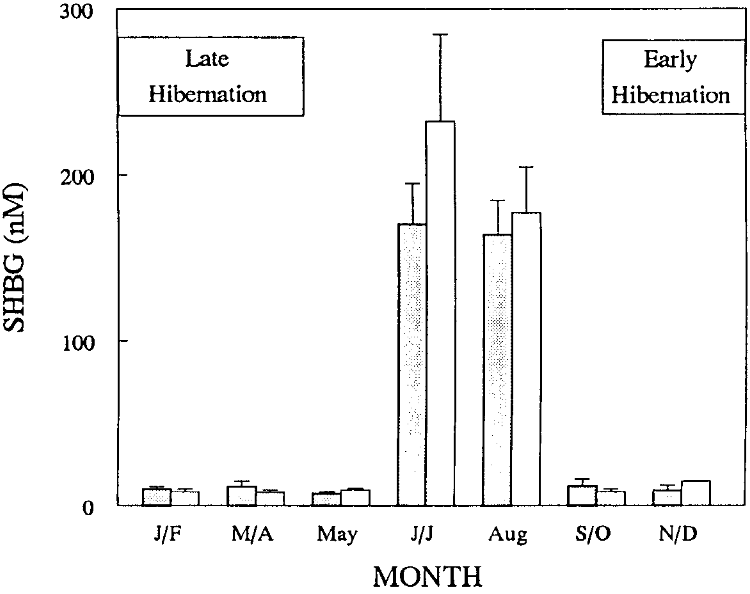

Figure 3.3 Annual plasma sex hormone-binding globulin (SHBG) concentrations in *M. lucifugus* adult males (solid bars) and females (open bars). Values are means ± SE. Normal periods of hibernation are indicated by the open horizontal bars.

exceed serum SHBG concentrations. However, the relationship of SHBG to accessory sex gland stimulation throughout the annual reproductive cycle of *M. lucifugus* cannot be simply explained by differences in the molar ratios of SHBG and T. In the fall, serum SHBG and T levels both decline and throughout hibernation SHBG concentrations are higher than those of serum T. Yet, androgen-dependent accessory sex gland weights remain maximal throughout hibernation.

Female *M. lucifugus* enter hibernation at the time when the single preovulatory follicle is maturing. Ovulation is then delayed and the ovum and follicle are held in meiotic arrest. Throughout hibernation, serum SHBG levels are low and changes in circulating SHBG levels do not appear to be necessary for the resumption of follicular development. Ovulation normally occurs at the time of arousal in April but plasma SHBG concentrations do not increase significantly until June. In this species, plasma levels of estradiol have not been measured. The mechanisms whereby seasonal changes in circulating SHBG can specifically modulate sex steroid action on target tissues remain to be determined.

3.3.4 Endocrine Control of SHBG and Relation to Reproduction

Given that SHBG modulates androgen- or oestrogen-mediated activation of target tissues, factors that control SHBG expression could serve to link environmental changes and reproductive function. A variety of studies have been conducted to identify endocrine factors responsible for the control of circulating SHBG concentrations.

Sex steroids

Based on studies in humans, oestrogens and androgens are frequently touted as the major factors regulating circulating SHBG concentrations. In both men and women, the administration of oestrogens increases circulating SHBG concentrations while androgen treatment reduces SHBG levels (Westphal, 1986). Women typically have higher serum SHBG concentrations than men and, consistent with the postulated regulatory role of sex steroids, these sex differences are eliminated by gonadectomy. In apparent contrast to humans, sex steroids appear to have little influence on circulating SHBG concentrations in bats. Male bats gonadectomized at the time of premature arousal from hibernation experienced arousal-induced increases in plasma SHBG levels that were not different from those of intact controls. The rise in SHBG levels occurred despite the fact that plasma estradiol and testosterone levels remained low or undetectable throughout the study (Damassa and Gustafson, 1984). Similarly in females, plasma SHBG concentrations remained low during the periovulatory period when circulating oestrogen levels are expected to be high. During late pregnancy, SHBG levels rise but this increase does not appear to be due to endocrine changes associated with pregnancy. Comparable increases in SHBG levels were seen in non-pregnant females and in males sampled at the same time. Although it has been suggested that changes in serum androstenedione concentrations may influence SHBG levels (Krishna and Singh, 1997), changes in androgen and oestrogen levels within physiological ranges do not appear to be major factors in the control of SHBG secretion in bats. The fact that SHBG is not influenced by changes in sex steroid levels in *Myotis* suggests that this protein could act to mediate extra-gonadal control of reproductive function.

Thyroid hormones

Studies in *M. lucifugus* have demonstrated that, unlike the sex steroids, thyroid hormones are important regulators of SHBG. Following premature arousal from hibernation, both males and females experience a 30-fold increase in plasma SHBG levels within three weeks. This increase in SHBG is completely abolished by thyroidectomy but can be fully reinstated with T_4 treatment (Damassa *et al.*, 1985). The positive actions of T_4 on SHBG appear to be specific and not related to metabolic effects. Both the naturally-occurring isoform of T_4, L-T_4, as well as the metabolically inactive D-isoform, restored the post-arousal increase in SHBG in thyroidectomized bats with equal potency.

These observations on the control of SHBG expression by T_4 at first inspection appear to be at odds with measurements of seasonal changes in plasma T_4. In both males and females, plasma T_4 levels begin to rise at mid-hibernation and reach maximal levels prior to normal arousal (see above). In addition, measurement of plasma T_4 concentrations in bats prematurely aroused from hibernation in April show that T_4 levels are high at the time of arousal but fall dramatically as SHBG levels increase during the three weeks following arousal (Damassa *et al.*, 1995). These apparently disparate observations lead to the conclusion that during normal arousal from hibernation thyroid hormone plays a permissive role in SHBG secretion. A certain threshold level of T_4 is required for SHBG expression but other down-stream factors seem to ultimately regulate SHBG expression. The identity of these distal regulatory factors remains elusive. To date, neither gonadectomy, short photoperiods nor partial food restriction have been found to influence plasma SHBG levels following premature arousal from hibernation in the spring (Damassa and Gustafson, 1984, 1985).

To make matters worse, the relative importance of various factors for regulating SHBG expression appears to change with the season. Bats aroused in the fall, shortly after the normal onset of hibernation, also show marked increases in circulating SHBG concentrations. At this time of year, plasma T_4 concentrations are at seasonally low levels and arousal-induced increases in T_4 directly correlate with increased plasma SHBG activity.

Thus, in *M. lucifugus* it seems that SHBG regulation is dictated by season. In the spring, T_4 appears to play only a permissive role in SHBG regulation while in the fall, plasma T_4 levels seem to directly determine circulating SHBG concentrations. The seasonal changes in SHBG levels do not appear to be simply a reflection of changes in environmental conditions. Housing bats collected in mid-summer under summer-like conditions of temperature, photoperiod and food availability did not prevent the decrease in plasma levels of T_4 and SHBG that normally occur in the fall (Damassa *et al.*, 1995). These results suggest that SHBG regulation is also influenced by an endogenous annual rhythm.

The complexity of the control of SHBG expression in bats may be a reflection of its importance as an integrator of environmental and internal signals. Thus, SHBG expression and the presumed alterations in the steroid-responsiveness of certain target tissues can be linked to specific seasonal and environmental factors known to influence reproduction.

Growth hormone, insulin and insulin-like growth factors (IGF)

Studies conducted primarily in humans have indicated that growth hormone as well as certain pancreatic hormones exert marked inhibitory effects on plasma SHBG concentrations. It has long been recognized that acromegalic men and women have reduced levels of SHBG (see Westphal, 1986). More recently, it has been shown that insulin or insulin-like peptides may also act to down-regulate SHBG secretion. Treatment of human liver (Hep G2) cells *in vitro* with insulin or IGF-I produces a marked decrease in the expression of SHBG (Plymate *et al.*, 1988; Crave *et al.*, 1995). A similar effect of insulin is also seen *in vivo*. Healthy women show a significant negative correlation between fasting insulin levels and plasma SHBG concentrations (Preziosi *et al.*, 1993). Likewise in normal men, there is a significant negative correlation between plasma SHBG levels and the insulin pulse frequency (Peiris *et al.*, 1993). These and other studies suggest an inverse relationship between plasma SHBG concentrations and pancreatic insulin secretion.

The role of growth hormone, insulin or the IGFs in regulating plasma SHBG concentrations in bats remains to be determined. In *Myotis lucifugus*, pancreatic insulin concentration and content increases significantly during the second half of hibernation but returns to low basal levels in June (Bauman, 1990). This fall in pancreatic insulin levels corresponds to the increase in plasma SHBG concentrations. To what extent feeding-induced changes in insulin secretion affects SHBG levels and reproductive processes remains to be determined. Moderate food restriction in *M. lucifugus* did not affect the normal post-arousal increase in plasma SHBG concentrations but insulin levels were not measured in this study (Damassa and Gustafson, 1985). The potential inhibitory effects of insulin or similar factors on SHBG production could provide an important link between food availability and the activation of the reproductive system.

3.4 SUMMARY

Many peripheral endocrine factors classically considered non-reproductive have been implicated in the timing and modulation of reproductive processes. These include hormones of the thyroid, adrenal and pancreas, and specific extracellular binding proteins for these hormones and sex steroids. Many of these endocrine factors have been identified in bats and undergo marked seasonal changes. Although much work has been done on the characterization of seasonal changes in endocrine gland morphology in bats, plasma hormone levels do not always reflect observed changes in morphological characteristics.

The thyroid gland appears to be an important modulator of reproductive activity in bats. In hibernating bats, peak seasonal levels of T_4 occur in the spring and precede arousal from hibernation. In addition, T_4 is required for the induction of post-arousal increases in plasma SHBG activity. These effects of T_4 on SHBG and seasonal reproduction appear to be permissive and depend on internal seasonal timing mechanisms. Further work is needed to delineate these mechanisms.

Very high levels of circulating glucocorticoids are found in certain bat species. Bats increase plasma glucocorticoid levels in response to standard stressors such as handling or restraint. In addition, arousal from hibernation may result in prolonged elevations in plasma glucocorticoids. Since stress and glucocorticoids are known to suppress reproductive function in mammals, the relationship between reproductive function and high levels of glucocorticoids in bats represents an exciting model for further studies of the role of the adrenal gland on timing and modulation of reproduction.

A high-affinity androgen- and oestrogen-binding protein (SHBG) has been characterized in bats. This protein was consistently identified in representatives of two distinct families of bats, the phyllostomids and the vespertilionids. However, no SHBG activity was detected in adult *Desmodus rotundus* or from several species of pteropodids. In common with primates, but unlike most other mammals, bat SHBG also shows relatively high affinity binding of oestradiol. Thus, in bats, SHBG has the potential for modulating the actions of both androgens and oestrogens. These species-associated differences in the expression of SHBG may reflect unappreciated divergence in the strategies developed for reproductive control.

The ability to activate certain steroid-dependent target tissues (e.g. testes) while maintaining others in an inactive state (e.g. sex accessory glands) is the hallmark of the asynchronous reproductive cycles observed in many bat species. Little is known about the specific actions of SHBG in bats but recent findings suggest that SHBG can differentially modulate steroid action at target tissues. This would provide a mechanism for circulating androgens and oestrogens to selectively activate certain reproductive organs and not others and possibly modulate feedback control systems. Defining the precise actions of SHBG in the control of reproduction in bats will depend on the eventual availability of bat SHBG and the development of appropriate *in vitro* systems to study SHBG action. In *M. lucifugus*, seasonal changes in SHBG levels do not appear to be simply a reflection of changes in environmental conditions. Expression of this protein is also influenced by a variety of endocrine factors as well as an endogenous annual rhythm. The complexity of the control of SHBG expression in bats may be a reflection of its importance as an integrator of environmental and internal signals in the control of reproduction.

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4

Anatomy, Physiology and Cyclicity of the Male Reproductive Tract

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Reproductive Biology of Bats
0-12-195670-9

4.1 INTRODUCTION

Considering the worldwide distribution and immense diversity exhibited by members of the order Chiroptera, remarkably limited attention has been given to reproduction in the male. The details of the structure of the reproductive system are generally not described. Even less is known about the function and physiological control of reproduction in the male.

This review brings together much of the current knowledge about the biology of male reproduction in Chiroptera. Special attention has been given to the functional cyclicity and gross and microscopic morphology of the primary, accessory and secondary sexual structures of the male reproductive tract. The general morphological plan of the organs that it comprises is relatively conservative in gross and microscopic structure. There is, however, variation in the complement and appearance of organs within and between taxonomic groups. Male bats also exhibit diversity in the timing and frequency of their reproductive cycles annually; in some species this may be expressed in a unique functional (dysynchronous) timing between primary (testes) and accessory (prostate, ampullary, seminal vesicles, urethral and Cowper's) sex glands. Permissive to this pattern can be the prolonged retention of viable spermatozoa in the caudae epididymidides and extended accessory sex gland function to effect additional intermittent winter mating by males who apparently retain their libido beyond the fall copulatory period.

Male reproductive activity may be accompanied by a wide variety of behavioral performances (vocalizations, body movements, special flight patterns, roost defense) and secondary sexual structural characteristics (i.e. odoriferous secretions from dermal glands, pelage adornments, often associated with dermal glands and urinary tract markings), all of which serve to broadcast sexual readiness and reproductive libido in the pursuit of female partners. These can be a significant part of the mating repertoire.

Bats vary widely in form, ecological requirements and reproductive adaptations. No other mammalian order exhibits greater niche diversity. Unique solutions have evolved for optimum timing of reproductive events coincident with these diverse nutritional needs. The male response to female reproductive cyclicity is expressed in the wide variety of reproductive patterns, the events of which are seemingly triggered by the female's sexual readiness. Those species which find their nutritional needs unrestricted by seasonal ecological events are more flexible in their reproductive pattern. An example of this is found in the hemophagous species, *Desmodus rotundus,* which is continuously reproductive. Those that face restrictive weather (i.e. northern and southern temperate regions) have evolved a monoestrous reproductive pattern with unique specializations to insure reproductive success (i.e. male and female sperm storage, a delay in ovulation, implantation, or embryogenesis). These schemes guarantee optimal timing of gestation, birth and lactation in relation to food supplies.

Tropical and subtropical species, both 'Old' and 'New' World, also express a wide range of reproductive patterns (monoestry, polyoestry (seasonally variably expressed) and continuous), also apparently timed in response to varying ecological conditions and resultant optimum nutritional opportunities. Here again, it appears that the male reproductive pattern is determined by female reproductive receptivity. Speculations relative to male reproductive events, however, mostly remain to be proven by comprehensive studies directed at defining these processes.

4.2 PUBERTY

In general, puberty is attained by most species of male bats in the year following their birth (1+ years) (Matthews, 1941; Ramakrishna, 1951; Brosset, 1962b; Short, 1961; Tuttle and Stevenson, 1982). Exceptions occur in the emballonurid *Coleura afra* (first year: Dunlop, 1997), the pteropids *Epomops buettikoferi* (eleven months) and *Micropteropus pusillus* (seven months: Thomas and Marshall, 1984) and in the vespertilionids *Myotis nigricans* (two to four months post-partum: Wilson and Findley, 1970; Wilson, 1971; Myers, 1977), *Pipistrellus ceylonicus* (nine months: Madhavan, 1971), *Tylonycteris pachypus* and *T. robustula* (three months: Medway, 1972), *Scotophilus temmincki* (nine months: Gopalakrishna, 1947), *S. wroughtoni* (six to seven months: Gopalakrishna, 1948) and possibly *Eptesicus furinales* (less than one year: Myers, 1977). Kofron (1997) reported that male *Cynopterus minutus* and probably also *C. brachyotis* obtain sexual maturity at about seven months.

4.3 REPRODUCTIVE ORGAN ANATOMY

The gross and microscopic structure of the primary (paired testes) and secondary (accessory glands) reproductive organs in bats follows the normal mammalian pattern (e.g. Pteropodidae (Figure 4.1): Wood Jones, 1917; Pal, 1984a; Rhinopomatidae (Figure 4.2): Banerjee and Karim, 1986; Emballonuridae (Figure 4.3): Murthy, 1969, 1972; Kitchener, 1973, 1976; Mokkapati and Dominic, 1976, 1977; Swami and Lall, 1979a, b, c; Bhardwaj and Lall, 1979; Pal, 1984b; Jolly and Blackshaw, 1987, 1988; Nycteridae (Figure 4.5): Matthews, 1941; Megadermatidae (Figure 4.6): Pal, 1984b; Rhinolophidae (Figure 4.7); Matthews, 1937, 1941; Gaisler and Titlbach, 1964; Brosset and Saint-Girons, 1980; Bernard, 1983, 1985, 1986; Pal 1983a; Krutzsch *et al.,* 1992; Phyllostomidae (Figure 4.8): Robin, 1881; Wood Jones, 1917; Vamburkar, 1958; Rajalakshmi and Prasad, 1970; Mokkapati and Dominic, 1976; Vespertilionidae (Figure 4.13): Miller, 1939; Gopalakrishna, 1948; Pearson *et* *al.,* 1952; Krutzsch, 1961, 1975; Sluiter, 1961; Racey and Tam, 1974; Gustafson, 1976, 1977; Myers, 1977; Richardson, 1977; Gaisler *et al.*, 1979; Bernard, 1980; Phillips and Inwards, 1985; Krutzsch and Crichton, 1986, 1990a; Bernard *et al.,* 1997; Swift, 1998; Natalidae (Figure 4.9): Broadbooks, 1961; Mitchell, 1965; Molossidae (Figure 4.14): Krutzsch and Crichton, 1985, 1987, 1990b; Vivier and van der Merwe, 1996). Information for the Furipteridae (*Amorphochilus schnablii*: Figure 4.10) and Myzopodidae (*Myzopoda aurita*: Figure 4.12) was generated for this review from alcohol-preserved specimens kindly made available for dissection by the late Barbara Lawrence, Curator of Mammals, Museum of Comparative Zoology, Cambridge, MA. A single specimen of *Noctilio leporinus* (Noctilionidae) and *Thyroptera tricolor* (Thyropteridae) was obtained by the late William A. Wimsatt in Mexico, and dissected for this review by P.H. Krutzsch (Figure 4.4 and 4.11). For the Mystacinidae, the general gross morphology of the male reproductive tract is known to me only from a drawing of *Mystacina tuberculata* kindly provided by Andrew P. Winnington. No descriptions or specimens were available to me for Mormoopidae or Craseonycteridae.

4.3.1 Testis

Gross anatomy

A survey of the published literature reveals some interesting differences (inter-familial and inter-species) in the seasonal and even daily location of the testes.

1. *Permanently abdominal.* It appears that the testes may be permanently abdominal in the Rhinopomatidae (e.g. *Rhinopoma kinneari* (= *R. microphyllum*): Anand Kumar, 1965; *R. hardwickei*: Karim and Banerjee, 1985, 1989) and in the molossid *Tadarida aegyptiaca* (Bernard and Tsita, 1995). In a single reproductively inactive male *Amorphochilus schnablii* (Furipteridae), the small, paired testes were attached to the posterior body wall and inferior pole of the kidney by a suspensory ligament. However, their location in this specimen does not necessarily dictate their permanent position. The same applies to the abdominal testes noted in the drawing of *Mystacina tuberculata.*
2. *Permanently inguinal or scrotal.* Permanently scrotal testes are found in *Taphozous longimanus* (Emballonuridae): Gopalakrishna, 1954, 1955; Krishna and Dominic, 1983a, *Noctilio leporinus* (Krutzsch, unpublished observations) and probably *Noctilio albiventris* (Noctilionidae) and in *Nycteris hispida* and *N. luteola* (Nycteridae) (Matthews, 1941).

   In the species in which no scrotum is present, the testes are inguinal (*Pteronotus parnelli*, (Mormoopidae): Garrido-Rodriguez and Lopez-Forment, 1981; Garrido-Rodriguez *et al.*, 1984).
3. *Migratory.* In other species, the testes migrate (daily or seasonally) from the abdomen to the scrotum via the inguinal canal (viz. some Pteropodidae: Baker and Baker, 1936; Ratcliffe, 1932). In *Taphozous georgianus* (Jolly and Blackshaw, 1988) and *T. hilli* (Kitchener, 1980, 1983) (Emballonuridae), the testes move from the abdomen to just peripheral to the external inguinal ring, where they appear to lie within an abbreviated non-pigmented fascial sac which extends from the

external oblique muscle fascia at the external inguinal ring. The testes of *Mormopterus planiceps* (Krutzsch and Crichton, 1987) and *Tadarida brasiliensis mexicana* (Krutzsch, 1955a) (Molossidae) occur from the abdomen to within the inguinal canal, or they may occupy an external position where they lie under the spermatic fascia alongside the base of the penis. The testicular position (intra-abdominal or scrotal) in *Tadarida* (= *Mops*) *condylurus* in Uganda showed no significant seasonal variation in sperm production (Mutere, 1973). However, in *Tadarida* (= *Chaerephon) hindei* in Uganda, spermatogenesis was not continuous in all males (Marshall and Corbet, 1959). Furthermore, in some cases where testes were intra-abdominal, spermatogenesis had ceased entirely. The epididymides, however, continued to retain sperm regardless of testicular position. In *Desmodus rotundus* (Phyllostomidae), Orsi *et al.* (1990) observed that the testes could be withdrawn into the abdomen at will.

4. *External*. In a number of species the testes may be found on the crest of the pubis lateral to the base of the penis (parapenial). This is true of active male *Natalus stramineus* (Natalidae: Broadbooks, 1961; Mitchell, 1965) and *Rhinolophus megaphyllus* (Rhinolophidae: Krutzsch *et al.*, 1992). In other species, the testes lie just external to the external inguinal ring viz. *Thyroptera tricolor* (Thyropteridae) and *Myzopoda aurita* (Myzopodidae). In *Macrotus californicus* (Phyllostomidae), they are held within a non-pigmented sacculation of the external spermatic fascia. This testicular pouch is not a true scrotum. It is covered by a thin, sparsely furred and highly vascularized skin in which the vessels are greatly engorged during the breeding season. In the non-breeding season this pouch is either lacking, not occupied by the testes (so not grossly visible), or it is withdrawn along with the testes into the inguinal canal; sometimes it is found tight against the external inguinal ring. Sample dissections of representative taxa of the families examined disclosed that the testes were usually attached to the posterior body wall at the kidney by a suspensory ligament which may function in testicular movement.

Microscopic anatomy

The cyclic process of spermatogenesis has been studied for a microchiropteran, *Rhinopoma kinneari* (Singwi and Lall, 1983) and the megachiropteran *Pteropus poliocephalus* (McGuckin and Blackshaw, 1987a). For *Rhinopoma*, 11 stages and 16 steps of spermiogenesis were described with the relative frequency of various cell associations differing significantly from other mammalian species, although the principal steps (e.g. acrosome and maturation phases) were essentially similar. In *Pteropus*, fewer (eight) stages were recognized but the duration of spermiogenesis was longer, approximating that of the human. The cellular associations more closely resembled the rat than the human or those seen in *R. kinneari*.

Some histochemical studies have been carried out by Swami and Lall (1979c), who reported that Sertoli and Leydig cells and the spermatocytes and sperm heads of breeding *Taphozous longimanus* were positive for acid phosphatase. They speculated that the hydrolytic action of this enzyme could produce end products utilized in the synthesis of molecules essential for the growth and differentiation of sperm, or it could be related to penetration of the oocyte during fertilization.

4.3.2 Epididymis

The testes deliver spermatozoa via the epididymal duct system and ductus deferens to the ampullary glands and then to the lumen of the prostatic urethra. The epididymides can be subdivided into caput, corpus and cauda regions. Histochemical studies by Swami and Lall (1979c) have identified glucose-6-phosphatase activity in varying degrees in the various epididymal regions of *Taphozous longimanus*. In the same species, Bhardwaj and Lall (1979) found acid phosphatase activity in the caput and cauda and relatively less in the corpus. The cauda epididymidis may store spermatozoa for periods of weeks to months, primarily in temperate dwelling, hibernating species, although some southern hemisphere tropical species do so as well. The interfemoral caudae epididymides are often enclosed in a heavily pigmented sheath (tunica vaginalis) whose role is not known, though it is speculated to be thermoregulatory and related to sperm viability.

From the cauda epididymidis, the tubular system straightens, becomes more muscular and forms the ductus deferens. In species whose testes are external, the ductus deferens passes into the pelvic cavity, along with the testicular nervous and vascular components (the spermatic cord), via the inguinal canal. The ductus then turns medially, looping over the ureter, to dilate terminally as the Ampulla of Henle. It then expands to form a large pear-shaped gland, the ampullary gland.

4.3.3 Ampullary Gland

Morphology

The luminal wall of the terminal dilation of the ductus (Ampulla of Henle) is thrown into numerous irregularly branching folds from the central duct to form the paired, compound tubuloalveolar ampullary glands, which typically lie dorsal and lateral to the base of the urinary bladder. These glandular-appearing alveolar spaces are lined by a simple columnar (when the glandular lumina are empty) or cuboidal to squamous (when they are full) secretory epithelium. The secretory products are generally granular and strongly eosinophilic (e.g. *Miniopterus schreibersii*: Krutzsch and Crichton, 1990a; *Scotophilus wroughtoni*: Gopalakrishna, 1949; *Pipistrellus pipistrellus*: Racey and Tam, 1974; *P. subflavus*: Krutzsch and Crichton, 1986; *Plecotus auritus*: Swift, 1998). The central duct of the ampullary gland is joined in the substance of the gland by the duct from the seminal vesicle, when that gland is present, to form the ejaculatory ducts which carry sperm and secretions into the lumen of the prostatic urethra just proximal to the crista urethra. The ampullary glands undergo seasonal changes in size and activity, presumably in response to circulating androgens. Their central duct seems to be a temporary storage site for spermatozoa in some species (e.g. *Cardioderma cor*: Matthews, 1941; *Miniopterus schreibersii*: Krutzsch and Crichton, 1990a) and to retain sperm throughout the year in others (*Rhinopoma kinneari*: Anand Kumar, 1965).

Physiology

The ampullary glands are known to secrete fructose (*Pipistrellus subflavus*: Krutzsch and Crichton, 1986; *Scotophilus heathi*: Mokkapati and Dominic, 1976, Krishna and Singh, 1997; *Myotis lucifugus* and *M. velifer*: Crichton *et al.*, 1981; *Nyctalus noctula*: Racey, 1974), citric acid (*S. heathi*: Mokkapati and Dominic, 1976; *Taphozous longimanus*: Mokkapati and Dominic, 1976) and protein-bound acidic mucins (*Megaderma l. lyra*: Pal, 1984b; *T. longimanus*: Pal, 1984c). Interestingly, Pal (1984b) described the ampullary gland of *M. l. lyra* as being histochemically similar to and located in the same position as the seminal vesicles of other bats.

Taxonomic distribution

Ampullary glands are present in most (but not all) species of chiropteran families that have been studied, including the furipterid, *Amorphochilus schnablii* (Krutzsch, unpublished observations). It seems that they are missing in the noctilionids and frequently lacking in pteropodids. They are also apparently lacking in the rhinolophid *Triaenops afer* (Matthews, 1941).

4.3.4 Seminal Vesicles

Morphology

Lying lateral to the ampullae and often very similar (even indistinguishable from each other) in gross external as well as microscopic morphology are the paired, elongated seminal vesicle glands, which most frequently lie cephalic to the prostate and dorsal to the urinary bladder.

Physiology

These glands greatly hypertrophy during the reproductively active state. Their alveolar lumina are filled with lightly granular, eosinophilic secretions that emanate from columnar epithelial cells with basal nuclei and vacuolated supranuclear cytoplasm. Reproductively inactive glands are much reduced in size and have less conspicuous glandular elements; the lining epithelium, however, remains columnar to high cuboidal with basal nuclei, but the supranuclear cytoplasm is nonvacuolated and agranular (Krutzsch, 1975), and the lumina contain little secretion. Histochemical studies have reported the presence of neutral mucins and protein-bound sialomucins (Gadegone and Sapkal, 1983) and fructose (e.g. *Taphozous longimanus*: Krishna and Dominic, 1982a; Mokkapati and Dominic, 1976; *Myotis lucifugus* and *M. velifer*: Crichton *et al.*, 1981; *Pteropus giganteus*: Rajalakshmi and Prasad, 1970; *Macrotus waterhousii* (= *californicus*): Krutzsch *et al.*, 1976). Interesting reports of Hunter *et al.* (1971a, b) described a protein in the seminal vesicles of *Myotis lucifugus* that, when injected into other mammals, diminished smooth muscle contractile frequency and tone as well as decreased circulating lymphocytes. The authors speculated this protein may block sperm transport and alter the phagocytic system so as to allow sperm retention (storage) in the female tract. In *Pteropus poliocephalus*, the seminal vesicles are thought to contribute to vaginal plug formation (Martin *et al.*, 1995).

Taxonomic distribution

Descriptions of these glands are available for a number of bat species (e.g. *Miniopterus schreibersii*: Krutzsch and Crichton, 1990a; *Pipistrellus hesperus*: Krutzsch, 1975; *P. dormeri*: Gadegone and Sapkal, 1983; *Mormopterus planiceps*: Krutzsch and Crichton, 1987). It appears they are absent in *Myzopoda aurita* (Myzopodidae: see Figure 4.12), *Thyroptera tricolor* (Thyropteridae: see Figure 4.11), *Hipposideros fulvus* (Patil, 1968), *H. speoris* (Pal, 1983a), *Scotophilus heathi* (Krishna and Singh, 1997) and *Rhinolophus capensis* (Bernard, 1985). They also appear to be lacking in the nycterids, rhinopomatids and natalids (Figures 4.2, 4.4 and 4.9).

4.3.5 Prostate Gland

Morphology

The prostate gland lies at the base of the bladder, where it either completely or incompletely (Nycteridae, Noctilionidae) surrounds the prostatic urethra into which it delivers its secretions via multiple small ducts. Grossly, it is an unpaired gland though often has several lobes. Microscopically, it is a compound tubuloalveolar gland that is enclosed by a fibromuscular capsule. The secretory epithelial cells that line the alveoli vary in profile, depending on the reproductive status of the gland.

Physiology

The prostate secretes a multi-sided, globular, eosinophilic, homogeneous product that contains glycogen and neutral and acidic mucins (*Taphozous longimanus*: Pal, 1984c; *Megaderma l. lyra*: Pal, 1984b; *Hipposideros speoris*: Pal, 1983a; *Pipistrellus dormeri*: Gadegone and Sapkal, 1983), acid phosphatase and adenosine triphosphate (*T. longimanus*: Swami and Lall, 1979a), fructose (*P. subflavus*: Krutzsch and Crichton, 1986; *Nyctalus noctula*: Racey, 1974; *Scotophilus heathi*: Mokkapati and Dominic, 1976; Krishna and Dominic, 1981; *Plecotus auritus*: Swift, 1998; *Myotis lucifugus* and *M. velifer*: Crichton *et al.*, 1981; *Pteropus g. giganteus*: Rajalakshmi and Prasad, 1970; *Taphozous longimanus*: Mokkapati and Dominic, 1976) and citric acid (*Scotophilus heathi*: Mokkapati and Dominic, 1976). Pressure from luminal contents appears to flatten the lining epithelial cells to a low cuboidal or squamous profile.

Taxonomic distribution

A prostate gland is present in every family for which there is information.

4.3.6 Cowper's (Bulbourethral) Glands

Morphology

These are small, pear-shaped glands which lie on either side of the anus close to the bulb of the urethra. They are enclosed in a fibromuscular capsule and are separated into lobules by connective tissue and skeletal muscle septae that enter the gland from the capsule. They consist of multiple acini which are lined by cuboidal secretory epithelial cells with basal or central nuclei and finely granular or clear, eosinophilic apical cytoplasm. The cytoplasm is filled with large, membrane-bound secretory granules (Krutzsch and Crichton, 1990a). Abundant microvilli are present in the intercellular spaces.

Physiology

Neutral and acidic (*Hipposideros speoris*: Pal, 1983a) and sialic acid-rich (*Pteropus g. giganteus*: Rajalakshmi and Prasad, 1970; *Taphozous longimanus*: Pal, 1984c) secretions are delivered via a single duct from each gland, which passes beneath the bulbospongiosum muscle to terminate in the bulb of the penile urethra distal to the prostate. Interestingly, in *Mormopterus planiceps* (Krutzsch and Crichton, 1987), these ducts are lined by a sebaceous rather than a low cuboidal epithelium, as is true in other chiropteran taxa studied. Like other sex accessory glands, the activity of Cowper's glands reflects the reproductive status of the individual. They become hypertrophied and secretory, with granular, columnar epithelium in synchrony with the elevated testicular steroid cycle and involuted with cuboidal epithelium and agranular cytoplasm, in the inactive testicular state (e.g. *H. speoris*: Pal, 1983a; *Scotophilus wroughtoni*: Gopalakrishna, 1949; *Pipistrellus hesperus*: Krutzsch, 1975; *P. subflavus*: Krutzsch and Crichton, 1986; *Miniopterus schreibersii*: Krutzsch and Crichton, 1990a; *Nyctalus noctula*: Racey, 1974). The nature of the secretory products of these glands is not well documented. Krutzsch and Crichton (1986) found fructose lacking in *Pipistrellus subflavus*. Gadegone and Sapkal (1983) demonstrated that these glands were histochemically positive for neutral sialic acid and sulfomucins in *P. dormeri*.

Taxonomic distribution

Cowper's glands appear to be present universally in the Chiroptera. However, they could not be found in a reproductively inactive male *Pteronotus parnellii*, the only mormoopid specimen that has been examined to date (Krutzsch, unpublished). These glands are almost always paired but are doubly paired in the vespertilionid genera *Corynorhinus* and *Plecotus*.

4.3.7 Urethral Gland

Morphology

This is a well-developed, pear- or carrot-shaped gland that encompasses the membranous urethra. It consists of a mass of convoluted, branched tubules that are lined by columnar epithelial cells whose secretory products drain into the urethral lumen via these tubules. Descriptions can be found in Matthews (1941) and Krutzsch *et al.* (1992). It appears that this gland may well be restricted to the rhinolophids and megadermatids, and it is not known if it is represented in all taxa within these families. Familial distribution needs further study.

Physiology

At least in some species, the secretory epithelium appears to contain two cell types. The first (urethral A cell) is columnar with a highly granular, strongly eosinophilic cytoplasm; the second (urethral B cell) is pyramidal in profile and basal in position with a less granular, lightly eosinophilic cytoplasm (Krutzsch *et al.*, 1992). Bernard (1986) described continuous secretory (blibbing) from type A cells throughout the year in *Rhinolophus capensis*, whereas type B cells only released their secretions seasonally. In *Hipposideros speoris* (Pal, 1983a), the secretions contained acidic mucins. Urethral gland secretions are thought to be responsible for the formation of the vaginal plug in some rhinolophids (Robin, 1881; Rollinat and Trouessart, 1897; Matthews, 1937; Gaisler and Titlbach, 1964; Gaisler, 1966). However, this plug in *R. capensis* (Bernard, 1985) and *H. caffer* (Bernard, 1983) appears to be primarily of vaginal epithelial cell origin. A similar origin for the vaginal plug has been described for *R. ferrumequinum* (Uchida and Mori, 1987) though others (see above) hold that it originates from the urethral gland. Krutzsch *et al.* (1992) did not find a vaginal plug in post-inseminated *R. megaphyllus*.

Taxonomic distribution

Although this gland has not been described in most chiropteran families, it is probably present in more families than is currently documented.

4.3.8 Urethral Glands (of Littre)

Morphology

These mucous secreting glands lie in the mucosal and muscular wall stroma of the urethra. Although prominent in the prostatic urethra, they may occur from the bulb to the penile urethra. The glands of Littre are small, tubular structures that are lined by columnar, secretory epithelial cells with spherical, central or basal nuclei.

Physiology

The secretory products are histochemically positive for mucins (Pal, 1983b, 1984a, b, c).

Taxonomic distribution

They have been reported in taxa from a number of families, viz. *Rousettus leschenaulti* (Pal, 1984a, 1989), *Megaderma l. lyra* (Pal, 1984b), *Miniopterus schreibersii* (Krutzsch and Crichton, 1990a) and probably exist in many more species that have not yet been critically visualized at the microscopic level.

4.3.9 Para-anal Glands

Morphology

These sebaceous glands, located on either side of the anal orifice, have been reported in male *Mormopterus planiceps* (Krutzsch and Crichton, 1987); they were described as appearing to undergo changes in size annually in synchrony with the active reproductive state. Their function is unknown, though they may be involved in territory marking and in the attraction of females.

4.4 PENIS

4.4.1 Morphology

Taxonomic variation exists in the posture of the penis, which may be cranially or caudally directed. However, the basic morphological architecture is essentially the same for all species. Paired, laterally placed, erectile crura of the corpora cavernosa peni (each enclosed by an ischiocavernosus muscle) join to form a central penile shaft. The erectile corpus cavernosum urethra (corpus spongiosum urethra) enclosed by the bulbospongiosum muscles runs the ventral length of the penile shaft, lying in the urethral groove formed by the juncture of the rounded crura of the paired corpora cavernosal bodies. The corpus spongiosum urethra terminates distally as the glans penis. This structure is often taxonomically distinctive in size and shape, and its epithelium often has dermal, basally directed spines; it is covered by a prepuce that is usually thick, externally hairy and well supplied with mucoid glands. In many species, the prepuce contains additional erectile tissue known as the accessory corpus cavernosus (Matthews, 1941; Wimsatt and Kallen, 1952; Krutzsch, 1975). The urethra opens terminally through a slit-like external urethral meatus in the distal glans penis. It is variably (depending on the species) capped by a bony ossicle (os penis) which is a highly generic, less often species-specific, structure embedded in the erectile tissue of the glans. The os penis (= baculum) is frequently attached at its base to the distal end of the fused corpora cavernosa. Its role in reproduction is conjectural. It may serve to support the penis during ejaculation (Krutzsch, 1975; Hill and Harrison, 1987), to stimulate the female and/or to prevent urethral closure during copulation. Courrier (1927) suggested a role in the penetration of the vaginal plug for those species that have such a structure.

4.4.2 Taxonomic Variation

1. *Pteropodidae*: A detailed description for *Cynopterus sphinx* (Vamburkar, 1958) is characteristic, in general, for the family. The penes of pteropids are strikingly similar to their primate counterparts. The penile skin, including the prepuce, is often pigmented and sparsely haired or glabrous, except at the base of the penis where the pubic skin is hairy (Jones, 1917; Davis, 1947). In *Pteropus*, the ventral surface of the glans is covered with dermal spines (Davis, 1947). An os penis is present and an extensive literature addresses variations in its morphology in a variety of taxa (Davis, 1947; Krutzsch, 1959b, 1962; Lanza, 1963, 1969; Didier, 1965; Bhatnagar, 1967).
2. *Rhinopomatidae*: Descriptions are available for *Rhinopoma microphyllum* (=*R. kinneari*) and *R. hardwickei* (Wassif and Madkour, 1972). Both have a baculum.
3. *Craseonycteridae*: *Craseonycteris thonglongyai* is reported to have a relatively large penis (Hill, 1974) but beyond this, there is no information.

4. *Emballonuridae*: The detailed morphology of the penis has been described only for *Taphozous longimanus* (Murthy, 1969). There are brief remarks on the penile posture and size for *T. nudiventris, T. mauritianus, T. perforatus* (=*T. sudani*) (Verschuren, 1957) and *T. georgianus* (Kitchener, 1973). An unusual feature of the penis of *T. longimanus* is an irregular blind space just posterior to the urethral meatus that extends about half the length of the body of the glans. Murthy (1969) speculated that this space might permit great distention of the glans during erection and, together with the large erectile accessory corpora cavernosa and numerous rows of proximately directed chitinoid spines, might act to prevent premature withdrawal during coitus.

   A baculum has been found in all taxa examined (*Taphozous longimanus*: Murthy, 1969; *T. perforatus, T. nudiventris*: Wassif and Madkour, 1972; *T. melanopogon*: Sinha, 1983; *Rhynchonycteris naso, Saccopteryx bilineata, Cornura brevirostris, Peropteryx macrotis, P. kappleri, Balantiopteryx plicata, B. io*: Brown *et al.*, 1971) and probably will be found throughout the family (Brown *et al.*, 1971). Interestingly, this structure is bipartite (united only at the base) in *T. longimanus*.
5. *Nycteridae*: The penes of *Nycteris luteola* and *N. hispida* have been described (Matthews, 1941). No accessory corpora cavernosa have been recognized. A baculum (5.0 mm long in *N. luteola* and 1.5 mm long in *N. hispida*) was present. In *N. luteola* it was associated with the corpus spongiosum urethra, whereas in *N. hispida*, the corpus spongiosum urethra, though well developed, provided little erectile tissue in the small glans of this species and was not associated with the baculum.
6. *Megadermatidae*: Descriptions are available for the penes of *Megaderma lyra lyra* (Pal, 1977; Murthy, 1979) and *Cardioderma cor* (Matthews, 1941). The penis of *M. l. lyra* is longer and its skin is heavily covered with hair, obscuring the separation of the glans from the penile shaft. The prepuce is thick, glandular and extends virtually the entire length of the glans. The latter lacks spinous projections in its epithelial covering. There is a small baculum but no accessory corpora cavernosa. The smaller penis of *C. cor* has a thin retractable prepuce. The glans is covered by spiniform tubercles. The walls of the deep gutter, formed where the glans joins the penile shaft, fuse below the glans to form a small, terminal papilla just dorsal to which is the urethral meatus. Accessory corpora cavernosa provide most of the erectile tissue of the glans and communicate proximally with the distal ends of the corpora cavernosa. The urethra is capped for most of its length by a distinctive, 2.0 mm long os penis.
7. *Rhinolophidae*: The rhinolophid penis has been described in a number of studies, viz. Matthews (1937, 1941); Topal (1958); Lanza (1959); Wassif and Madkour (1972); Khajuria (1982); Yoshiyuki (1989); Krutzsch *et al.* (1992). The penis is directed cranially in some species (*Rhinolophus ferrumequinum*: Matthews, 1937) and caudally in others (*R. lepidus, Hipposideros fulvus, H. durgadasi*: Khajuria, 1982; *H. caffer, Triaenops afer*: Matthews, 1941). The penile shaft is usually hairy and varies in length between species (5.0–7.0 mm long for *T. afer*, and 8.0 mm long for *H. caffer*: Matthews, 1941). The prepuce may be thin, hairless and retractable to its attachment at the glans–penile shaft juncture (*H. caffer, T. afer*: Matthews, 1941), and lacking prepucial glands

(*T. afer*: Matthews, 1941). In other rhinolophid species, the prepuce is thick and hairy (*R. lepidus, H. fulvus* and *H. durgadasi*: Khajuria, 1982) and there are prepucial glands. The glans penis varies between taxa in its length, shape, surface markings and epithelial specializations. The glans may be subconical (*Rhinolophus ferrumequinum*: Matthews, 1937), oblong and dorso-ventrally flattened (*R. lepidus*: Khajuria, 1982), elongate-broad in the middle with pointed tip (*H. fulvus*: Khajuria, 1982), large and strongly flattened dorso-ventrally (*T. afer*: Matthews, 1941) or cylindrical (*R. hipposideros*: Matthews, 1937). The paired accessory corpora cavernosa form the greater part of the body of the glans and provide its primary erectile tissue. The erectile corpus spongiosum is small and surrounds the urethra primarily in the shaft, where the urethra lies ventral to the fused corpora cavernosa. The external surface of the glans can be unmarked or thrown into prominent, longitudinal grooves and ridges, which can be either on the lateral and/or ventral surface. In most species, the surface epithelium of the glans is smooth; however, in some the glans is covered with parallel rows of proximally directed, keratinized spines (copulatory spurs) (Khajuria, 1982).

A baculum has been found in all rhinolophid species examined and likely occurs family-wide. In view of the variation recorded between species in its size and shape, it may be a significant element in the determination of taxonomic affinity or distinction.

8. *Noctilionidae*: The penis of *Noctilio leporinus* is large, pendulous and characterized by a well-developed prepuce. Both Brown *et al.* (1971) and Krutzsch (unpublished observations) found no baculum. A baculum is also absent in *N. labialis* (Brown *et al.*, 1971).
9. *Mormoopidae*: The gross morphology of the penis of *Pteronotus parnellii* has been studied (Brown *et al.*, 1971). The penile shaft and glans together averaged 5.3 mm in length. The penile skin was pubescent almost to the distal end of the retractable prepuce. The surface of the glans was smooth with a ventral groove formed between prominent folds that diverged distally to provide a well-marked ridge on either side of the terminal urethral meatus. The os penis is apparently lacking in Mormoopidae. Brown *et al.* (1971) examined *P. parnellii* (n=5), *P. gymnonotus* (= *suapurensis*) (n=10), *P. davyi* (n=8), *P. personatus* (n=4), *Mormoops megalophylla* (n=3) and Krutzsch (unpublished) studied the penes from *P. davyi* (n=3), *P. parnellii* (n=10), *P. personatus* (n=4), *M. megalophylla* (n=4) and *M. blainvillii* (n=3) and none had an os penis.
10. *Phyllostomidae (including Desmodontidae)*: Characteristic of phyllostomids is the absence of a baculum (Brown *et al.*, 1971). The penis has been described in *Macrotus californicus* (Krutzsch *et al.*, 1976). It is a lightly pigmented, sparsely haired, somewhat inflexible (as a result of a thick collagenous tunic investing the penile shaft) organ. The average length of the penis is 7.0 mm (6.5–7.5 mm, n=30), about twenty per cent of which is occupied by the glans. A thick prepuce extends beyond the tip of the glans and is retractable to its base. In *Desmodus rotundus*, the penis is unusually long (12.0 mm) with a retractable prepuce (Orsi *et al.*, 1990).
11. *Natalidae*: Only one species, *Natalus stramineus* has been examined (Brown et al., 1971; P.H. Krutzsch, unpublished). The average penile length was 6.2 mm

(5.9–6.35 mm, n=15), and it tapers at its tip to form a tiny glans. The penile skin is haired to the tip of the prepuce which covers the glans entirely. The prepuce is easily retracted to its attachment at the base of the glans. The ventral penile urethra narrows distally and appears to lack erectile tissue in the glans where it terminates via a slit-like opening. A tiny, ventrally concave baculum is present; its base (0.025 mm) is wider than its tip (0.010 mm) and it is 0.1 mm long (P.H. Krutzsch, unpublished observations).

12. *Furipteridae*: Little is known about the penis in this family. A single specimen of *Amorphochilus schnablii* appeared to contain a tiny baculum capping the urethra at the tip of the glans (P.H. Krutzsch, unpublished observations), confirming the observations of Ibanez (1985) who measured the ossicle (n=4) as 0.54 mm long (0.48–0.61 mm) and 0.19 mm wide (0.18–0.21 mm).
13. *Thyropteridae*: Few data are available on the gross structure of the penis of *Thyroptera tricolor*. In this species, the penile skin is lightly pubescent. The prepuce extends distally almost to the tip of a small glans which contains a small, rod-like baculum (0.5 mm long) dorsal to the urethra (Krutzsch, unpublished). Wilson (1978) noted that the penis of *T. discifera* was about 8.0 mm long and appeared not to have a baculum.
14. *Myzopodidae*: Thomas (1904) described the penis of *Myzopoda aurita* as short, slender and pointed with a styliform glans (about 2.0 mm long) covered by a wrinkled, lightly pubescent prepuce. There was no baculum.
15. *Vespertilionidae*: The vespertilionid penis is usually small, semi-pendulous and cylindrical. It frequently has a genicular profile and can be directed caudally (e.g. *M. lucifugus*: Wimsatt and Kallen, 1952; *M. mystacinus, P. pipistrellus* and *Nyctalus noctula*: Matthews, 1937; *P. hesperus*: Krutzsch, 1975) or cranially (*M. nattereri, Plecotus auritus*: Matthews, 1937). The overlying skin is usually pigmented and sparsely haired. The glans is most often small (Matthews, 1937; Wimsatt and Kallen, 1952; Krutzsch, 1975), cylindrical (e.g. *M. nattereri*: Matthews, 1937), subconical (e.g. *P. pipistrellus* and *Plecotus auritus*: Matthews, 1937), hemispherical (e.g. *Myotis mystacinus*: Matthews, 1937), club-shaped (e.g. *P. mimus*: Khajuria, 1982) or flattened dorsoventrally with two or four laterally placed lobes (e.g. *Antrozous pallidus* and *A. dubiaquercus*: Pine *et al.*, 1971; *Scotophilus kuhli*: Khajuria, 1982; *Otonycteris hemprichii*: Bates and Harrison, 1997). The glans is smooth or thrown into parallel longitudinal folds with grooves and ridges. Proximally directed keratinized spines are present in some species. A boundary between the glans and the terminal shaft may or may not be visible. The prepuce is most often variably pigmented, thick, vascularized (*P. hesperus*: Krutzsch, 1975) and hairy. It may contain accessory erectile tissue and numerous vascular spaces that permit engorgement by blood. In *Myotis lucifugus*, the prepuce contains an accessory cavernous body (Wimsatt and Kallen, 1952).

The penis (including its vascularization) has been studied in detail in two vespertilionid species (*Myotis lucifugus*: Wimsatt and Kallen, 1952; *Pipistrellus hesperus*: Krutzsch, 1975). Although the autonomic innervation was not addressed, Wimsatt and Kallen (1952) suggested that there were basic mammalian similarities in pelvic plexuses and ganglia that occurred around the accessory organ complex. The somatic nervous supply to the penis is likely from the paired pudendal nerves, dorsal penile branches of which are visible beneath the penile skin and subcutaneous fat and fascia alongside the dorsal artery and veins. Krutzsch (1975) also observed that these nerves arborized into the cavernous tissue of the glans.

With the exception of *Miniopterus* (Matthews, 1937; Krutzsch and Crichton, 1990a) and *Mimetillus* (Hill and Harrison, 1987), a baculum is present in all vespertilionids examined for this structure (e.g. Hamilton, 1949; Atallah, 1970; Pine *et al.*, 1971; Brown *et al.*, 1971). Its ontogeny is described in *Scotophilus* by Harrison and Brownlow (1978). An extensive literature discusses its significance in establishing taxonomic distinction and/or affinity in the Vespertilionidae (Hill and Harrison, 1987; LaVal, 1973; McKean and Price, 1978; Harrison and Brownlow, 1978; Nader and Hoffmeister, 1983).

16. *Mystacinidae*: Pierson (1986) recorded that a baculum was lacking in *M. tuberculata* but did not otherwise describe the penile morphology.
17. *Molossidae*: Variation exists in this family in the morphology of the glans penis (Ryan, 1991a, b) and in the presence or absence of a baculum as well as in the morphology of this ossicle (Krutzsch and Vaughan, 1955; Long and Jones, 1966; Brown, 1967; Krutzsch and Crichton, 1987). Ryan (1991a) noted several morphological factors that are common features for molossids. These include the presence of an elongated and conical glans that is often invested with keratinized spines, a thin retractile prepuce, fused corpora cavernosa extending well into the glans and a poorly developed corpus spongiosum that often does not contribute to erection. An unusual feature is seen in the penis of the long penile form of *Mormopterus planiceps*, the glans of which is well developed and attenuated, being horizontally bifid and occupying some ninety percent of the shaft of the penis. Not only does this taxon have a particularly long, pendulous penis (9.4 mm) but also an unusually long (7.9 mm) baculum located in the upper primary division of the bifid penis (Krutzsch and Crichton, 1987).

4.5 SPERMATOZOA

Chiropteran spermatozoa are classically mammalian with inter-familial distinctions.

4.5.1 Pteropodidae

The morphology of the spermatozoa is known for only the following taxa: *Pteropus poliocephalus*, *P. scapulatus*, *P. conspicillatus*, *P. alecto* and *Syconycteris australis* (Rouse and Robson, 1986; Cummins *et al.*, 1988). While similar to one another, they differ from microchiropteran sperm in their relatively long acrosome, unique anvil-shaped subacrosomal space, redundant nuclear envelope and in the origin of the central singlets of the axoneme. The latter, with central singlets arising distal to the outer doublets, is atypical for mammals. Coarse fibers 1, 5, 6 and 9 are larger than the others. This is similar to the Microchiroptera (also insectivores and primates).

4.5.2 Rhinopomatidae

Some information is available on the sperm of *Rhinopoma kinneari* (Bedwal and Mathur, 1983) and *R. hardwickei* (Mathur and Kumar, 1962; Singwi and Lall, 1983). For both, a sexual dimorphism has been described; however, it seems likely that the authors are describing lateral and flat profiles of the sperm rather than two different morphological types.

4.5.3 Rhinolophidae

A comprehensive study of the fine structure of the sperm of a rhinolophid is lacking. The literature available considers either post-ejaculatory sperm stored in the female reproductive tract (*Rhinolophus hipposideros*, *R. ferrumequinum*: Racey, 1975, 1979, 1982; Uchida and Mori, 1972; *R. clivosis*: Bernard, 1988) or epididymal sperm retention or clearance from the caudae epididymides (*R. capensis*: Bernard, 1984, 1985, 1988). It is noteworthy that sperm stored in the female reproductive tract show no fundamental differences, other than the absence of a cytoplasmic droplet, from viable epididymal sperm (*R. cornutis* and *R. ferrumequinum*: Uchida and Mori, 1972).

4.5.4 Noctilionidae

Forman *et al.* (1989) described the light microscopic morphology of ethanol-fixed sperm of *Noctilio albiventris*. Two-thirds to three-fourths of the extremely large head is occupied by the acrosome which is broadly rounded at the apex. The neck piece is either absent or (more likely) not visible in these preparations. The middle piece is described as tapering gradually to the tail. Phillips *et al.* (1997) described the ultrastructure of the acrosome of *N. leporinus* and speculated that the unique acrosomal ridges may strengthen the sperm head during transport.

4.5.5 Phyllostomidae (Including Desmodontidae)

Using light microscopy, the gross morphology of the epididymal spermatozoa of 35 species of phyllostomids has been reported (Forman and Genoways, 1979). Subtle variation exists between species. The differences are primarily in the acrosome and head morphology. The ultrastructural characteristics of the development of the acrosome of *Desmodus rotundus* have been described (Orsi *et al.*, 1992); it seems to be generally similar to other mammals (Fawcett and Ito, 1965).

4.5.6 Vespertilionidae

There is a growing literature concerning spermatozoan morphology of vespertilionid bats. Initially, researchers using phase contrast light microscopy determined the basic morphology of the sperm of a variety of species (Orr, 1954; Hirth, 1960; Forman, 1968; Breed and Inns, 1985). Genera differed primarily in relative size and shape of the head and acrosome and in the arrangement and symmetry of the tail components. A number of studies at the electron microscope level have considered the sperm of various species in relation to their storage environment in the male or female tract (*Myotis lucifugus* and *Eptesicus fuscus*: Fawcett and Ito, 1965; Wimsatt *et al.*, 1966; Krutzsch *et al.*, 1982; Racey *et al.*, 1987; *M. velifer*: Krutzsch *et al.*, 1982; *Miniopterus schreibersii*: Bernard and Hodgson, 1988; *Tylonycteris* sp.: Racey *et al.*, 1975; *Pipistrellus pipistrellus*: Racey and Potts, 1970; *P. kuhlii*: Andreuccetti *et al.*, 1984; *P. abramus*, *P. savii* and *P. endoi*: Son *et al.*, 1988).

4.5.7 Molossidae

The ultrastructural morphology of molossid spermatozoa is known from the cauda epididymidal sperm of four genera and five species (*Mormopterus planiceps*, *Chaerephon jobensis*, *Tadarida australis* (Breed and Leigh, 1985), *T. brasiliensis mexicana* and *Molossus major* (P.H. Krutzsch, unpublished). The fine structure of the sperm of these taxa differs only slightly, but they are all morphologically distinct from the sperm of other chiropteran families reported upon to date. These molossid sperm have an elongated nucleus which is dense throughout except for a few, small, clear vacuoles which vary in number and location. The distal two-thirds of the nucleus is capped by an acrosome which is uniquely characterized by projecting processes (referred to as acrosomal blisters, Breed and Leigh, 1985); these arise principally from the apical and main acrosomal segments, on their external surface. In *T. b. mexicana* and *M. major*, P.H. Krutzsch (unpublished) found that the acrosome terminates in a three-pronged tip, the processes of which differ slightly in length between these two taxa. The acrosome, however, is distinct morphologically from the sperm of other families of Chiroptera heretofore described. Also, in these two taxa, the subacrosomal space is very narrow and terminates at the tip of the head as a pencil-like projection. The connecting piece and the proximal centriole, which appear to be joined by the coarse fibers from the middle piece, are similar to other mammalian sperm. A classic appearing membranous scroll is present in many, but not all, sperm of both species. The scroll apparently arises from a redundant portion of the nuclear envelope as described in other chiropteran sperm (*Pteropus* sp.: Rouse and Robson, 1986; *Myotis lucifugus*: Fawcett and Ito, 1965; Wimsatt *et al.*, 1966). The middle piece is short with only 36 (*Molossus major*) to 38 (*Tadarida b. mexicana*) paired, spiral mitochondrial gyres and, according to Breed and Leigh (1985), 38 to 42 in the species they report. This is a markedly reduced mitochondrial number as compared to other chiropteran species reported (i.e. *Pteropus* sp. (80): Rouse and Robson, 1982; *Myotis lucifugus* (114–120): Fawcett and Ito, 1965; *M. nattereri* (135); *M. macrodactylus* (117); *Pipistrellus abramus* (138); *Rhinolophus cornutus* (130); *R. ferrumequinum* (160); *Miniopterus schreibersii* (78): Uchida and Mori, 1972). Molossids are, in general, non-hibernators and have synchronized male–female reproductive cycles and, therefore, do not require prolonged sperm storage. These observations may add credence to Racey's (1979) speculation that the increased mitochondrial number characterizes the sperm of hibernating bats and provides a useful source of metabolizable phospholipids for their stored sperm.

P.H. Krutzsch (unpublished observations) found that the sperm of molossid bats, when viewed in cross-section at the ultrastructural level, include a classical mammalian middle piece axial filament complex of nine course (dense) fibers, nine doublets and a central pair of fibrils. Just proximal to the middle piece–principal piece juncture a typical tubular and vesicle-filled cytoplasmic droplet projects laterally from the middle piece of cauda epididymidal sperm. In longitudinal section, at the junction of the middle and principal pieces, a small annulus is visible and appears related to the fibrous sheath. Distal cross-sections of the principal and end pieces disclose typical mammalian reduction in the compliment of axoneme fibers. The dense fibers in the principal piece are reduced in number and are lost entirely in the end piece where only the nine doublets and the central pair remain.

There is no published information on the spermatozoa of the Mormoopidae, Emballonuridae, Craseonycteridae, Myzopodidae, Nycteridae, Natalidae, Megadermatidae, Mystacinidae, Thyropteridae or Furipteridae.

4.6 REPRODUCTIVE PATTERNS

Relative to the female, male bats have been the subject of many fewer investigations. Most assignments of their reproductive status are based either upon evaluations of externally visible changes in testicular and epididymal size, which are thought to signal reproductive readiness, or upon events occurring in the female (monoestry or polyoestry) which may correlate with the reproductive activity (or lack thereof) in the male.

Secondary sexual characteristics, where prominent, are offered as additional evidence of active male sexual function (libido). They cover a broad range of morphological (e.g. skin gland=olfaction) and behavioral features (e.g. vocal, audible and high frequency emissions, body posturing and flight displays). Such characteristics are well represented in the Chiroptera where they undoubtedly play an enormous role in insuring the meeting of the sexes and in pronouncing the reproductive status of the individual thus leading to successful copulation. The many examples that have been recognized to date are too numerous to enumerate here, and many are addressed in Chapter 9. However, some specific examples in each family, where appropriate, will serve to highlight the magnitude and complexity as well as the importance of this highly developed aspect of reproduction in male bats.

Even more rare are attempts to define mediating mechanisms that control and direct seasonality. Chapters 1, 2 and 3 address information that has been gathered to date on the roles of the central nervous system (including the pineal gland), the primary sex organs and other endocrine organs.

Taken together, the information suggests that male bats fall into two broad categories. There are those in which testicular recrudescence and atrophy take place once seasonally, in concert with the monoestrous cycle of the female. In others, there is either bimodally recurring (in concert with bimodal polyoestry) or continuous reproductive capability (in concert with more than two female oestrous cycles annually), although within the latter category, it may well be that at any one time throughout the year not all males in the population are reproductively active at the same time.

An additional aspect of male reproduction in bats concerns the lack of synchrony, in many species, between the primary and accessory reproductive organs. In most cases, these taxa dwell in temperate latitudes, where a period of hibernation interrupts the reproductive cycle.

In large part, bats fall into one or the other of these categories as a function of latitude and its impact on the environment. Temperate dwelling species generally attain reproductive status only once a year, for a short period, while tropical dwelling species are generally reproductively active more often in the year. There are, however, frequent exceptions to this generalization, particularly within the latter group.

While the terms used to categorize females are technically inapplicable to males (Krutzsch, 1979), it is likely that within any taxon, the reproductive readiness of males is in synchrony with that of females; therefore it remains pertinent to consider males from this aspect, especially in cases when there has been nothing published on their reproductive cyclicity. An overview of male reproduction in Chiroptera is best presented family by family.

4.6.1 Pteropodidae

The pteropodids cover an extensive geographic distribution which encompasses much of the tropics and subtropics of the Old World including all but the eastern-most islands of the central and south Pacific Ocean (Koopman, 1970; Koopman and Jones, 1970; Hill and Smith, 1984; Nowak, 1994; Rainey and Pierson, 1992). A number of studies have addressed seasonal changes in structure and function of the male tract of pteropids (e.g. Baker, 1947; McGuckin and Blackshaw, 1987a, b, 1991; Martin *et al.*, 1987; O'Brien, 1993; Martin *et al.*, 1995). While female pteropids are either monoestrous or polyoestrous, for the most part males appear to retain (almost) year-round fecundity and libido, as determined by observations on testicular and accessory gland dimensions and activity and by displays of hormonally mediated overt behavioral and morphological secondary sexual characteristics. While some species display reduced behavioral aggression with the onset of pregnancy, others may continue to copulate throughout the year and certainly well into female pregnancy (e.g. *P. poliocephalus*).

The majority of the monoestrous pteropid species belong to the genus *Pteropus*. Data are available on *Pteropus scapulatus*, *P. alecto* (= *P. gouldi*) and *P. poliocephalus* (Ratcliffe, 1932; Nelson, 1965a, b; McGuckin, 1988; McGuckin and Blackshaw, 1985, 1987a, b, 1991, 1992; Martin *et al.*, 1987, 1995; O'Brien, 1993; O'Brien *et al.*, 1993), *P. eotinus* (Baker and Baker, 1936), *P. giganteus* (Marshall, 1947; Neuweiler, 1969; Rajalakshmi and Prasad, 1970; Sandhu and Gopalakrishna, 1984; Sastry and Karim, 1995), *P. geddei* (Baker and Baker, 1936), *P. medius* (Jones, 1917; Groome, 1940), *P. comorensis* (Kingdon, 1974), *P. voeltzkowi* (Kingdon, 1974), *P. rodricensis* (West, 1985). Other species that also fall into this category are *Eidolon helvum* (Rosevear, 1965; Mutere, 1967, 1968b; Jones, 1972; Fayenuwo and Halstead, 1974; Kingdon, 1974; *Chironax melanocephalus* (Medway, 1978; Bergmans and Rozendoal, 1988), *Haplonycteris fisheri* (Heideman, 1988, 1989), *Nanonycteris veldkampi* (Bernard and Cumming, 1997), *Nyctimene rabori* (Heideman, 1995) and also *Rousettus aegyptiacus* at more temperate latitudes (Herzig-Straschil and Robinson, 1978; Bernard and Cumming, 1997).

Pteropus scapulatus, *P. alecto* and *P. poliocephalus* have been studied in detail in Australia by Nelson (1965a, b), O'Brien (1993), O'Brien *et al.* (1993), McGuckin and Blackshaw (1987a) and Towers and Martin (1985). They found, year to year, that the timing of the male testicular cycle was relatively stable. Captive bats varied little in their cyclicity or sexual behavior from bats obtained in the wild. However, in wild populations, timing of breeding events varied as a function of latitude (Ratcliffe, 1932). In populations studied in southern Queensland, spermatogenesis and circulating testosterone peaked in the early fall (March and April: *P. poliocephalus* and *P. alecto*) or early summer (*P. scapulatus*) and births occurred six to seven months later. Sperm were present in the female tract of *P. poliocephalus* and *P. alecto* well into pregnancy (June), suggesting continued sexual activity in males beyond oestrus even though seminal vesicle activity did not appear to persist this long (McGuckin, 1988). Furthermore, in *P. poliocephalus*, male–female associations began while females were lactating, and mating commenced about two months prior to conception. However, Martin *et al.* (1995) noted that early ejaculations contained only sperm; apparently testosterone production and seminal vesicle gland activity are delayed until closer to the time of conception.

The monoestrous species *Haplonycteris fischeri* was studied in the Philippines by Heideman (1988). There is apparently no synchronized mating time in this species. Copulations occurred throughout most of the year and sperm were present in the testes and epididymides of adults in all months, apparently to serve the several month span of time in which females come into oestrus and to reinseminate those that failed to become pregnant or suffered foetal loss.

In polyoestrous species, females have two or more well-defined oestrous cycles per year. For the most part, their male pteropid counterparts appear to remain sexually active with no variation in testicular size, and spermatozoa are present throughout the year (e.g. *Cynopterus minutus*: Kofron, 1997). Some continue to solicit females even during pregnancy (e.g. *Epomops buettikoferi* and *Micropteropus pusillus*: Thomas and Marshall, 1984; Kofron and Chapman, 1994). For the latter two species, which display lek behavior, Thomas and Marshall (1984) speculated that it is advantageous for males to maintain aggressive, territorial behavior. Other species have a bimodal cycle of testicular recrudescence although sperm are present year-round (e.g. *C. brachyotis*: Kofron, 1997) to serve the two or three annual pregnancies (Lim, 1970). *Cynopterus sphinx* (Sreenivasan *et al.*, 1974) and *M. pusillus* (Kofron and Chapman, 1994) appear to be continuously fecund in some parts of their range but display bimodal testicular spermatogenesis in other parts (Krishna and Dominic, 1983a, b; Vamburkar, 1958; LeKagul and McNeely, 1977; Sandhu and Gopalakrishna, 1984; Jones, 1972; Bergmans, 1979). In *Epomophorus anurus* and *Rousettus aegyptiacus* (Mutere, 1968a, b; Okia, 1974b), the male reproductive cycle apparently is in synchrony with the female (Bergmans, 1979; Jacobson and DuPlessis, 1976; Kingdon, 1974; Kulzer, 1958) since they appear to undergo biseasonal testicular hypertrophy in West Africa.

Details of the male reproductive events are lacking for many of the species for which we have information on the female cycle, e.g. *Pteropus molossinus* and *P. tonganus* (Pierson and Rainey, 1992), *Rousettus leschenaulti* (Gopalakrishna and Choudhari, 1977; Noronha and Bhiwgade, 1995), *Myonycteris torquata* (Brosset, 1966b; Kingdon, 1974; Bergmans, 1976), *Harpyionycteris whiteheadi* (Heideman, 1995), *Hypsignathus monstrosus* (Kingdon, 1974; Thomas and Marshall, 1984), *Epomops franqueti* (Okia, 1974a), *Epomophorus gambianus* (Thomas and Marshall, 1984), *E. wahlbergi* (O'Shea and Vaughan, 1980), *Nanonycteris veldkampi* (Marshall and McWilliam, 1982; Wolton *et al.*, 1982) and *Ptenochirus jagori* (Heideman, 1995).

For species in which pregnant or lactating females are present in the population in all months of the year, adult males sampled throughout the year reveal that numerous, but not all, have enlarged spermatogenic testes and hypertrophied, secretion-filled accessory glands, indicating that while males are fecund population-wide, they enter periods of inactivity individually (Fleming, 1988). Examples are *P. m. mariannus* (Wiles, 1987), *P. m. yapensis* (Falanruw, 1988), *P. m. paganensis* and *P. m. volanus* (Wiles, 1992), *P. pumilus* (Heideman, 1987) and *P. tonganus* (Wodzicki and Felten, 1975, 1980; Pierson and Rainey, 1992), *Epomophorus wahlbergi* (Bernard and Cumming, 1997), *Syconycteris australis papurana* (Flannery, 1992), *Rousettus lanosus* (Kingdon, 1974), *R. aegyptiacus* in some parts of its range (Bernard and Cumming, 1997), *Stenonycteris lanosus* (Kingdon, 1974), *Cynopterus minutus* (Kofron, 1997), *Eonycteris spelaea* (Beck and Lim, 1973), *Macroglossus minimus* (McKean, 1972a, b; Hood and Smith, 1989), *M. sobrinus* and *Balionycteris maculata* (Davidson, 1992).

An interesting adjunct to the reproduction of the pteropid *Dyacopterus spadiceus* is lactation in the male. The histology of the male mammary glands showed them to be similar to those of lactating females. Francis *et al.* (1994) offer some possible explanations, the most plausible of which is that the glands hypertrophy under the influence of elevated dietary oestrogens (phytoestrogens), in which case the response would be better termed galactorrhoea. A more exact physiological explanation awaits a careful study measuring the circulating hormones in the plasma to determine the mediating mechanisms inducing milk expression.

Secondary sexual characteristics are well represented in the Pteropodidae. The most conspicuous specializations are the bulbous muzzle and gigantically hypertrophied larynx in the strikingly larger male hammer-headed bat, *Hypsignathus monstrosus*. These are utilized as resonating spaces to produce loud calls to which females are attracted for breeding in their lek mating system (Bradbury, 1977). Courtship vocalizations (calling territories) are also part of the male mating pattern in *Epomophorus wahlbergi* (Wickler and Seibt, 1976), *Epomops buettikoferi* and *Micropteropus pusillus* (Thomas and Marshall, 1984). Other specializations include shoulder tufts or epaulets in *Epomops*, *Epomophorus*, *Megaloglossus*, *Micropteropus* and *Nanonycteris* (Rosevear, 1965; Marshall and McWilliam, 1982) for which roles in both display and olfactory signalling have been suggested (Wickler and Seibt, 1976), and neck-chest ruffs in male *P. conspicillatus* (Spencer and Flick, 1995), *Rousettus* (some species), *Nanonycteris*, *Megaloglossus* and *Eidolon* (Rosevear, 1965). In *P. conspicillatus*, males were seen to anoint their neck ruffs with a cranberry-red secretion produced from the urogenital tract. This secretion was found to contain high concentrations of long chain fatty acids (Spencer and Flick, 1995).

4.6.2 Rhinopomatidae

Occupying the arid and semi-arid, more temperate parts of northern Africa, southwestern Asia and India to Thailand and Sumatra (Hill and Smith, 1984), two (*Rhinopoma microphyllum* [= *R. kinneari*] and *R. h. hardwickei*) of the three species studied to date demonstrate that this monogeneric group is monoestrous, breeding in spring upon arousal from winter torpor. Following a three-month gestation, birth and lactation period, females enter anoestrus until the following spring. Males, with their permanently abdominal testes, are spermatogenic in winter at decreased body temperatures (Anand Kumar, 1965; Racey, 1982; Singwi and Lall, 1983; Karim and Banerjee, 1985) rather than in the late summer as in most bat species that hibernate. In *R. hardwickei*, testicular involution can precede the cessation of oestrus; however, copulation is effected one month later by epididymal sperm stores (Karim and Banerjee, 1985). Sperm also persist in the epididymides and ampullary glands of *R. kinneari* for much of the year (Anand Kumar, 1965). The accessory sex gland cycle is in synchrony with the testicular cycle.

4.6.3 Craseonycteridae

There is no information on the reproductive cycle of *Craseonycteris thonglongyai*, the only representative of this recently discovered monogeneric, monotypic family that is restricted to western Thailand (Hill, 1974; LeKagul and McNeely, 1977; Hill and Smith, 1984; Nowak, 1994).

4.6.4 Emballonuridae

Primarily tropical in distribution in both the New and Old World (Koopman, 1970; Koopman and Jones, 1970; Hill and Smith, 1984), the extensive range of this family gives rise to a variety of reproductive strategies. Females are either monoestrous or seasonally polyoestrous. Virtually nothing has been published on the reproduction of the New World genera *Cormura*, *Centronycteris* or *Cyttarops*. There is a dearth of information on male emballonurids.

Monoestrous species include *Taphozous melanopogon* (Brosset, 1962b; Khaparde, 1976), *T. nudiventris* (= *T. kutchensis*) (Al-Robaae, 1968; Krishna and Dominic, 1982a), *T. georgianus* (Kitchener, 1973, 1976; Jolly and Blackshaw, 1987, 1988); *T. hilli* (Kitchener, 1980, 1983), *T. hildegardeae* (McWilliam, 1982, 1988c), *Saccolaimus flaviventris* (Chimimba and Kitchener, 1987), *Peropteryx macrotis*, *P. leucoptera*, *Balantiopteryx io*, *B. plicata* (Lopez-Forment, 1981).

The male reproductive cycle is known for very few of these. Male *B. plicata* are known to have epididymal sperm throughout the year with sperm numbers increasing at the onset of the highly stable, synchronized breeding season and decreasing at its termination (Lopez-Forment, 1981). *Taphozous nudiventris* is a winter hibernator in part of its geographic range (southern Iraq). As in many temperate dwelling hibernating species, females store spermatozoa which presumably effect conception upon arousal (Al-Robaae, 1968). One wonders if males also store epididymal spermatozoa during hibernation. There is no information either on the male cycle for this species in the parts of its range where it does not hibernate (Brosset, 1962a; Krishna and Dominic, 1982a).

Male reproduction is well documented for *T. georgianus* in Australia (Kitchener, 1973, 1976; Jolly and Blackshaw, 1987, 1988). The testes are actively spermatogenic year-round with peak activity in the autumn; however, there is a marked asynchrony between the testicular and accessory gland (spring) cycles. The extended parturition season of five months suggests that there is a prolonged mating season during which receptive females are inseminated. This is made possible by epididymal sperm stores. *Taphozous hilli* (Kitchener, 1980, 1983) also stores epididymal sperm throughout the year; however, copulation, ovulation and fertilization are apparently restricted to a brief period in this species. Movement of the testes between the scrotum (summer, presumably spermatogenic), inguinal canal (late autumn–early winter) and abdomen (mid-winter–spring, likely not spermatogenic) presumably reflects the gametogenic cycle. *Taphozous hildegardeae* males display a seasonally bimodal increase in testicular weight which correlates with the two annual rainy seasons in Kenya; however, the second, much smaller hypertrophy, which is not matched by hypertrophy of the accessory gland complex, does not result in conception. McWilliam (1988c) speculated that this behavior is a relic of an ancestral polyoestrous pattern of reproduction. It may also be significant to the maintenance of harem polygyny.

Polyoestrous species include *Taphozous longimanus* (Krishna and Dominic, 1982a, b, 1983a; Gopalakrishna, 1954, 1955), *T. perforatus* (Harrison, 1958a; Rosevear, 1965), *Saccolaimus* (= *Taphozous*) *peli* (Lang and Chapin, 1917; Kingdon, 1974), *Emballonura monticola* (Medway, 1978), *E.* (= *Mosia*) *nigrescens* (McKean, 1972a, b), *Coleura seychellensis* (Nicoll and Suttie, 1982), *Peropteryx kappleri* (Giral *et al.*, 1991), *Rhynchonycteris naso* (Bradbury and Vehrencamp, 1976, 1977; Dalquest, 1957). Some are seasonally bimodal while others are continuously polyoestrous and some (e.g. *Taphozous longimanus* in India: Krishna and Dominic, 1982a, b, 1983a; Gopalakrishna, 1954, 1955) can be either, depending on latitude. A third annual peak in testicular hypertrophy in this species at more northerly latitudes apparently does not result in conception (Krishna and Dominic, 1982a). Nothing is known of the male cycle in more northerly latitudes where parturitions occur throughout the year. Gopalakrishna (1954) reported that male *T. longimanus* around Nagpur were spermatogenic in all months of collections (none made in April and May), and Krishna and Dominic (1982a) found that *T. longimanus* had epididymal sperm throughout the year. Male fecundity in *P. kappleri* may be a biannual event in synchrony with the bimodal oestrous cycle (Giral *et al.*, 1991). Plumpton and Jones (1992) reviewed data on male testicular size for a variety of localities and dates for *R. naso* and concluded that there was no consistent spermatogenic pattern in this species.

Some species of emballonurids are monoestrous in parts of their range and polyoestrous in others. These include *Taphozous mauritianus* (Allen *et al.*, 1917; Lang and Chapin, 1917; Smithers, 1971; Anciaux de Faveaux, 1973; Kingdon, 1974; O'Shea and Vaughan, 1980; Happold and Happold, 1990; Nowak, 1994), *T. perforatus* (Harrison, 1958a; Brosset, 1962a, b; Kock, 1969; McWilliam, 1982), *Coleura afra* (Kock, 1969; McWilliam, 1987a), *Saccopteryx leptura* (Bradbury and Emmons, 1974; Nowak, 1994) and *S. bilineata* which is primarily monoestrous (seasonal or aseasonal) over its geographic range except in Costa Rica where it is polyoestrous (Young, 1972; LaVal and Fitch, 1977). Some observations on *C. afra* were made in Kenya where McWilliam (1987a) reported that this species has two reproductive cycles annually. Testicular activity increased at the time of greatest body mass at the end of the two annual rainy seasons when copulation occurs. In Ghana, where this species is also polyoestrous, there is only one annual rainy season; however, it is of sufficient length (seven months) to permit two birth periods. There is no information on the male at this location or in the Sudan where the species has one annual cycle.

Secondary sexual characteristics are well developed in the emballonurids. They include ventral throat (gular) glands (described in detail in *T. longimanus*: Pondey and Dominic, 1987), jaw, chin or sternal skin glands that cycle seasonally in concert with the male reproductive cycle. In most cases, the glands are functional only in the male and may be undeveloped or absent entirely in females and in young, prepubertal males. Males make use of the thick, odoriferous, sebaceous secretion from these glands to mark their territory, self and/or females, presumably to defend and enhance their breeding success. Geographic variation is evident in the gular sac in *T. mauritianus*. In Nigeria and Mozambique it is only present in males (Smithers and Tello, 1976; Happold, 1987), while in West Africa the males have a functioning sac and females have a vestigial pouch (Rosevear, 1965). In the Sudan a functioning gular sac is present in both sexes, though more highly developed in the male (Koopman, 1975). In male *T. (= Saccolaimus) flaviventris* (Hall and Gordon, 1982) the gular pouch has no glandular tissue nor connection to the traditional glandular region deep to the throat skin; however, the proximity of a tubercle duct opening from a nearby sebaceous gland suggests the possibility that its fatty secretion might enter the throat pouch. New World emballonurids lack the gular pouch, but a number of species have glandular sacs or pouch-like structures in the antebrachial membrane which open either on the dorsal or ventral surface of the wing (Goodwin and Greenhall, 1961; Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1976; Lopez-Forment, 1981). These structures, which are far better developed in the male, are used in association with wing movements, vocal, flight and olfactory displays and are employed by males to attract and retain harem females during courtship and in defending territory.

4.6.5 Nycteridae

This monogeneric family is primarily tropical in distribution, although some species occur in the grassy savanna and arid regions of Africa and southwestern Asia (Koopman and Jones, 1970; Bernard, 1982; Hill and Smith, 1984). Within this large area of ecological diversity, nycterids show relative homogeneity in their reproductive strategies.

The species about which most is known, *Nycteris thebaica*, is monoestrous in the subtropical, temperate regions of South Africa with a longer than usual gestation of five months and a three-month anoestrus (Bernard, 1982). Male and female cycles are synchronized; spermatogenesis commences in late summer and the epididymides contain mature sperm from autumn to summer (Bernard, 1976). Both reproductively active and inactive males were found concurrently by Verschuren

(1957). This may reflect age (adult v. subadult) rather than a dysynchrony in the testicular cycle.

Most nycterid species are polyoestrous with two or more (continuous) cycles per year, a postpartum oestrus and often no anoestrus. These include *Nycteris luteola* (Matthews, 1937, 1941), *N. hispida* (Lang and Chapin, 1917; Verschuren, 1957), *N. aethiopica (= N. macrotis)* (Adams and Hubert, 1976; Anciaux de Faveaux, 1978), *N. javanica* (LeKagul and McNeely, 1977), and *N. grandis* (Verschuren, 1957; Kingdon, 1974). Information on the males of these species is fragmentary and it is not known whether they are continuously breeding or not. Matthews (1937) noted that male *N. luteola* collected at the time of post partum oestrus were reproductively functional. Matthews (1941) recorded that male *N. luteola* had active testes throughout female oestrus and that copulation followed shortly after parturition in *N. hispida*. Anciaux de Faveaux (1978) noted copulation in *N. macrotis* in August and October in Zaire and Rwanda, Africa. His summary data on *N. thebaica* and *N. macrotis* from various African states also recorded that breeding occurred at least twice a year. In *N. macrotis*, where as many as three litters may occur in succession, he thought it logical to suspect continuous male fecundity.

4.6.6 Megadermatidae

This family has a large geographic range in the Old World, wherein they are primarily found in more arid, tropical and subtropical locations (Koopman, 1970; Hill and Smith, 1984; Nowak, 1994). With the exception of the polyoestrous *Cardioderma cor* (Matthews, 1941; McWilliam, 1987b), this family is seasonally monoestrous.

Details are known for males of the monoestrous species *Megaderma lyra* (Ramakrishna, 1951; Ramaswamy, 1961; Pal, 1977, 1984b) which exhibits seasonal breeding; the testicles are aspermatic for six months of the year. Spermatogenesis occurs in summer and cauda epididymidal sperm are present for the following six months, during which time (fall) insemination occurs, following about one month after maximum testicular weight (which is indicative of the presence of sperm). As is characteristic for a number of tropical and subtropical Chiroptera, the onset of the breeding season varies in a north–south cline in India, occurring later in the southern regions and earlier in the north (Douglas, 1967).

In the polyoestrous species *C. cor*, Matthews (1941) noted that the ampullary glands were filled with secretions and spermatozoa when females were pregnant. Male secondary sexual features that are well developed in this family include dorsal skin glands in *Lavia frons* (Rosevear, 1965), vocalizations in *C. cor* (Vaughan, 1976; McWilliam, 1987b) that have yet to be correlated to the individual's sex or reproductive status, and territory defence in the form of flight antics, patrols and vocalizations exhibited by male *L. frons* (Vaughan and Vaughan, 1986).

4.6.7 Rhinolophidae

This widely distributed Old World family (sub-families Rhinolophinae and Hipposiderinae) covers both temperate and tropical habitats from the British Isles, through Africa to the Indo-Australian archipelago and New Hebrides (Koopman and Jones, 1970). However, little variation occurs in the basic reproductive pattern of the male.

Monoestry appears to be the rule and male and female gamete cycles are often not synchronized. In male rhinolophids inhabiting northern, temperate latitudes spermatogenesis occurs in the spring–summer and concludes in the late summer–early fall when testicular regression occurs in concert with release of sperm to the epididymides and hypertrophy of the accessory sex glands. Dysynchrony between primary and accessory sex organs is similar (but not as marked) to that occurring in the vespertilionids (Bernard, 1986). Sperm are stored in the caudae epididymidides until spring arousal from hibernation. They are then cleared from the male tract. Mating occurs in the fall and can continue during hibernation with torpid females until their arousal and ovulation in spring.

Examples of taxa in this category are *Rhinolophus ferrumequinum* (Courrier, 1927; Vignoli, 1930; Saint Girons *et al.*, 1969) and *R. hipposideros* (Courrier, 1923b, 1927; Gaisler and Titlbach, 1964; Gaisler, 1966). Several studies on the latter species have addressed the annual secretory function of the accessory sex glands (e.g. Pal, 1983a). Two sympatric, southern hemisphere (South African) rhinolophids, *Rhinolophus capensis* (Bernard, 1985) and *R. clivosus* (Bernard, 1983, 1988) and the Australian *R. megaphyllus* (Krutzsch *et al.*, 1992) follow a similar pattern of late spring/summer/early fall spermatogenesis. Only in *R. clivosus*, however, does copulation occur in the fall followed by sperm storage in the female until arousal and conception in the spring. In *R. megaphyllus* copulation takes place during the winter when spermatogenesis is waning, and sperm storage does not occur in the female. In *R. capensis* copulation occurs towards the conclusion of winter rather than in the preceding autumn, and sperm are stored only in the male reproductive tract (Bernard, 1985). Interestingly, *R. megaphyllus* males retain epididymal sperm for at least four months beyond conception and beyond the annual secretory life span of the accessory glands which begin to involute late in winter; nevertheless, the ampullary–seminal vesicle complex retains luminal secretions and sperm through winter.

In other (mainly tropical) rhinolophids, both sexes produce gametes synchronously. Ovulation and immediate fertilization follow copulation with no sperm storage in the female. Sperm may or may not be cleared from the epididymides soon after copulation. Information is available for male *R. rouxi* (Sreenivasan *et al.*, 1973; Ramakrishna, 1976; Gopalakrishna and Rao, 1977), *R. landeri* and *Hipposideros caffer* (Menzies, 1973), *H. fulvus* (Madhavan *et al.*, 1978) and *H. speoris* in some parts of its range (Brosset, 1962a). In other parts of its range in India, the breeding season of *H. speoris* is more prolonged; sperm persist in the epididymal tubules for some time following conception and may be useful for later insemination of prepubertal individuals who attain sexual maturity after the adult mating season (Gopalakrishna and Bhatia, 1980, 1983). The accessory glands remain secretory throughout this extended time. *Hipposideros caffer* is somewhat of an enigma, being clearly monoestrous in some parts of its range but possibly bimodally or continuously polyoestrous in other parts (Bernard and Cumming, 1997).

The only departure recorded from the monoestry pattern is that reported by Medway (1969) for three Malaysian species which have two periods of parturition. These are *R. affinis, R. trifoliatus* and *H. armiger*. There is no information on the males of these species.

Secondary sexual characteristics specific to male rhinolophids include a variety of skin glands that secrete odoriferous substances (Kingdon, 1974). These glands (e.g. para-anal-penile glands of *Hipposideros cyclops*, shoulder glands of *R. landeri*, axillary pseudonipples of *R. alcyone*, bulbous forehead glands of *H. cyclops* and *H. commersoni*) are often associated with specialized hair adornments (e.g. tufts and plumes). In several instances it has been observed that the activity of these various glands correlates with the male active reproductive season (e.g. Mainoya, 1979).

4.6.8 Noctilionidae

This small family of two species is restricted to the neotropics where it is widely distributed from Sinaloa and southern Vera Cruz, Mexico, the West Indies, middle and South America to northern Argentina (Koopman and Jones, 1970; Koopman, 1970; Hill and Smith, 1984; Nowak, 1994).

Although there have been no detailed studies on male reproduction in this family, the many reports stemming from field observations suggest that females of both species are monoestrous. This certainly holds true for *Noctilio albiventris* in which autumn–winter insemination coincides with ovulation; birth occurs in spring (Anderson and Wimsatt, 1963; Davis *et al.*, 1964; Hooper and Brown, 1968; LaVal and Fitch, 1977; Rasweiler, 1979; Hood and Pitocchelli, 1983). Rasweiler (1977) noted a high incidence of uterine sperm in non-pregnant females sampled from nature over a period of several weeks; he speculated on the possibility of extended sperm survival, prolonged receptivity to males or slow elimination of sperm from the female tract; however, the significance of this observation remains unknown. Limited data promote the possibility that *N. leporinus* conceives a second litter/year by virtue of a postpartum oestrus (Hood and Jones, 1984), a scenario that is supported by observations of a virtually continuous presence of lactating females in Cuba (Silva Taboada, 1979). The male sexual cycle is not known for either species.

An interesting secondary sexual characteristic in this family has been reported in males of both species of *Noctilio*. Inguinal (Studier and Lavoie, 1984) or parascrotal (Dunn, 1934) folds of skin on the lateral side of the scrotum or in the inguinal region only become visible when the testes are descended into the scrotum which occurs apparently during the breeding season (Dunn, 1934). These ridges produce a strong, musky odor and as such are thought to serve a secondary sexual function. Studier and Lavoie (1984), however, determined that these structures were not glandular and that the odor was the product of bacterial metabolism. No direct sexual function has yet been ascribed to them but they may have a hedonic role.

4.6.9 Mormoopidae

This small family of eight species (Koopman, 1993) has an extensive range in neotropical regions of the New World (from Brazil northward to Mexico, southeastern Arizona and southwestern and central Texas, West Indies and most of the Bahamas) where it occupies habitats ranging from hot and humid to dry, semi-arid and arid (Hill and Smith, 1984).

The mormoopids are monoestrous, the timing of breeding activity varying geographically irrespective of season (Carter, 1970; Fleming *et al.*, 1972; Wilson, 1973; Garrido-Rodriguez *et al.*, 1984; Rezsutek and Cameron, 1993; Lancaster and Kalko, 1996). Information on the male reproductive cycle is scant. Numerous reports on testicular dimensions document that their length varies throughout the year, and perhaps this information can be used to estimate reproductive cyclicity and readiness. These data have been used to demonstrate the coincidence of maximal testicular length with January mating of *Pteronotus davyi* (Jones, 1966; LaVal, 1972; Jones *et al.*, 1973); males collected later in the year had smaller testes (Adams, 1989). Garrido-Rodriguez and Lopez-Forment (1981) and Garrido-Rodriguez *et al.* (1984) recorded testes length in *P. parnellii* and noted that only those 3.0 mm or longer contained mature sperm. Epididymal sperm were noted from December through February. Observations of accessory gland hypertrophy have been used to estimate the time of copulation (January) in this species (Garrido-Rodriguez *et al.*, 1984). Together, these scant observations suggest a single annual male cycle that coincides with that of the female.

No special secondary sexual characteristics have been reported for the mormoopids, probably because so little is known about this family. It may also be true that such characteristics are not well marked in a species that resides in sexually segregated colonies. When the sexes congregate together they do so in large breeding colonies where mating is random and secondary sexual clues are perhaps less important.

4.6.10 Phyllostomidae (including Desmodontidae)

Widely distributed in the New World, they occur in temperate to tropical areas (Koopman and Jones, 1970; Hill and Smith, 1984; Nowak, 1994). A range of reproductive strategies has been recorded in this large family of some 142 species, representing 49 genera (Koopman, 1993). Based on the occurrence of oestrus and pregnancy they, like the Pteropodidae, can be sorted into three basic schemes (monoestrous and bimodally and continuously polyoestrous). Data suggest that the annual reproductive cyclicity of the male probably parallels that of the female with synchronized gamete production (Krutzsch *et al.*, 1976; Taddei, 1976; Wilson, 1979; Fleming, 1988; Handley *et al.*, 1991).

Monoestrous species include *Macrotus californicus, Anoura geoffroyi, Leptonycteris curasoae, Choeronycteris mexicana* and *Phyllostomus hastatus* (Fleming *et al.*, 1972; Wilson, 1973, 1979; Krutzsch *et al.*, 1976; Taddei, 1976; Willig, 1985b; Graham, 1989; Brosset and Charles-Dominique, 1990; Heideman *et al.*, 1992; Baumgarten and Vieira, 1994; Martino *et al.*, 1998). Males, in concert with females, appear to have a single annual period of libido following which the primary and accessory sex organs synchronously involute. In *M. californicus*, for which there is detailed information on the male, the primary and accessory male cycles are closely synchronized; levels of testosterone correlate with spermatogenesis as do changes in steroid synthesizing organelles of the Leydig cells (Krutzsch *et al.*, 1976). In *A. geoffroyi*, Heideman *et al.* (1992) found that maximum testicular size coincided with an annual mating in July and August in Trinidad. Data on seasonal changes in testicular size are not available for other parts of the range of this species where females also appear to be monoestrous (Willig, 1985b; Graham, 1989; Brosset and Charles-Dominique, 1990; Baumgarten and Vieira, 1994). Testicular descent as well as size (McCracken and Bradbury, 1981) is a clue to the onset of male fecundity; these coincided with the annual period of oestrus and copulation between late October and early December in *P. hastatus* in Trinidad. In Brazil, testicular activity and epididymal sperm were seen only between May and July; females were reproductively active only in the second half of the year (Taddei, 1976).

Polyoestrous species include *Desmodus rotundus* (Wimsatt and Trapido, 1952; Burt and Stirton, 1961; Goodwin and Greenhall, 1961; Greenhall, 1965; Mann and Aulagnier, 1995), *Stenoderma rufum* (Gannon and Willig, 1992), and *Sturnira lilium* (Sanchez *et al.*, 1986). This pattern may pertain in part of the geographic range of some other species which are elsewhere in their range bimodally polyoestrous. These include *Glossophaga soricina* (Taddei, 1976; Wilson, 1979; Willig, 1985b; Graham, 1989), *Artibeus lituratus* (Goodwin and Greenhall, 1961; Tamsitt and Valdivieso, 1963, 1965; Tamsitt, 1966; Taddei, 1976), *Artibeus planirostris* (Taddei, 1976; Graham, 1989), *Vampyrops* (= *Platyrrhinus*) *lineatus*, *Anoura caudifera* and *Carollia perspicillata* (Taddei, 1976). Data concerning the male cycle of these species are fragmentary (Wimsatt and Trapido, 1952; Dalquest, 1955; Burt and Stirton, 1961; Hall and Dalquest, 1963; Greenhall, 1965; Mann and Aulagnier, 1995; Villa-R, 1966). Males in full sexual activity are found in all seasons; however, it is not clear for most species whether continuous fecundity is an individual or population-wide scenario. For *A. lituratus*, although there is seasonal variation in testicular size, adult males were found to be fully sexually active with sperm-filled epididymides throughout the year (Tamsitt and Valdivieso, 1963, 1965). The mean diameter of seminiferous tubules and the height of the epithelium remained unchanged seasonally. Leydig cells were always small and few in number. Tamsitt and Valdivieso (1965) speculated that their hormone production is constant throughout the year and at a level to maintain spermatogenesis. They did not examine the steroid-dependent accessory glands to determine their size or secretory state, critical factors in determining male reproductive activity.

Most phyllostomids are bimodally polyoestrous. These include *Micronycteris megalotis*, *Phyllostomus hastatus* (part of its range, i.e. Columbia), *P. discolor, Tonatia silvicola*, *Trachops cirrhosus*, *Anoura candifera*, *Glossophaga soricina* (part of its range), *Leptonycteris curasoae* (= *sanborni*), *Carollia brevicauda*, *C. castanea*, *C. perspicillata*, *C. subrufa*, *Artibeus anderseni*, *A. cinereus*, *A. fraterculus*, *A. glaucus*, *A. jamaicensis*, *A. lituratus* (part of its range), *A. phaeotis*, *A. planirostris*, *A. watsoni*, *Chiroderma trinitatum*, *Platyrrhinus* (= *Vampyrops*) *helleri*, *Sturnira bogotensis*, *S. erythromos*, *S. lilium*, *S. magna*, *Uroderma bilobatum*, *Vampyressa nymphaea*, *V.* (= *Mesophylla*) *macconnelli*, *V. pusilla*, *Vampyrodes caraccioli* (Fleming *et al.*, 1972; Wilson, 1973, 1979; Graham, 1989). Most of these species have had their reproductive periodicity and seasonality established by observation of the female cycle where conspicuous events such as pregnancy, lactation, or concurrent pregnancy and lactation clearly mark their reproductive cycle. Geographical variation exists in the timing of mating and birth (Wilson, 1979). Evidence defining the male reproductive cycle is less readily recognized grossly and it requires histological confirmation. Details of the male reproductive cycle and its controlling factors for the polyoestrous phyllostomids are thus very poorly understood.

Fleming *et al.* (1972) employed testicular length as an indicator of the reproductive state of male *A. jamaicensis*, *Uroderma bilobatum*, *C. perspicillata* and *G. soricina*. They found that those individuals with testes 6.0 mm or greater in length were in active spermatogenesis. These observations were confirmed by histological examination; however, for *A. jamaicensis* at least, the testes remained over 6.0 mm in length in the non-reproductive period, so such a gross observation as an indicator of function may not be reliable for this species. The onset and termination of the testicular gametic cycle, though poorly defined by their small sample, appeared to correlate with female sexual receptivity and pregnancy. For example, in adult reproductively active female *A. jamaicensis* (July–August and March), *U. bilobatum* (September–October and February–March) and *C. perspicillata* (October and March), the male testicular size was largest just preceding female receptivity and smallest when females were pregnant or sexually inactive. For *C. perspicillata*, Fleming (1988) speculated that because there were at least some males with enlarged testes in all months of the year that the population contained sexually competent males the year-round. Testis size in adult males, as related to age and season, was investigated with the thought that older males were more likely to have the largest testes and therefore sire offspring. His research also suggested that the dominant territorial males (which are the oldest) have the greatest success in inseminating females. Male copulatory, as well as acoustic and territorial behavior, has been described by Porter (1979a, b) and male–male and male–female interactions have been reviewed by Fleming (1988).

Observations by Handley *et al.* (1991) on *A. jamaicensis* in Panama confirmed those of Fleming *et al.* (1972) and Wilson (1973, 1979) that adult males have enlarged spermatogenic testes when females are receptive in postpartum oestrus. The degree of asynchrony in parturition indicates that males may be capable of insemination for a relatively extended period, overlapping both parturition peaks.

Secondary sexual characteristics are variably expressed in the reproductively active and/or harem defensive males of a number of phyllostomid taxa. Skin glands that are most often sebaceous and function only in the male, are found in the throat or sternal area of *Phyllostomus discolor* and *P. hastatus* (Goodwin and Greenhall, 1961; Valdivieso and Tamsitt, 1964); their activity correlates with the male reproductive cycle. Goodwin and Greenhall (1961) have also described lappets of loose skin on the chin of male *Centurio senex* that probably contain scent glands. Davis *et al.* (1964) and Goodwin and Greenhall (1961) have remarked on odoriferous glands in the shoulder patches of male *Sturnira lilium* and *S. mordax*. A large number of behavioral patterns have also been attributed to the reproductive process in this family including vocal emissions, elaborate wing movements, nosing and tongue extension. Many of these are associated with harem formation and maintenance (see Chapters 8 and 9).

4.6.11 Natalidae

This family occurs in the tropical lowlands of the New World from northern Mexico and southern Baja, California southward to Brazil. It achieves its greatest diversity in the West Indies where it is represented by all five of its currently recognized species (Koopman, 1993; Nowak, 1994). Since there is relative ecological stability in the areas it inhabits, it is likely, though not yet demonstrated, that there is little variation in reproductive strategies among taxa.

There has been little published on male reproductive events since the review of this apparently monoestrous, monogeneric family by Krutzsch (1979). Testes that are hardly visible without the aid of a dissecting microscope during the inactive reproductive period in *Natalus stramineus* begin to increase in size in late September–October to reach about 2.4 mm long × 1.5 mm wide during the breeding season (December and January: Mitchell, 1965). The caudae epididymidides also increase in size and appear to be engorged with sperm at this time, remaining so until early April, several months after breeding. The accessory glands exhibit a parallel functional cycle to that of the testes, gradually involuting to the resting state in March–April. This chain of events conforms well to the onset of the extended delayed embryonic development characteristic of the female gestation cycle. It is likely that other *Natalus* taxa express a similar male cycle as evidenced by Goodwin's (1970) report on *N. major (= N. stramineus*) and *N. micropus* from Jamaica. Confusing, but of interest, is the presence of cauda epididymidal spermatozoa well into the extended gestation period. This is a seemingly redundant phenomenon unless it serves unfertilized females or young adults entering the reproductive pool during the prolonged period of delayed embryogenesis exhibited by the major segment of the adult female population.

Male secondary sexual characteristics, other than the unique 'natalid organ' (Dalquest, 1950), have not been recorded. This organ (13.0 mm long × 7.0 mm wide × 2.0 mm thick) is a bell-shaped, flattened mass that covers the frontal region of the skull of *N. mexicanus*. The organ consists of a mass of tubules separated by layers of connective tissue. Although it shows evidence of sensory and glandular capabilities, no function has been ascribed to it.

4.6.12 Furipteridae

These bats are primarily distributed in tropical South America, Trinidad, West Indies, Panama and Central America (Koopman and Jones, 1970; Hill and Smith, 1984; Nowak, 1994).

Little is known regarding the reproductive biology of this family. Pregnant females with a single foetus have been recorded in June, August and October in Peru (Graham, 1989) and November in Ecuador (Ibanez, 1985). Nothing has been published on the male reproductive cycle or the presence of secondary sexual features.

4.6.13 Thyropteridae

This small monogeneric family of two species occurs in the tropical American mainland from southern Mexico to southern Brazil and in Trinidad (Koopman and Jones, 1970; Hill and Smith, 1984; Nowak, 1994).

Almost nothing is known of the reproductive biology of the disc-winged bats. Circumstantial information on female *Thyroptera tricolor* suggests it is likely polyoestrous (Wimsatt and Enders, 1980; Rasweiler, personal communication) with at least two breeding cycles per year. This strongly indicates the likelihood of two (or more) spermatogenic cycles in the male or continuous spermatogenesis. To date this has not been substantiated. A histological study of the male reproductive tract of museum specimens might provide an answer to this speculation and could be a productive undertaking by future students of chiropteran reproductive biology.

4.6.14 Myzopodidae

This monogeneric and monotypic family is endemic to Madagascar (Koopman and Jones, 1970).

There is no published information concerning the reproductive biology of either sex of *Myzopoda aurita*.

4.6.15 Vespertilionidae

This, the largest chiropteran family, is also the most widespread, occurring on all continents to the limits of forests in the subarctic and subantarctic and reaching many oceanic islands (Koopman and Jones, 1970; Hill and Smith, 1984). Not surprisingly, a large variety of reproductive strategies have evolved.

Most temperate-dwelling northern and southern hemisphere vespertilionid bats of both the Old and New World that have been studied are monoestrous. Males likewise have one annual reproductive cycle. Spermatogenesis commences in the late spring and summer and ceases prior to the autumn breeding season. Interstitial cells involute (‘*Myotis* Pattern’: Gustafson, 1979) or are reduced in function (‘*Pipistrellus* Pattern’: Gustafson, 1979) at the onset of mating. Females enter hibernacula either pregnant (*Miniopterus schreibersii, M. australis*; Richardson, 1977) or with stored sperm. A cauda epididymidal (and sometimes an ampullary gland) sperm store is also retained by all males in this category until cleared from the reproductive tract following their arousal from hibernation in the spring. Upon the apparent waning of the closely synchronized testicular-androgenic cycle in the autumn, the accessory sex glands hypertrophy and become secretory, remaining so throughout hibernation (except in the ‘*Miniopterus* Pattern’: Gustafson, 1979) and allowing additional inseminations during this period. The accessory glands quickly regress upon arousal from hibernation in spring (Christian, 1956; Gustafson, 1976, 1979; Krutzsch, 1961, 1975; Krutzsch and Crichton, 1986; Miller, 1939; Pearson *et al.*, 1952; Racey, 1974; Racey and Tam, 1974). The apparent asynchrony between the testicular and accessory gland cycles of Holarctic hibernating vespertilionids has been a topic of extended discussion, particularly with regard to the androgenic (interstitial cell) control of accessory gland function when the testes seem involuted. The role of sex steroid binding proteins in the availability of circulating testosterone has been discovered and defined (e.g. Gustafson, 1975, 1987; Gustafson and Shemesh, 1976; Damassa *et al.*, 1982, 1983, 1985; Gustafson and Damassa, 1984, 1985, 1987; Gustafson *et al.*, 1985). This subject is addressed in detail in Chapter 2.

Monoestrous species include *Pipistrellus hesperus* (Krutzsch, 1975), *P. subflavus* (Krutzsch and Crichton, 1986), *P. kuhli* (Andreuccetti *et al.*, 1984), *P. pipistrellus* (Racey and Tam, 1974), *Nyctalus noctula* (Kleiman and Racey, 1969; Racey, 1974, Gaisler *et al.*, 1979), *Lasiurus intermedius* (= *Dasypterus floridanus*) (Sherman, 1939), *Antrozous pallidus* (Hermanson and O'Shea, 1983; Beasley *et al.*, 1984; Beasley and Zucker, 1984), *Myotis lucifugus* (Guthrie, 1933a, b; Miller, 1939; Wimsatt, 1960, 1969; Gustafson, 1976, 1977, 1979), *M. austroriparius* (Rice, 1957), *M. velifer* (Krutzsch, 1961; Hayward, 1970; Kunz, 1973), *M. californicus* (Krutzsch, 1954), *M. thysanodes* (O'Farrel and Studier, 1973), *M. volans* (Druecker, 1972), *M. sodalis* (Guthrie, 1933a, b; Miller, 1939), *M. grisescens* (Miller, 1939), *M. myotis* (Sluiter, 1961), *M. chiloensis* (Pearson and Pearson, 1989), *M. capaccini* (Courrier, 1927), *M. blythi* (= *M. oxygnathus*) (Koceva, 1970), *Lasiurus ega* (Myers, 1977), *Chalinolobus morio* (Kitchener and Coster, 1981), *C. gouldii* (Kitchener, 1975), *Vespadelus regulus, Nyctophilus geoffroyi* and *N. major* (Hosken *et al.*, 1998), *N. gouldi* (Kitchener, 1975; Tidemann, 1982; Phillips and Inwards, 1985), *Eptesicus fuscus* (Christian, 1956), *E. nilssonii* (Rydell, 1993), *E. vulturnus* (Tidemann, 1982), *E. pumilus* (Green, 1965; Tidemann, 1982), *E. regulus* (Kitchener and Halse, 1978), *Barbastella barbastellus* (Rydell and Bogdanowicz, 1997), *Miniopterus schreibersii* (Courrier, 1923a, b, 1927; Richardson, 1977; Pal, 1983b; Krutzsch and Crichton, 1990a; Bernard and Cumming, 1997), *Plecotus auritus* (Swift, 1998; Entwistle *et al.*, 1998), *P.* (= *Corynorhinus rafinesquei*) *townsendii* (Pearson *et al.*, 1952), *P.* (= *Corynorhinus*) *rafinesquei* (Jones, 1977) and *Vespertilio superans* (Oh, 1977).

A number of tropical-dwelling vespertilionids are also monoestrous. These include *Pipistrellus ceylonicus* (Madhavan, 1971; Bates and Harrison, 1997), *P. rusticus* (van der Merwe and Rautenbach, 1990), *Nycticeius schlieffeni* (van der Merwe and Rautenbach, 1987), *Miniopterus schreibersii* (Baker and Bird, 1936; Dwyer, 1963b; Richardson, 1977), *M. australis* (Baker and Bird, 1936; Dwyer, 1963a, 1968; Medway, 1971; Richardson, 1977), *Scotophilus wroughtoni* (Gopalakrishna, 1947, 1948, 1949), *S. heathi* (Bates and Harrison, 1997), *Eptesicus capensis* (van der Merwe, 1994), *Histiotus montanus* (Pearson and Pearson, 1989) and *Lasiurus ega* (Myers, 1977). In these species, in contrast to temperate-dwelling vespertilionids, copulation (usually in spring) is immediately followed by ovulation and fertilization. Characteristic of some of these species is the presence of spermatozoa in the epididymides often for several months prior to and following conception. In some tropical taxa, sperm are also stored in the female reproductive tract following copulation, e.g. *Tylonycteris pachypus, T. robustula* (Medway, 1972), *Scotophilus heathi* (Bates and Harrison, 1997), and *Pipistrellus nanus* (Bernard *et al.*, 1997). *Pipistrellus nanus* may be polyoestrous in other parts of its range (Anciaux de Faveaux, 1973; LaVal and LaVal, 1977) as also is *Rhogeessa minutilla* (Sosa *et al.*, 1996). In *Lasiurus ega*, epididymal sperm are available some three months prior to breeding and persist for several months beyond (Myers, 1977). The testicular gametic and interstitial gland cycles begin to decline prior to conception. In the South African *Miniopterus minor* (Bernard and Cumming, 1997), sperm are first present in the testes in early autumn (March) and remain in the cauda epididymidis until early winter (July). In Kenya, McWilliam (1988b) found that *M. minor* underwent testicular recrudescence, accompanied by hypertrophy of the accessory sex glands, during the 'long rains' (April–June) (= autumn). All reproductive organs involuted following mating in July (early winter) and remained inactive until the following April (autumn).

A departure from the dominant monoestrous vespertilionid pattern is expressed in many tropical and subtropical species where bimodal polyoestry and/or continuous reproduction are recognized. A number of species are bimodally polyoestrous in one area of their range and continuously breeding elsewhere, e.g. *Myotis adversus* (Dwyer, 1970a, b) and *Eptesicus pumilus* (Maddock and McLeod, 1976), probably in response to ecological cues (Dwyer, 1970a). Male *M. adversus* show two peaks of reproductive activity in areas where females produce two litters a year, whereas in areas where three litters are born (September, December and March), the epididymides remain enlarged from June to September and from December to March. *Eptesicus pumilus* is thought to have three reproductive periods but continuous male fecundity has not yet been confirmed (Maddock and McLeod, 1974, 1976).

Details of the male reproductive cycle in other vespertilionids that have been reported as polyoestrous are fragmentary. These include *Glauconycteris variegata* (Kingdon, 1974) and *Scotophilus nigrita* (Smithers, 1971). Male *Mimetillus moloneyi* is reported (Kingdon, 1974) to have enlarged testes in January and June, and females parturate in February/March and in August. Myers (1977) reported on the reproduction of four New World southern hemisphere, non-hibernating vespertilionids in Paraguay (22°–25°S). Three of these, *Myotis albescens, Eptesicus furinalis* and *M. nigricans* are bimodally polyoestrous with a possible third cycle occurring in *M. albescens*. Male *E. furinalis* have two periods of testicular recrudescence and accessory gland hypertrophy a year, and the period of male libido may be extended by epididymal sperm storage. Though not marked, seasonal changes in Leydig cell size occur. Their nuclei are largest during the two periods of copulation (March–June and September–October). Male *M. albescens* appear to behave somewhat differently, having an extended period of spermatogenesis and accessory gland hypertrophy (autumn to spring) that could well be permissive to two or more well defined breeding periods (May and October and possibly January). *Myotis nigricans* is aseasonally polyoestrous in Paraguay and in Panama and Mexico (Wilson and Findley, 1970, 1971). Either testicular and epididymal spermatozoa are present throughout the year (except in the dry season, September–November, in Panama when females do not reproduce) or male fecundity is extended by epididymal sperm storage in the wake of the cessation of spermatogenesis (Mexico). Of interest is the fact that the epididymides of Paraguayan and Panamanian populations were not as packed with sperm as were those of *E. furinalis, M. albescens* or *L. ega*. It is possible that this reflects constant turnover (ejaculation) of spermatozoa. Notable in this species is the small size of the testes relative to body weight. Testicular interstitial cells showed little seasonal change, and small changes in accessory gland size likely reflect little variation in circulating androgens.

The widespread, tropical, polyoestrous species *Pipistrellus mimus* shows plasticity in the timing of its reproductive cycle, breeding throughout the year at Mahareshtra, India (19°N: Gopalakrishna *et al.*, 1975) and producing three litters annually at Varanasi, India (25°N: Krishna, 1985). At Varanasi, adult males have three peaks of testicular weight and spermatogenesis, between which the testes involute, although the epididymides remain full of viable spermatozoa. Changes in the weight of the accessory glands parallel the gametic activity of the testes.

There are a variety of secondary sexual morphological characteristics that serve in male recognition and sexual function, e.g. seasonally enlarged anal glands (*Vespertilio murinus*) and facial glands (*Plecotus auritus*) that are thought to be utilized in the marking of potential roosting sites (Horacek, 1975). Strongly odoriferous urine coincides with peak testicular function in reproductively active male *Miniopterus minor* (McWilliam, 1988b, 1990) and is thought to produce an 'odor plume' which acts as an 'olfactory lek' for the recruitment of oestrous females. Behavioral factors that include vocal and flight displays (often accompanied by songs) may be employed by breeding males to attract and retain receptive females during courtship and/or male territorial defense. Such behavior enhances dominant male or male groups enjoy primary access to harem or other free-foraging females (Gaisler *et al.*, 1979). Harem behavior has been reported for a number of tropical and temperate vespertilionids (e.g. *Myotis adversus*: Dwyer, 1970a; *M. myotis*: Gaisler, 1979; *M. blythi*: Horacek and Gaisler, 1986; *Pipistrellus nanus*: O'Shea, 1980) (see Chapters 8 and 9).

Male copulatory behavior has been described for a number of vespertilionids, e.g. *Plecotus* (= *Corynorhinus*) *townsendii* (Nader and Hoffmeister, 1983), *C. rafinesquei* (Pearson *et al.*, 1952), *Antrozous pallidus* (Orr, 1954), *Myotis lucifugus* (Miller, 1939; Wimsatt, 1945; Findley and Jones, 1967; Racey *et al.*, 1987), *M. grisescens* (Miller, 1939), *M. sodalis* (Hall, 1962), *M. nigricans* (Wilson and Findley, 1970; LaVal, 1973), *M. myotis* (Gaisler, 1979). In general, mating most often seems random and promiscuous with the males seeking out the females. However, Racey *et al.* (1987) reported uninseminated *Myotis lucifugus* females attempting to elicit copulation from torpid males by positioning themselves against the males and uttering copulation calls.

4.6.16 Mystacinidae

This family of two species occurs only in New Zealand (Koopman and Jones, 1970; Hill and Smith, 1984; Nowak, 1994). *Mystacina robusta* is thought to be extinct and *M. tuberculata* endangered.

The few data that exist for this family suggest that *M. tuberculata* is monoestrous; mating in autumn is followed by a delay in fertilization, implantation or development during winter torpor. Gestation begins or continues in spring, and a single young is born in summer (Daniel, 1979). The greater short-tailed bat (*M. robusta*) may have been polyoestrous with parturitions occurring twice a year in spring and autumn (Daniel, 1979). Regretfully, there is no information on the reproductive cycle or on secondary sexual characteristics of the male of either species. However, male courtship behavior has been described for *M. tuberculata* (Daniel and Pierson, 1987). This taxon appears to have a lek mating system. Four to eight males were observed to occupy clustered tree hollows in autumn. Males took up their stations at the mouths of small tree hollows at dusk, advertising their presence with intense warbling calls. Such occupied trees were vigorously defended against other males. Females made brief visits for copulation.

4.6.17 Molossidae

The free-tailed bats occur in the Old World, from southern Europe and Asia through Africa (except the Sahara), Malaysia, Indonesia, Australia and the Fiji Islands, and in the New World, from southern and western United States, through the West Indies, Mexico and Central and South America (Koopman and Jones, 1970; Nowak, 1994). Interestingly, smaller members of this group stand apart from the remainder of the Microchiroptera in escaping the restraints that affect reproduction in most species. This may be facilitated by their shorter gestation lengths and greater foraging flexibility (Bernard and Cumming, 1997). Some species migrate rather than persist and hibernate during winter. The great ecological diversity represented by their geographic distribution has resulted in the evolution of a number of reproductive strategies. Molossids are either monoestrous or polyoestrous.

New and Old World temperate-dwelling molossids are primarily seasonally monoestrous with male libido and female oestrus occurring concurrently. These include *Tadarida brasiliensis mexicana* (Krutzsch, 1955a, 1959a; Short, 1961; Davis *et al.*, 1962), *T. b. cynocephala* (Sherman, 1937), *Eumops perotis californicus* (Krutzsch, 1955b; Cockrum, 1960), *E. underwoodi* (Barbour and Davis, 1969), *Nyctinomops* (= *Tadarida*) *femorosaccus* (Cockrum, 1955; Kumirai and Jones, 1990), and *N.* (= *Tadarida*) *macrotis* (Barbour and Davis, 1969; Milner *et al.*, 1990). The male reproductive cycle is characterized by late winter–early spring testicular recrudescence, spermatogenesis and androgenesis that, in turn, initiate accessory sex gland hypertrophy and secretory activity. Oestrous females are inseminated in the spring, fertilization follows immediately and birth occurs in early summer. Following the breeding season, the male tract involutes to the resting state. With the exception of *Molossus fortis* (Krutzsch and Crichton, 1990b) and *Mormopterus planiceps* (Krutzsch and Crichton, 1987), there is no recorded storage of sperm in the cauda epididymidis of molossids. The male cycle of *M. planiceps* includes a protracted gametic cycle from spring to autumn when mature sperm are available and copulation occurs. The cycle includes subsequent cauda epididymidal sperm storage and extended accessory gland function which continue beyond the gametic phase, suggesting extended fecundity and possible inseminations from autumn to spring (Krutzsch and Crichton, 1987).

A few subtropical and tropical molossids are also monoestrous. Those taxa for which there is information exhibit classical mammalian male–female synchrony of reproductive cycles. Examples are *Otomops martiensseni* (Mutere, 1973; Kayanja and Mutere, 1978), which demonstrate peak testicular weight and spermatogenesis concurrently with oestrus and pregnancy, and *O. wroughtoni* (Brosset, 1962c) and *T. aegyptiaca*, whose synchronized male and female reproductive cycles culminate in a short breeding period in late June (Kashyap, 1980) in India. *Tadarida aegyptiaca* is also monoestrous in South Africa (33 °S), where testicular sperm are present in June and breeding occurs in August (winter), following which the accessory sex gland complex involutes (Bernard and Tsita, 1995). Another monoestrous, tropical molossid, *Neoplatymops (= Molossops) mattogrossensis*, displays complete male–female reproductive synchrony (Willig, 1985a). In a population from the Caatingas of northwest Brazil, males were reproductively active (based on testis size) from May to August with parturition ending in

December. *Molossus sinaloae* has been reported to be monoestrous in the Yucatan, Mexico (Heideman *et al.*, 1990) but polyoestrous in Costa Rica (LaVal and Fitch, 1977); there is no information on the male cycle.

New and Old World tropical and subtropical taxa are primarily polyoestrous, varying in the number of breeding cycles undergone annually. These include *Tadarida (= Mops) condylurus* (Braestrup, 1933; Mutere, 1973; Kingdon, 1974; O'Shea and Vaughan, 1980; Happold and Happold, 1989; Vivier and van der Merwe, 1996), *T. limbatus* and *T. websteri* (Braestrup, 1933), *Tadarida (= Mops) nanulus* (Kingdon, 1974), *Tadarida (= Mops) midas* (Nowak, 1994), *Tadarida (= Mops) longica* (Nowak, 1994), *Molossus ater* (LaVal and Fitch, 1977; Marques, 1986; Rasweiler, 1987, 1992; Nowak, 1994), *M. fortis (= M. molossus)* (Krutzsch and Crichton, 1985, 1990b; Graham, 1989), *M. pretiosus* (Jones *et al.*, 1971; LaVal and Fitch, 1977; Dolan and Carter, 1979), *M. sinalaoe* (LaVal and Fitch, 1977), *M. bondae* (Gardner *et al.*, 1970; LaVal and Fitch, 1977; Dolan and Carter, 1979), *Promops centralis* (Graham, 1989), and *Eumops glaucinus* (Gardner *et al.*, 1970; Silva Taboada, 1979; Best *et al.*, 1997).

Other species are remarkably fecund, undergoing three oestrous cycles per year. The best known of the virtually continuously breeding molossids are *Chaerephon (= Tadarida) hindei* from equatorial Uganda (Marshall and Corbet, 1959) and the widespread African species *Chaerephon (= Tadarida) pumila*. The latter exhibits variation in the number of annual oestrous cycles in various areas of its extensive geographic range, from two (Nigeria: Harrison, 1958b; Botswana: Smithers, 1971; Kenya and Sudan: Kingdon, 1974) to three or more (Somalia: Harrison, 1958b; Happold and Happold, 1989; Ghana: McWilliam, 1988a; Uganda: Marshall and Corbet, 1959; Mutere, 1973; Zambia: Ansell, 1986; South Africa: van der Merwe, 1987; van der Merwe *et al.*, 1987). For most of these species, data concerning the male reproductive cycle are fragmentary and primarily observational. McWilliam (1987a, c) noted that in Ghana, Africa, male *T. (= Chaerephon) pumila* were spermatogenic throughout the year. Reporting on the same species in Uganda, Mutere (1973) concluded that the testes showed no significant seasonal variation in weight; nor did the testes of *T. (= Mops) condylurus*, regardless of their position (intra-abdominal or scrotal). For *T. (= Chaerephon) hindei*, Marshall and Corbet (1959) recorded that spermatogenesis was not continuous in all males and that in some cases, where both testes were intra-abdominal, spermatogenesis had ceased entirely, although the epididymides contained spermatozoa all year. Their findings clearly suggest that even though the male population may not be constantly spermatogenic, a store of epididymal spermatozoa provides for successful inseminations. In *M. fortis*, Krutzsch and Crichton (1990b) found little annual variation in testis or accessory gland size, and sperm were present in the male tract in all months except in specimens obtained in October and December. Furthermore, these individuals may have been subadults since an adult male from November had scattered spermatozoa in the testes, epididymides and ductus deferens. Males of this species may well be continuously fecund to serve the bimodally polyoestrous females (Krutzsch and Crichton, 1985). In captive female *M. ater*, Rasweiler (1992) noted the presence of sperm in vaginal smears for extended periods following the end of oestrus, indicative of either sperm retention or continuous fecundity of males housed with them.

Male secondary sexual characteristics are variably expressed and serve to define reproductive cyclicity. Pelage characteristics of the male, though rarely distinct from the female, can be uniquely specialized in some breeding males, e.g. special odoriferous hair crests in breeding male *Tadarida (= Chaerephon) chapini* (Allen *et al.*, 1917; Fenton, 1983; Racey, 1988), *T. (= Chaerephon) pumila* (Braestrup, 1933; McWilliam, 1988a), *T. (= Chaerephon) hindei* and *T. (= Chaerephon) limbatus* (Braestrup, 1933). Also present in many species is a sexually dimorphic gular gland which is well marked in the male but most often small and non-functional in the female. Males exhibit hypertrophy and active secretory function of this sebaceous-sudoriparous gland (Krutzsch and Crichton, 1990b) during the breeding season (Herreid, 1960; Davis *et al.*, 1962; Werner and Lay, 1963; Gutierrez and Aoki, 1973; Mutere, 1973; Dapson *et al.*, 1977; Rasweiler, 1987, 1992; Heideman *et al.*, 1990). Its activity is apparently mediated by androgens as has been indirectly demonstrated by castration experiments (Horst, 1966). The active gland of *M. bondae* produces a greasy, oily, sebaceous, odoriferous exudate in which unsaturated fatty acids are prominent (Dapson *et al.*, 1977). Breeding males have been observed smearing this substance on the dorsal, interscapular surface of females (Heideman *et al.*, 1990; Rasweiler, 1992) and females have been noted to be attracted to the gular gland of the male; the emission of high-pitched vocalizations by the female sometimes accompanied this activity which often resulted in coitus (Rasweiler, 1987, 1992). Other functions suggested for these secretions are the marking of territories (Heideman *et al.*, 1990) or as an aid to the location of roosts (Horst, 1966).

4.7 CONCLUSIONS AND FUTURE DIRECTIONS

There are many gaps in our basic knowledge of the morphology and physiology of the male reproductive system of Chiroptera. Only a few taxa have been examined over an extended period of time (one or more annual cycles) in which seasonal changes in the system have been recorded and related to organ structure and physiological function. A very limited number of species have had seasonal reproductive variations correlated with female reproductive events. Gradually data are being accumulated which document variation in reproductive organ complement in the male reproductive tract and distinguish their morphological and functional seasonal changes. In a few representative species, primarily vespertilionids, rhinolophids and pteropodids, the details of the reproductive process and the controlling mechanisms have been more critically examined and described. The light microscopic morphology of the accessory sex glands remains poorly known, and in virtually none of the Chiroptera has the ultrastructure of the glands been examined and correlated with their secretory cycle. Little is known regarding the chemical composition of the accessory sex gland secretions. Those species that have been studied have primarily been examined histochemically, although citric and sialic acid, and fructose values have been reported following chemical assay in a few species.

Published accounts concerning the specialized male secondary sexual organs and associated behavior indicate that these factors exert a significant role in successful mate attraction and retention. However, little is known about the physiological events mediating their function and cyclicity. Characterization of the controlling mechanisms and associated behavioral patterns would be a productive area for future study.

The functional role, if any, of the os penis remains open for resolution, though it has been suggested that it may play a part in successful coitus. This bony element is morphologically species specific and has been considered of value in chiropteran taxonomy. Interestingly, the os penis is absent from the penes of Emballonuridae, Noctilionidae, Phyllostomidae, Myzopodidae and Mystacinidae, apparently none of which store sperm.

Planned studies directed at describing the annual male reproductive events are needed. Neotropical fruit bats (Phyllostomidae) are particularly noteworthy in this respect. Considerable data exist concerning the female reproductive cycle and annual seasonality; however, other than *Macrotus californicus*, little has been recorded as to the components and function of the male reproductive tract. Such data should reveal the relationship between male and female reproductive events and the impact, if any, that female reproductive events exert on the timing of the male reproductive cycle(s).

A similar paucity of data regarding the male reproductive pattern and tract structure and function exists for the majority of multi-annual reproductive species. Future investigators considering such species must first establish the reproductive tract structure and physiological function and its annual pattern. Following characterization of the male reproductive tract structure and function, controlled laboratory studies to determine the physiological controlling mechanisms of critical events would be ideal.

ACKNOWLEDGMENTS

I am grateful for the advisory remarks of reviewers which vastly improved the final product. I am also particularly indebted to my late colleagues and dear friends who provided critical representative fluid-preserved specimens required for dissection and illustration: Kunwar P. Bhatnagar, Department of Anatomy, University of Louisville School of Medicine; Charles O. Handley, Jr, Division of Mammals, Smithsonian Institution; Barbara Lawrence, Division of Mammals, Museum of Comparative Zoology, Harvard College; Joseph C. Moore, Division of Mammals, Field Museum of Natural History; Andrew Winnington, New Zealand and the late William A. Wimsatt, Department of Zoology, Cornell University.

Special thanks are due to Dr Elizabeth G. Crichton who, through many years, has tolerated my eccentricities and buoyed my continuing enthusiasm for this project.

As always, I am thankful to my wife, Dorothy, who has joined in the effort of doing and re-doing the drafts of the manuscript; without her tenacity and support, it is likely that it would not have reached fruition.

Lastly, thanks are due to the many unnamed people who helped me in ways too numerous to mention.

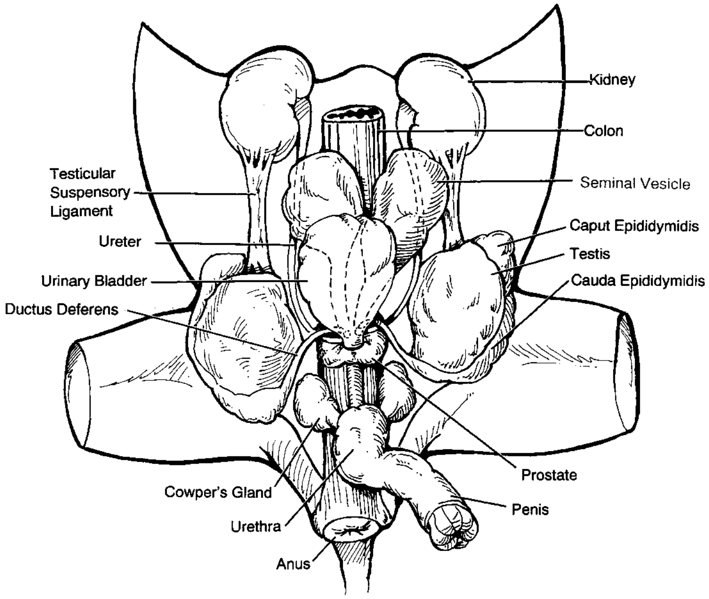

Figure 4.1 Family Pteropodidae: the male reproductive tract of *Rousettus leschenaulti* drawn from an adult specimen obtained at Johuba, India by Kunwar Bhatnagar. (Figures of the representative reproductive tracts were rendered from dissected specimens that were preserved in either 10% ethanol or formalin. In each, the anterior abdominal wall and the pubic symphysis were removed, and the reproductive tract was viewed from the ventral aspect. The hidden parts of the tract are shown by dotted lines. The intent is to provide a three-dimensional visualization of the reproductive tract more or less in situ.)

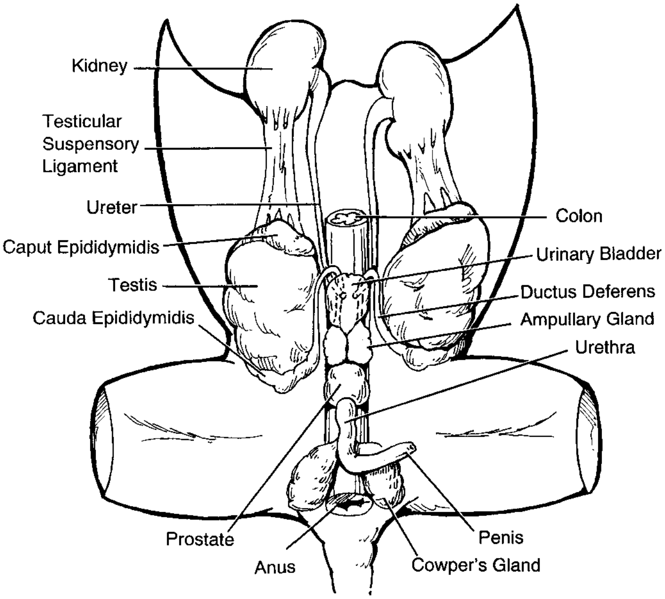

Figure 4.2 Family Rhinopomatidae: the male reproductive tract of a non-breeding male *Rhinopoma kinneari* (= *R. microphyllum*) drawn from a specimen obtained at Guana, India by Kunwar Bhatnagar.

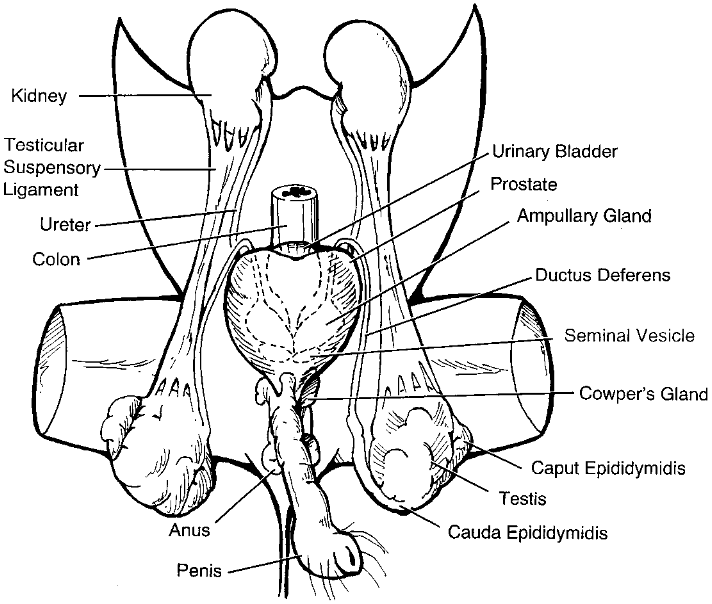

Figure 4.3 Family Emballonuridae: the reproductive tract of an adult male *Rhynchonycteris naso* obtained near Catamaco, Veracruz, Mexico by William A. Wimsatt.

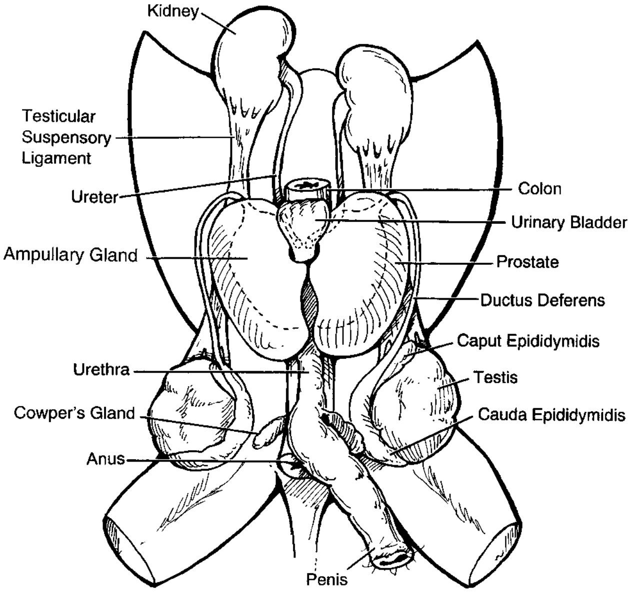

Figure 4.4 Family Noctilionidae: the reproductive tract of an adult male *Noctilio leporinus* obtained 2 km east of Catamaco, Veracruz, Mexico by William A. Wimsatt.

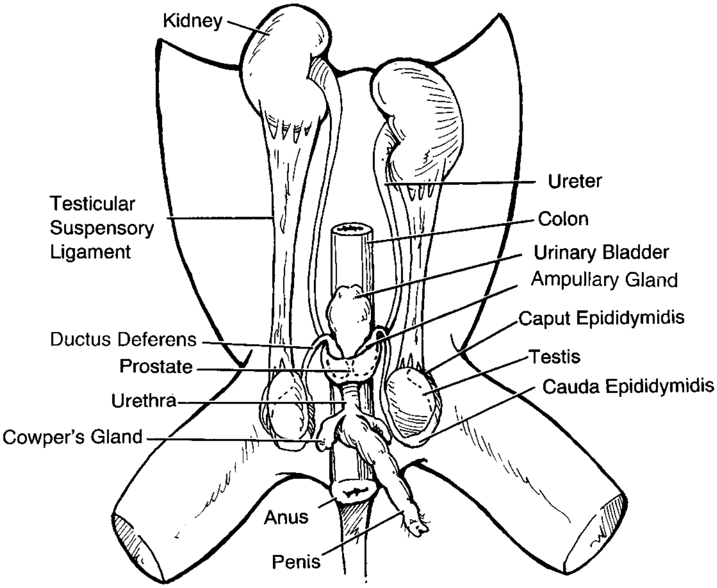

Figure 4.5 Family Nycteridae: the reproductive tract of a non-breeding male *Nycteris thebaica* from Minufiya province, Egypt. Smithsonian Institute, #312331.

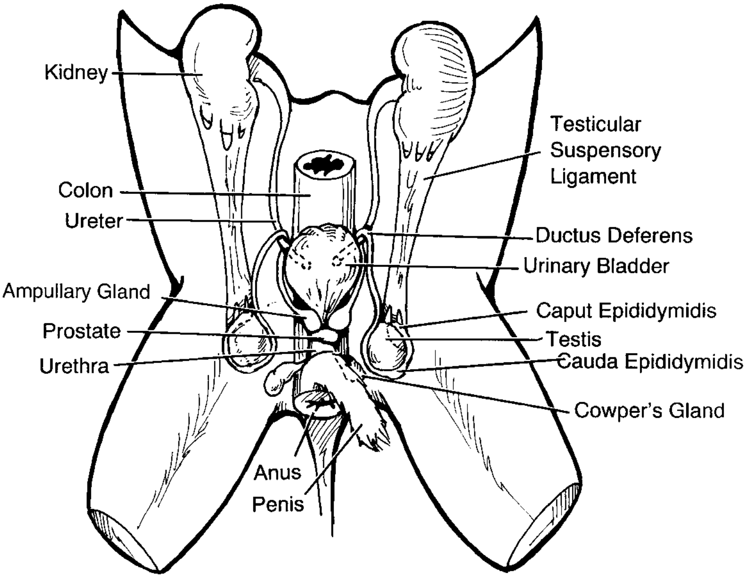

Figure 4.6 Family Megadermatidae: the reproductive tract of a non-breeding male *Megaderma lyra* obtained at Gana, India by Kunwar Bhatnagar.

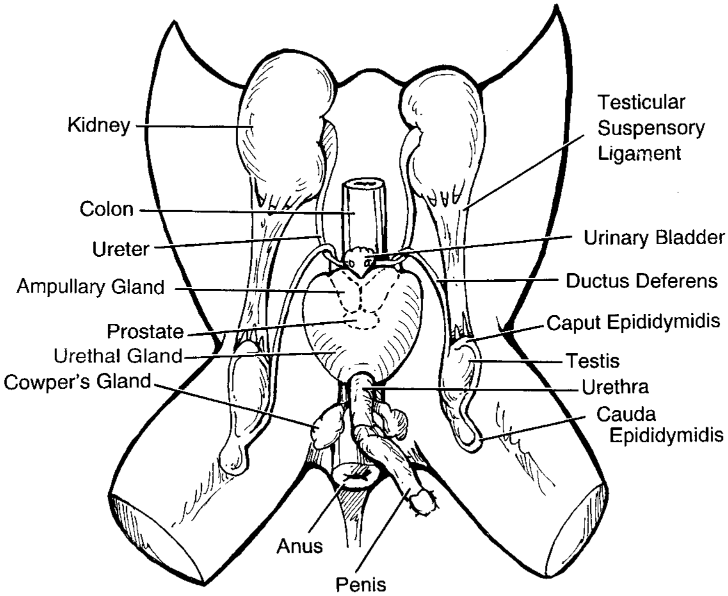

Figure 4.7 Family Rhinolophidae: The reproductive tract of an adult male *Rhinolophus ferrumequinum* from Japan.

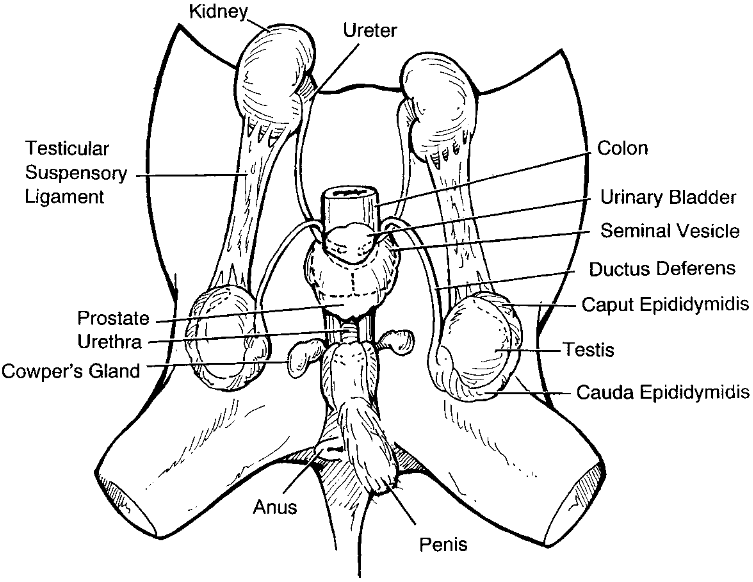

Figure 4.8 Family Phyllostomidae: The reproductive tract of an adult male *Macrotus californicus* obtained near Tucson, Pima County, Arizona, USA.

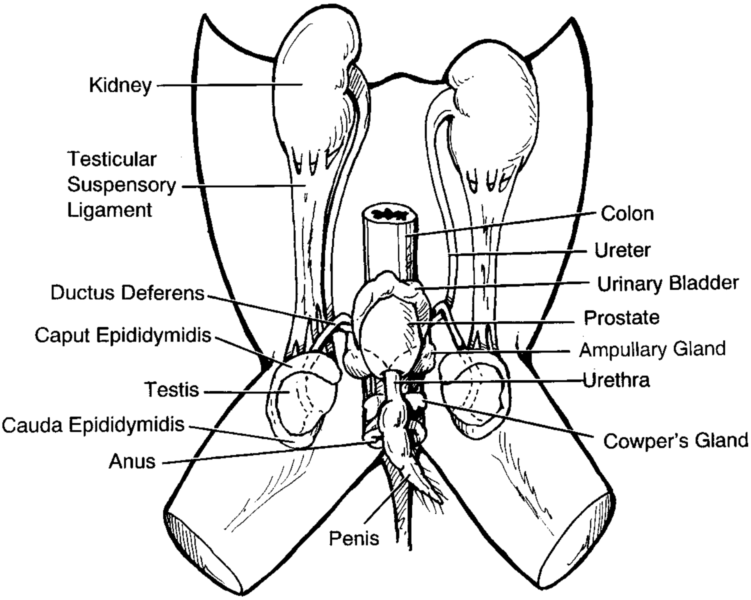

Figure 4.9 Family Natalidae: the reproductive tract of an adult male *Natulus stramineus* obtained near Alamos, Sonora, Mexico by William A. Wimsatt.

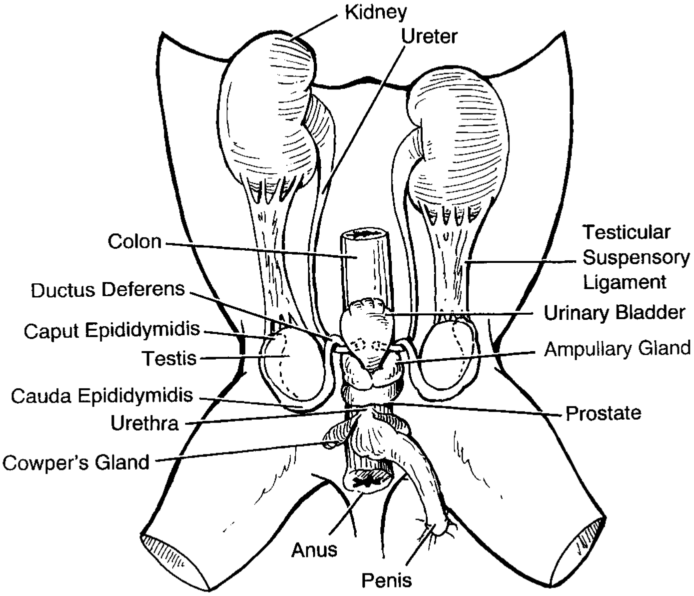

Figure 4.10 Family Furipteridae: the reproductive tract of an adult male *Amorphochilus schnablii* obtained between Chosica and Lima, Peru. Museum of Comparative Zoology, Harvard College, #33187.

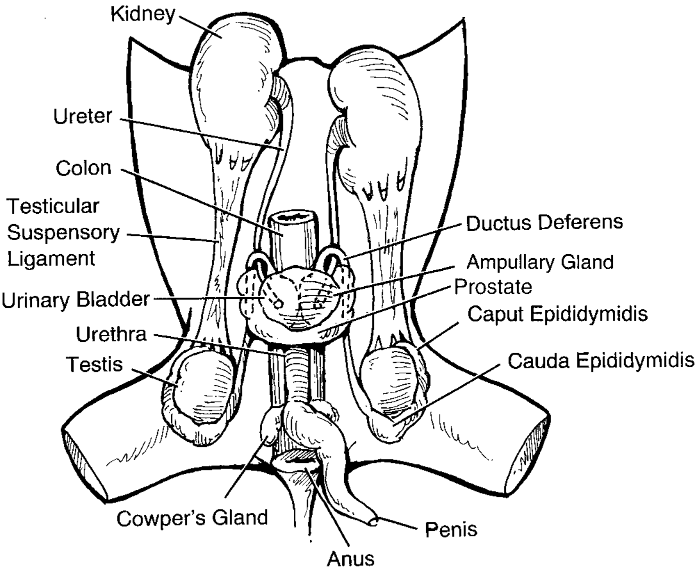

Figure 4.11 Family Thyropteridae: the reproductive tract of an adult male *Thyroptera tricolor* obtained near Sontecomapan, Veracruz, Mexico by William A. Wimsatt.

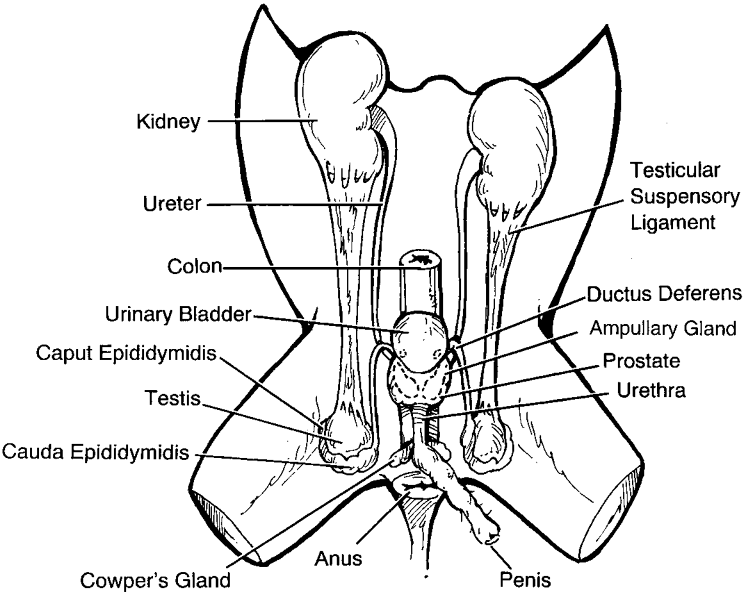

Figure 4.12 Family Myzopodidae: the reproductive tract of an adult male *Myzopoda aurita* obtained 72 km north of Tulcar, Madagascar. Chicago Field Museum of Natural History, #92833.

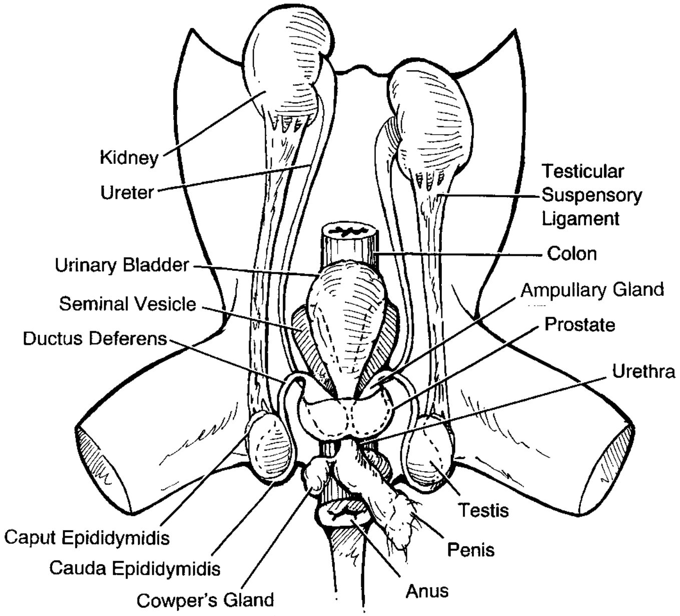

Figure 4.13 Family Vespertilionidae: the reproductive tract of an adult male *Lasiurus borealis* obtained near Pittsburgh, Allegheny County, Pennsylvania, USA.

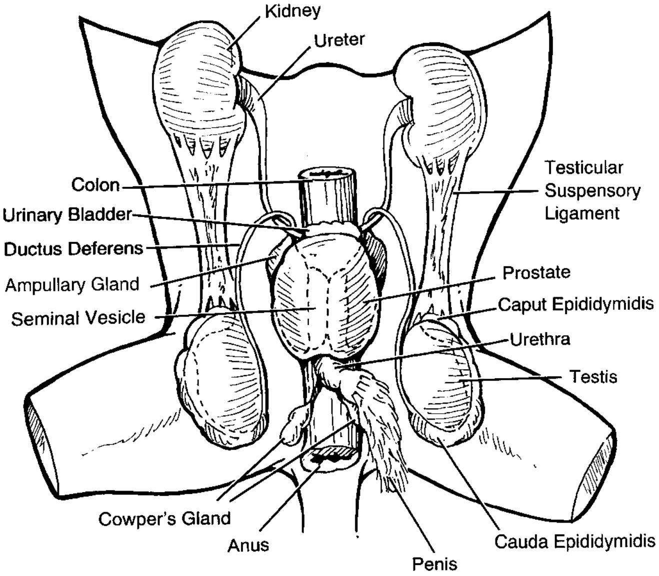

Figure 4.14 Family Molossidae: the reproductive tract of an adult male *Tadarida braziliensis mexicana* obtained at Davis Blow Out Cave, Blanco County, Texas, USA.

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5

Anatomy and Physiology of the Female Reproductive Tract

John J. Rasweiler IV and Nilima K. Badwaik

5.1 INTRODUCTION

By any measure, bats must be considered highly successful mammals. They are second only to the rodents in number of species and are represented on all major land masses with the exception of Antarctica. Particularly in tropical areas of the world, they are extremely abundant and probably exceed the rodents there in absolute numbers. Bats also vary greatly in habits, form and function. The hog-nosed bat, *Craseonycteris thonglongyai*, is one of the smallest living mammals (weight: 1.7 to 2.0 grams; wingspan: 130–145 mm), while the Malayan flying fox, *Pteropus vampyrus* (wingspan: 1400–1500 mm) and the great black flying fox,

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Acerodon jubatus (wingspan: 1510–1600 mm) can reach more than a kilogram. Their ability to fly has enabled bats to utilize a wide variety of roosts, many of which would be quite inaccessible to other mammals. Furthermore, some of these are characterized by extreme environmental conditions, e.g. the near-freezing temperatures of winter hibernacula and the heat of attic roosts in the tropics. Similarly, no order of mammals exhibits a greater diversity of dietary specializations. Among the foods regularly taken by different bats are insects (both volant and nonvolant), scorpions, spiders, flower parts, nectar and pollen, fruits, smaller vertebrates (including rodents, other bats, birds, frogs, lizards and fish) and blood (both mammalian and avian). These have, in turn, required the development of a variety of solutions to the problems of food procurement, nutrition, excretion and the optimal timing of breeding activities.

In view of this remarkable adaptive radiation, it is perhaps not surprising that bats also exhibit considerable diversity in their reproductive characteristics. These are of interest and importance for several reasons. Because many bat species are both small and abundant, it is feasible to collect specimens exhibiting major reproductive states and to conduct thorough comparative investigations. The latter can, in turn, provide insights into how and why particular characteristics may have evolved. This would be much more difficult or impossible with many other mammalian groups (e.g. primates) because of animal cost and/or availability problems. This can provide a perspective on the evolution of mammalian reproduction that one might have trouble in acquiring through comparative studies of unrelated groups (e.g. rodents and primates) or within other common but less diversified orders. A better knowledge of how bats reproduce is important to the proper management or rescue of wild populations increasingly threatened by human activities, and to the successful propagation of captive populations in zoological parks for educational purposes. Finally, bats exhibit reproductive specializations which are sometimes either uniquely similar to those of primates or unusual when compared to those operative in more commonly studied mammals. These include true menstruation and a variety of functional asymmetries of the reproductive tract. The history of biomedical research indicates that we have often learned a great deal from studies of unusual and innovative model systems. As it has now been established that some bats can be maintained, bred and worked with in a controlled, captive environment, representatives of this order may provide useful animal models in reproductive biology.

With the passage of time, the species' names have been changed for some of the bats discussed in this chapter. When this has happened, the nomenclature used by Koopman (1994) has generally been substituted for that incorporated in the original studies.

5.2 ANATOMY OF THE REPRODUCTIVE TRACT

5.2.1 General Organization

As in other eutherian mammals, the female tract in bats consists of two ovaries, two oviducts, the gestational portion of the uterus, the uterine cervix and the vagina.

Within this basic framework, however, there exist many anatomical and functional specializations of the tract. When ovulation consistently occurs from the same ovary, in at least some species that ovary is usually larger, even in the absence of a Graaffian follicle or corpus luteum (CL). Some bats exhibit differential stimulation of the oviducts and/or uterine horns, which is reflected in the relative size of the ducts on either side during the periovulatory period and early pregnancy (prior to implantation). But uteri also vary considerably in anatomical type – being duplex, bicornuate or simplex (Figure 5.1). Finally, bats generally exhibit implantation at localized, predetermined sites, and microscopic studies have established that some of these sites have distinctive morphological features (Badwaik and Rasweiler, this volume).

The characteristics of each uterine type as described generally for mammals by Mossman (1977, 1987) are as follows:

1. *Duplex*. The duplex type (Figure 5.1A) has two separate tubes, often joined externally at the cervical ends, but always opening independently into two cervical canals. These canals usually open separately into the vagina, but in some species may join within the cervical region and open into the vagina via a single external os.
2. *Bicornuate*. The bicornuate type (Figures 5.1B, C) has two cornua (horns), the lumina of which join caudally to form at least a short unpaired uterine corpus (body) that communicates with the vagina via a single cervical canal.
3. *Simplex*. The simplex type (Figure 5.1D) has a single, unpaired corpus externally. Most of the corpus has a single lumen, but small rudiments of the paired lumina of the embryonic cornua persist as endometrial-lined, intramural uterine cornua at the cranial (cephalic) end of the main lumen in some species. The main lumen communicates with the vagina via a single cervical canal.

The anatomical types of uteri observed in representatives of the various chiropteran families are summarized in Table 5.1. It should be cautioned that, for many species, the available information is often based upon the examination of a small number of specimens, the size and mucosal characteristics of the uterine corpus (when it exists) have frequently been imprecisely characterized, and the distribution of a mucus-secreting cervical epithelium has not always been determined through the use of histological stains for carbohydrate moieties. Within a species, uterine anatomy can vary markedly with physiological state and possibly reproductive history. For example, in some phyllostomid bats, implantation normally occurs in one of the intramural uterine cornua interposed between the end of each oviduct and the main uterine cavity (Rasweiler, 1974; Rasweiler and Bonilla, 1992; Rasweiler and Badwaik, 1999a). These segments may be absent or very short in the uteri of menstrual, recently menstrual, recently parturient, and possibly pubertal animals. Another phyllostomid, *Diaemus youngi*, has a uterus with extremely short cornua; however, these were not even evident on some enlarged uteri from recently parturient bats which instead appeared simplex (Badwaik *et al.*, 1998). For reasons which are not yet clear, the length and completeness of the septum which internally divides the uterine corpus of the noctilionid bat *Noctilio albiventris* is quite variable. Possibly this is affected by the parity of the females. Finally, cervical mucus-secreting cells may be difficult to

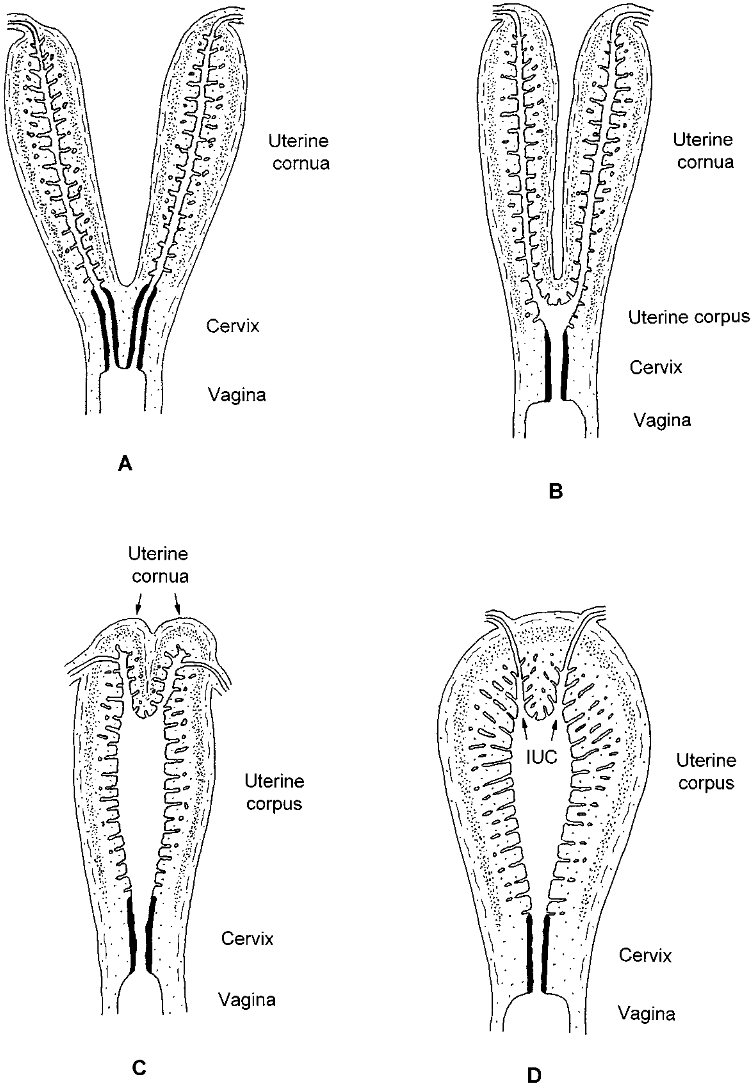

Figure 5.1 Examples of the types of uteri observed in bats. (A) duplex; (B) long bicornuate; (C) short bicornuate; (D) simplex with intramural uterine cornua (IUC) which are usually the site of implantation in some species. Many species with bicornuate uteri possess cornua and corpora intermediate in length between those shown in B and C, and the cornua in most cases are not fused externally to the degree shown in C. All simplex uteri in bats do not possess intramural uterine cornua.

recognize in histological sections stained with routine overview procedures, particularly if they contain reduced numbers of mucigen granules. This sometimes occurs near the junction with the gestational endometrium and may vary with reproductive state.

These considerations are important because, when used correctly, uterine anatomy would appear to have a valuable role to play in assessing phylogenetic relationships. For example, based upon uterine anatomy and foetal membrane characteristics, Wimsatt and Enders (1980) suggested that the family Thyropteridae might bear closer affinities to the superfamily Phyllostomatoidea (now considered to be the Noctilionoidea; Koopman, 1994) than to the Vespertilionoidea where they have generally been classified. We have pointed out elsewhere (Badwaik and Rasweiler, this volume) that some of the foetal membrane and placental similarities between *Thyroptera tricolor* and the phyllostomatid bats that they relied upon in drawing this conclusion are either of uncertain significance or now appear much less phyllostomatoid-like in the light of additional evidence. A further analysis of reproductive anatomy also strongly supports continued placement of the thyropterids in the superfamily Vespertilionoidea. *Thyroptera tricolor* does possess a uterus with unusually short cornua and a long corpus. This is quite different from the bicornuate uteri of many vespertilionid bats, and it does approach the simplex form characteristic of many phyllostomid bats. However, the uteri of two other vespertilionoid bats – *Furipterus horrens* (family Furipteridae) (Figure 5.2) and *Natalus tumidirostris* (family Natalidae) – have now been examined (Table 5.1). The uterus of *T. tricolor* is far more similar to these in both gross and microscopic appearance than to any of the phyllostomid bats (e.g. *G. soricina*, *C. perspicillata*, *P. discolor*, *D. rotundus*, *Diaemus youngi*) studied by the authors. Comparative observations on ovarian microanatomy (see below) also strongly support the placement of the Thyropteridae in the superfamily Vespertilionoidea.

Uterine anatomy and other reproductive features also appear to be of some value in assessing the phylogenetic position of the lesser short-tailed bat (*Mystacina tuberculata*; family Mystacinadae) which is native to New Zealand. In recent years, most authorities have assigned the mystacinids to the superfamily Vespertilionoidea (Hill and Smith, 1984; Koopman, 1994; reviewed by Kirsch *et al.*, 1998); however, comparative studies of plasma proteins (Pierson *et al.*, 1986), hyoid morphology (Griffiths, 1997) and DNA structure (Kirsch *et al.*, 1998) all indicate that *M. tuberculata* is more closely allied to the neotropical superfamily Noctilionoidea. This seems to be supported by our histological studies of two reproductive tracts obtained from mature female *M. tuberculata* (the left ovary in each tract contained a CL). These were removed by the senior author from museum specimens preserved by Mike J. Daniel of the New Zealand Department of Scientific and Industrial Research. The uterine anatomy of *M. tuberculata* is quite similar to that of *Noctilio albiventris*. Both species have long bicornuate uteri in which there is considerable external fusion of the cornua. Internally, however, separate lumina extend along much of the length of the uterus, and the common corpus, lined by a gestational type endometrium, is short (although variable in length in *N. albiventris*). One of the *M. tuberculata* also carried a zona pellucida-enclosed, oviductal blastocyst. Among bats, embryonic development to this stage in the oviduct has only been observed in *N. albiventris*, several phyllostomids, *Peropteryx kappleri* (an emballonurid) and *Rousettus* sp. (pteropodids), while the embryo enters the uterus at significantly earlier stages in all of the vespertilionoid bats in which this characteristic has been examined. Unfortunately, we do not know whether the blastocyst of *M. tuberculata* loses its zona pellucida in the oviduct or the uterus. Among microchiropterans, oviductal loss of the zona has only been observed, thus far, in the noctilionid and phyllostomid bats (Badwaik

Table 5.1 Types of uteri in selected species of bats

Family and species	Uterine type	Notes	References
Family Pteropodidae			
Balionycteris maculata, *Cynopterus* sp., *Dobsonia* sp., *Eonycteris spelaea, Epomops franqueti, Hypsignathus monstrosus*, *Macroglossus* sp., *Megaloglossus woermanni, Melonycteris melanops*, *Micropteropus pusillus, Notopteris macdonaldi, Nyctimene* sp., *Pteropus* sp., *Rousettus amplexicaudatus*	Duplex		Hood, 1989
Family Emballonuridae			
Coleura afra	Long bicornuate		Matthews, 1941
Peropteryx kappleri	Long bicornuate		Rasweiler, 1982
Taphozous melanopogon, *Taphozous longimanus*	Duplex		Robin, 1881; Gopalakrishna *et al.*, 1979; Hood and Smith, 1983
Family Furipteridae			
Furipterus horrens	Short (partially) bicornuate	Cornua are short and fused externally, while corpus is long and endometrial-lined; smooth muscle is present in septum between two cornua; uterotubal junction is on lateral side of each cornu; cervix is lined by a mucus-secreting columnar epithelium	Rasweiler and Badwaik, unpublished observations
Family Megadermatidae			
Megaderma sp.	Probably bicornuate		Robin, 1881; Gopalakrishna, 1950
Family Molossidae			
Chaerephon plicata	Bicornuate	Uterine cornua open into a short corpus lined by endometrium	Hood and Smith, 1983
Molossus ater	Bicornuate	Uterine cornua open separately into a single cervical canal lined by a mixture of ciliated and mucus-secreting columnar epithelial cells	Rasweiler, 1990

Mormopterus planiceps	Duplex	Vagina and uterine corpus is lined by a stratified squamous epithelium; only the uterine cornua are lined by endometrium	Crichton and Krutzsch, 1987
Tadarida brasiliensis	Bicornuate	Similar to *M. ater* but the cervix is also apparently lined by a stratified squamous epithelium	Stephens, 1962
Family Mormoopidae			
Pteronotus parnellii	Short (partially) bicornuate	Cornua are relatively short, while corpus is large and endometrial-lined	Hood and Smith, 1983
Family Mystacinidae			
Mystacina tuberculata	Long bicornuate	Cornua are fused externally over most of their length but have separate lumina; short corpus is lined by gestational endometrium; myometrial smooth muscle is present in the wall of each cornua where fused	J.J. Rasweiler, N.K. Badwaik and M.J. Daniel, unpublished observations
Family Natalidae			
Natalus tumidirostris	Short (partially) bicornuate	Cornua are short and lateral, while corpus is large and endometrial-lined	L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations
Family Noctilionidae			
Noctilio albiventris	Bicornuate	Cornua are relatively short, while corpus is long and endometrial-lined; a mid-sagittal septum, sometimes split into dorsal and ventral septa, extend(s) along a portion to nearly all of the length of the corpus; smooth muscle is present in these septa. Prominent endometrial ridge which is the site of implantation runs longitudinally along the lateral and antimesometrial side of each cornua	Anderson and Wimsatt, 1963; Rasweiler, 1979a, unpublished observations; Hood and Smith, 1983

Table 5.1 (continued)

Family and species	Uterine type	Notes	References
Family Nycteridae			
Nycteris hispida, Nycteris macrotis	Duplex	Parallel cervical canals open separately into the vagina	Matthews, 1941
Family Phyllostomidae			
Desmodus rotundus, Diaemus youngi, Macrotus californicus, Micronycteris sp., *Trachops cirrhosus*	Short (partially) bicornuate	Cornua are extremely short in the species other than *Desmodus*; in some recently postparturient *Diaemus* the uterus appears simplex	Wimsatt, 1954; Hood and Smith, 1983; Badwaik *et al.*, 1998
Anoura geoffroyi, Artibeus jamaicensis, Carollia perspicillata, Centurio senex, Glossophaga soricina, Leptonycteris curasoae, Lonchophylla robusta, Phylloderma stenops, Phyllonycteris aphylla, Phyllostomus sp., *Sturnira lilium, Uroderma bilobatum, Vampyrops helleri*	Simplex externally	In some of these species (e.g. *G. soricina* and *C. perspicillata*), short endometrial-lined segments (intramural uterine cornua) are interposed between the end of the oviducts and the main uterine cavity; in the event of pregnancy, these are usually the site of initial blastocyst attachment Intramural uterine cornua are absent from the uteri of bats belonging to the subfamily Stenoderminae (e.g. *A. jamaicensis, C. senex, S. lilium, U. bilobatum* and *V. helleri*); when initial blastocyst attachment has been observed, it was at the extreme fundic end of the uterine lumen	Rasweiler, 1974, 1979a; Hood and Smith, 1983; Rasweiler and Badwaik, 1999a
Family Rhinolophidae			
Hipposideros caffer	Duplex	Uterine corpus and caudal ends of cornua are lined by a stratified squamous epithelium; uterine glands are present in the mucosa at the caudal ends of the cornua	Bernard and Meester, 1982
Hipposideros fulvus	Duplex	Similar but the mucosal structure in the caudal half of the cornua is similar to that of the vagina	Karim, 1973a

Hipposideros speoris	Bicornuate	Cornua join a corpus lined by a similar, columnar epithelium; this gradually changes distally into a stratified squamous epithelium	Jeevaji, 1979; Gopalakrishna and Bhatia, 1983
Rhinolophus sp.	Bicornuate	Size of the corpus lined by an endometrial mucosa is often unclear	Caffier and Kolbow, 1934; Matthews, 1937; Gopalakrishna and Rao, 1977; Krutzsch *et al.*, 1992
Triaenops persicus	Long bicornuate		Matthews, 1941
Family Rhinopomatidae			
Rhinopoma hardwickei	Bicornuate		Karim and Fazil, 1987
Family Thyropteridae			
Thyroptera tricolor	Short (partially) bicornuate	Cornua are short and partially fused, while corpus is long and endometrial-lined; smooth muscle is present in septum between two cornua; cornua curve laterally, so that uterotubal junction is on lateral side of each cornu; cranial cervix is lined by a columnar epithelium with some mucus-secreting cells	Wimsatt and Enders, 1980; J.J. Rasweiler and N.K. Badwaik, unpublished observations
Family Vespertilionidae			
Barbastella barbastellus, *Chalinolobus* sp., *Eptesicus regulus*, *Miniopterus* sp., *Myotis* sp., *Pipistrellus* sp., *Plecotus townsendii, Scotophilus kuhli*, *Vespertilio murinus*[a]	Bicornuate	Size of the corpus lined by an endometrial mucosa is often unclear	Duval, 1895; Nolf, 1896; Caffier and Kolbow, 1934; Guthrie, 1933; Reeder, 1939; Wimsatt, 1944; Matthews, 1941; Gopalakrishna, 1949; Pearson *et al.*, 1952; Gopalakrishna *et al.*, 1975; Karim, 1975; Kitchener, 1975; Krutzsch, 1975; Richardson, 1977; Kitchener and Halse, 1978; Kitchener and Coster, 1981; Hood and Smith, 1983; Gopalakrishna *et al.*, 1985

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[a] In some cases the bats referred to as *V. murinus* by earlier investigators may in fact have been *Myotis myotis* (discussed briefly by Allen, 1939).

Figure 5.2 Frontal sections of the reproductive tract from a recently parturient *Furipterus horrens*. (A) In this section, the arrowhead indicates the junction between one of the short uterine cornua at the top and the long uterine corpus (C). (B) This section depicts the same uterine cornu, the distal end of the other cornu (*), the septum (S) between the fused portions of the cornua, and the lateral position of the uterotubal junction (UTJ). Ov = right ovary. O = right oviduct. Haematoxylin and eosin. Bars = 200 µm.

and Rasweiler, this volume). Finally, the ovarian microanatomy of *M. tuberculata* is more like that of the noctilionoid than the vespertilionoid bats (see below).

5.2.2 Microscopic Anatomy of the Ovaries

Bats exhibit a variety of ovarian characteristics that are unusual and/or may be useful in assessing phylogenetic relationships.

Interstitial gland tissue

The type and relative abundance of interstitial gland tissue (Mossman and Duke, 1973) varies between bats and seems to be helpful in the latter regard. Many of the vespertilionoid bats, e.g. *Antrozous pallidus*, *Myotis* sp., *Eptesicus* sp., *Miniopterus schreibersii*, *Pipistrellus mimus*, *Plecotus townsendii*, *Tylonycteris* sp. (Wimsatt, 1944; Pearson *et al.*, 1952; Medway, 1972; Myers, 1977; Richardson, 1977; Oxberry, 1979; Krishna, 1985), *Molossus ater* (Figure 5.10) (Rasweiler, 1988), *Scotophilus heathi* (Krishna and Dominic, 1981), *Furipterus horrens*, *Natalus tumidirostris*, and *Thyroptera tricolor* have ovaries that typically possess an abundance of stromal type interstitial gland tissue (Figure 5.3). This differs markedly from the situation in the noctilionoid bats, *Carollia perspicillata*, *Noctilio albiventris* and *Glossophaga soricina*. Interstitial gland tissue is not distinctive in the ovaries of

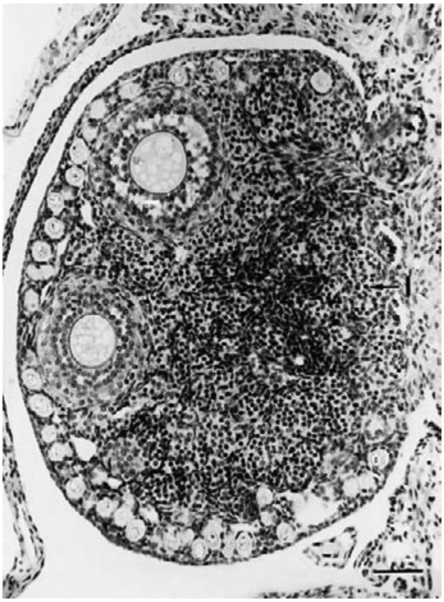

Figure 5.3 Section of the right ovary from a recently parturient *Furipterus horrens*. Abundant interstitial gland tissue (I) is present in both the cortex and medulla. Amylase-PAS-haematoxylin. Bar = 50 μm.

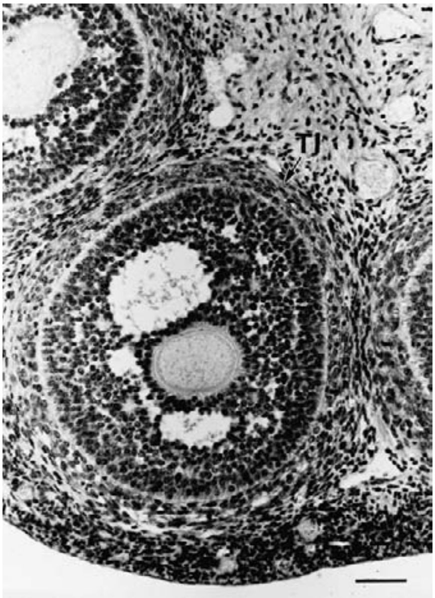

Figure 5.4 Section of ovary from a preovulatory *Carollia perspicillata* obtained on day 1 *postcoitum*. This shows portions of three atretic follicles, each surrounded by a theca interna (TI). The adjacent stroma lacks abundant, epithelioid interstitial gland tissue. Haematoxylin and eosin. Bar = 50 μm.

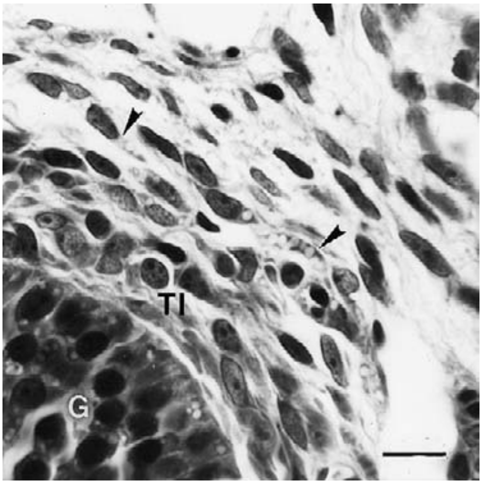

Figure 5.5 Section of the ovary from a *Glossophaga soricina* killed on day 7 of her nonpregnant cycle. This shows a portion of the granulosa (G) of an atretic follicle and its adjacent theca interna (TI). Some interstitial gland cells which are more highly vacuolated (e.g. at arrowheads) are evident on the periphery of the theca, but the remainder of the stroma is composed of typical fibroblasts. Masson's trichrome stain. Bar = 10 μm.

periovulatory or early pregnant *C. perspicillata* (Figure 5.4) and *N. albiventris*. In *G. soricina*, a small amount of interstitial gland tissue is sometimes evident immediately adjacent to the thecae internae of atretic follicles (Figure 5.5); however, its abundance is highly variable, even in animals examined at about the same stage of their nonpregnant cycle (Rasweiler, 1970). Also, it is found only around atretic follicles or their remnants, and it does not occupy much of the ovarian stroma. This suggests that the interstitial gland tissue of *G. soricina* is either derived from the theca or induced by the theca to differentiate from the adjacent stroma. The type and abundance of interstitial gland tissue in *T. tricolor* simply does not support the suggestion that the thyropterids might be more closely allied to the phyllostomids than the vespertilionoids (Wimsatt and Enders, 1980).

In the New Zealand short-tailed bat, *M. tuberculata*, interstitial gland tissue was abundant in the left ovaries of the two adult females. Each of these ovaries also contained a well-developed CL. The interstitial gland tissue usually surrounded atretric follicles or contained their remnants and was not abundant in the ovarian medulla. It was also absent from the right ovaries, which were smaller and exhibited only the early stages of development of a small number of follicles. These observations suggest that the interstitial gland tissue in this species may be of thecal, rather than stromal, origin. In contrast, in the vespertilionoid bat *M. ater*, the interstitial gland tissue is of the stromal type and packs much of the cortex and medulla in both the functional (right) and smaller, nonfunctional (left) ovaries (Rasweiler, 1988). This characteristic would seem to provide further evidence that the mystacinids may be more closely related to the noctilionoid bats than to the vespertilionoids.

Graafian follicles of hibernation

Female vespertilionid bats inhabiting the temperate latitudes have an annual cycle in which much of the reproductive season overlaps the period that these animals spend in hibernation. During the weeks immediately preceding the entrance into hibernation, significant follicular growth becomes evident in the ovaries and mating activity begins. By the onset of hibernation, the number of growing vesicular follicles is reduced to one or only a few, depending upon the species. The surviving follicle(s) then persist(s) in the ovaries throughout the hibernating period, which can last for many months. At the end of hibernation in the spring, the same follicle(s) undergo(es) preovulatory growth and finally ovulation. The female tract remains in an oestrous state during hibernation, and this is also conducive to the prolonged storage of spermatozoa. Although additional copulations may occur in the wild, the experimental isolation of inseminated females from males has established that some of the stored spermatozoa retain the ability to successfully fertilize ova (Wimsatt and Parks, 1966; Wimsatt, 1969; Racey, 1979).

The Graafian follicles that are present in the ovaries of these bats during hibernation typically have a small antrum, a large multilayered cumulus, and exhibit marked vacuolation of the cumulus cells in sections stained with routine histological procedures. Histochemical and/or ultrastructural studies have established that this vacuolation is attributable to the presence of large glycogen deposits in these cells in *Antrozous pallidus, Eptesicus fuscus, Murina leucogaster, Myotis* sp., *Pipistrellus* sp., and *Plecotus rafinesquei* (Nakano, 1928; Wimsatt, 1944, 1960; Wimsatt and Kallen, 1957; Wimsatt and Parks, 1966; Oxberry, 1979; Son *et al.*, 1987, 1988; Mōri *et al.*, 1989). Wimsatt and Kallen (1957) suggested that this may constitute a reserve to meet the energy requirements of the surviving follicle(s) during the prolonged period of hibernation. This glycogen may also play a role in providing for some of the energy requirements of the oocyte and early embryo after ovulation. Wimsatt and Parks (1966) noted that the glycogen content of the oocyte in *Myotis lucifugus* increases dramatically immediately prior to ovulation, and that the sizeable mass of cumulus cells released into the oviduct with the oocyte still possesses a significant content of glycogen. Exactly how the glycogen in the oocyte and granulosa cells is utilized around this time requires further study.

Although enlargement of the cumulus and the synthesis of large amounts of glycogen by its cells were initially viewed as adaptations contributing to the prolonged survival of follicles during hibernation, a number of investigators have since reported that the Graafian follicles of a variety of nonhibernating, tropical vespertilionids exhibit the same specializations (Medway, 1972; Gopalakrishna *et al.*, 1974a; Kitchener and Halse, 1978; Krishna, 1985). In most cases the observations were purely histological, and one is left with the impression that the degree of vacuolation of the cumulus cells may be less than in many of the hibernating vespertilionids. Krishna and Dominic (1982b) established, by means of histochemical tests, that large amounts of glycogen are present in the Graafian follicles of *Scotophilus heathi*, but even that may be less than what is synthesized by the cumulus cells in the surviving follicles of hibernating vespertilionids.

Published photomicrographs of the latter also suggest that all of the glycogen may not have been preserved and stained histochemically (Wimsatt and Kallen, 1957; Wimsatt, 1960; Wimsatt and Parks, 1966). Finally, while there is no question that such surviving follicles have an unusual appearance, the Graafian follicles of some nonhibernating insectivores exhibit many similarities, e.g. having little or no antrum, a large multilayered cumulus, and pronounced vacuolation of the cumulus (see especially Graafian follicles of the Madagascar hedgehog, *Setifer setosus*, and the star-nosed mole, *Condylura cristata*, in Mossman and Duke, 1973). Further comparative work is required to clarify which ovarian characteristics are truly unique adaptations associated with prolonged hibernation.

The hibernating rhinolophid bats (e.g. *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros*) also exhibit prolonged sperm storage in the female and an extended period of oestrus that overlaps the hibernation period. Graafian follicles present in the ovaries during hibernation do not resemble the surviving follicles of hibernation in many of the vespertilionids in either cumulus morphology or glycogen content. Furthermore, Wimsatt (1969) noted that while vesicular follicles are present in the ovaries of the European rhinolophids during hibernation (Matthews, 1937; Gaisler, 1965), no one had demonstrated that the follicle destined for ovulation persists throughout that period. Gaisler, in fact, states that formation of the Graafian follicle takes place shortly after the end of hibernation. The situation in Japanese *R. ferrumequinum* is different in that a single, large Graafian follicle is maintained in the right ovary throughout hibernation (Oh *et al.*, 1985). Although many lipid droplets were observed in the oocyte and cumulus cells of such follicles, abundant glycogen was not.

The Graafian follicles of a variety of bats belonging to other families also do not resemble those of many vespertilionids and have, instead, large antra and relatively smaller cumuli oophori (Figure 5.8) (Wimsatt and Trapido, 1952; Gopalakrishna, 1955; Anand Kumar, 1965; Rasweiler, 1972, 1977, 1982, 1988; Bonilla and Rasweiler, 1974; Gopalakrishna *et al.*, 1974a; Quintero and Rasweiler, 1974; Ramakrishna *et al.*, 1981; Crichton and Krutzsch, 1985; Heideman, 1989; Martin *et al.*, 1995; Heideman and Powell, 1998).

Ovarian zonation

In most mammals, as well as many bats, the ovary is divided into two major zones – an outer cortex and an inner medulla. In adults the cortex contains the follicles, CLs (when present), some types of interstitial gland tissue, degenerate forms of these elements and stromal cells. The medulla contains the major intraovarian blood and lymphatic vessels, nerves, medullary cords and their derivatives, interstitial gland tissue, and the ovarian rete. A surface or 'germinal' epithelium envelops the exterior of the cortex.

At least some bats deviate from this general pattern. In the phyllostomid *Glossophaga soricina*, primordial follicles are congregated in a cap-shaped zone on the medial side of the ovary, and the surface epithelium covers only this area (Figure 5.6). When follicles are selected for further development, they are recruited from the primordial follicles along the inner side of this zone. When ovulation occurs, follicular rupture also always appears to occur through this zone based

Figure 5.6 Section of the ovary from a *Glossophaga soricina* killed on day 22 of her nonpregnant cycle. All of the primordial follicles are restricted to a zone on one side of the ovary. Follicles are recruited for development from the inner side of this zone (e.g. at arrowheads). Ovulation always occurs through this zone. This is indicated in this section by tapering of the regressing corpus luteum (CL) to the point (*) at which follicle rupture had presumably occurred. Haematoxylin and eosin. Bar = 125 μm.

upon the distribution of stigmata (Rasweiler, 1972, unpublished observations). This is very similar to the ovulation pit that occurs in armadillos, the cuis (*Galea musteloides*) – a caviid rodent, donkeys, horses and zebras (Mossman and Duke, 1973; Weir and Rowlands, 1974). This anatomical organization of the ovary is of interest because it would seem to provide a novel model system in which to examine the local factors that promote the long-term storage of oocytes, their recruitment into the developing population, and follicular rupture.

Primordial follicles have a similar, restricted distribution in several other phyllostomid bats, *Leptonycteris curasoae* (Hood and Smith, 1983), *Phyllostomus discolor* and *Phyllostomus elongatus* (J.J. Rasweiler, unpublished observations), as well as some of the pteropodids such as *Otopteropus cartilagonodus, Ptenochirus jagori* and *Pteropus* sp. (Heideman *et al.*, 1993; Pow and Martin, 1994, 1995; Heideman and Powell, 1998). This is not the case, however, in *Carollia* sp. or *Desmodus rotundus* (Wimsatt and Trapido, 1952; Bonilla and Rasweiler, 1974; Quintero and Rasweiler, 1974) which are also phyllostomids.

Figure 5.7 Section of the ovary from a *Peropteryx kappleri* showing an everted corpus luteum (CL). This animal carried an oviductal morula. PAS-haematoxylin. Bar = 125 μm.

Everted corpora lutea

Although most bats have the usual, intraovarian form of CL, some species belonging to the superfamilies Emballonuroidea and Rhinolophoidea instead exhibit CLs that are partially or completely everted (Figure 5.7). Among the emballonuroids, *Peropteryx kappleri* (family Emballonuridae), *Rhinopoma hardwickei* and *Rhinopoma microphyllum* (family Rhinopomatidae) have everted CLs (Anand Kumar, 1965; Rasweiler, 1982; Banerjee and Karim, 1985; Gopalakrishna and Badwaik, 1988), while *Taphozous georgianus* and *Taphozous longimanus* do not (Gopalakrishna, 1955; Kitchener, 1973). In *P. kappleri* CLs sometimes form that are not everted (Figure 5.11). Among the rhinolophoids, *Megaderma lyra* (family Megadermatidae), *Nycteris thebaica* (family Nycteridae), several *Rhinolophus* sp. and several *Hipposideros* sp. have partially or completely everted CLs (Matthews, 1937; Gopalakrishna *et al.*, 1970; Ramakrishna, 1976; Ramakrishna *et al.*, 1981; Bernard, 1982; Bernard and Meester, 1982; Gopalakrishna and Badwaik, 1988, 1989; Krutzsch *et al.*, 1992). The degree of eversion probably varies with the functional state of the CL, but this has not been defined well for any species.

5.3 FUNCTIONAL ASYMMETRIES OF THE FEMALE TRACT

5.3.1 Asymmetry Patterns Observed

In an earlier review of this subject, Wimsatt (1979) summarized it well when he stated that 'Bats are noteworthy in that anatomical and/or functional asymmetries of [their] female reproductive organs are far more frequent and profound than recorded for any other mammalian order.' Depending upon the species, these may involve the partial or complete dominance of one ovary, the alternation of successive ovulations, differential stimulation of the oviducts and uterine horns, and/or the dominance of one uterine horn (Table 5.2). It should be cautioned that, for some of the species listed, the sample sizes were small. Also, the data were not always rigorously quantified, and/or subjected to statistical testing.

Brief but interesting observations are now available for two additional species. When the reproductive tracts from two *M. tuberculata* (family Mystacinidae) discussed previously were examined histologically, single CLs (apparently recently formed) were present only in the left ovary. In each case, the right ovary was much smaller, and contained only primordial, primary and a few small secondary follicles without antra. Interstitial gland tissue was also moderately abundant in the left ovary but absent from the right. These observations indicate that, at the least, the responsiveness of the ovaries in *M. tuberculata* is asymmetrical. They also raise the possibility that advanced follicular development and ovulation in this species may be limited to the left side (Rasweiler, Badwaik and Daniel, unpublished observations).

When six pregnant leaf-chinned bats, *Mormoops megalophylla* (family Mormoopidae), were examined, the discoidal chorioallantoic placenta was, in each case, on the left side of the uterus and the CL in the left ovary. On the basis of such a small sample, it is impossible to conclude anything about the functional capabilities of the right side of the tract in this species; however, ovulations and placental development clearly are not restricted to the right side as appears to be the case in another mormoopid bat, *P. parnellii* (Table 5.2). Similar species' differences in the functioning of the left versus the right side of the reproductive tract have been observed in the rhinolophid bats.

5.3.2 Physiological Basis for the Patterns of Asymmetry and the Capability of the Nonfunctional Side

Dominance of one ovary

The factors that are responsible for the functional ovarian asymmetries exhibited by some bats are presently unclear. In the case of the molossid *M. ater* (which normally ovulates only from the right ovary), the smaller left ovary was observed to have a much less prominent intraovarian vascular supply than the right. This difference existed even when large follicles and CLs were absent from the right ovary, and it might limit the ability of follicles to grow in the left ovary (Rasweiler, 1988). Crichton and Krutzsch (1985) speculated that the functional asymmetry occurring in the phyllostomid *M. californicus* might be

Table 5.2 Functional patterns observed in the female reproductive tracts of bats

Pattern observed/ Family and species of bat	Comments	References
Both ovaries capable of functioning but usually only one egg is released; implantation in the ipsilateral uterine cornu		
Family Pteropodidae		
Cynopterus sphinx, Haplonycteris fischeri, Pteropus giganteus, *Rousettus leschenaulti*	Ovulation can occur from either ovary; evidence for alternation of successive ovulations; unilateral endometrial reaction has been observed in some species	Marshall, 1947, 1949, 1953; Ramakrishna Iyer, 1950; Gopalakrishna and Murthy, 1960; Gopalakrishna, 1964, 1969; Gopalakrishna and Karim, 1971; Gopalakrishna and Choudhari, 1977; Gopalakrishna *et al.*, 1979, 1986; Sandhu and Gopalakrishna, 1984; Heideman, 1989; Badwaik and Gopalakrishna, 1990
Family Emballonuridae		
Taphozous longimanus	Ovulation can occur from either ovary; suggested to exhibit alternation of successive ovulations; however, only 50% of females that were simultaneously lactating and pregnant had remnants of an old corpus luteum on the nonpregnant side; unilateral endometrial reaction observed	Gopalakrishna, 1954, 1955; Gopalakrishna and Murthy, 1960; Gopalakrishna *et al.*, 1979; Krishna and Dominic, 1982a; Badwaik and Gopalakrishna, 1990
Family Noctilionidae		
Noctilio albiventris	Ovulation can occur from either ovary; evidence for alternation of successive ovulations; unilateral oviductal and endometrial reactions; unilateral bloating of the bursa surrounding the ovary containing the new corpus luteum	Rasweiler, 1978, 1979a, b; Rasweiler and Badwaik, 1999a, unpublished observations
Family Phyllostomidae		
Desmodus rotundus	Ovulation can occur from either ovary; evidence for alternation of successive ovulations; unilateral oviductal reaction	Wimsatt and Trapido, 1952; Quintero and Rasweiler, 1974; Rasweiler, 1979b
Carollia sp., *Glossophaga soricina*	Ovulation can occur from either ovary; evidence for alternation of successive ovulations (weak effect in *G. soricina*); unilateral oviductal reaction; implantation on side of simplex uterus ipsilateral to corpus luteum	Rasweiler, 1972, 1974; 1979a,b; Bonilla and Rasweiler, 1974; Badwaik and Rasweiler, this volume; Rasweiler and Badwaik, 1999a

Family Rhinopomatidae		
Rhinopoma hardwickei, Rhinopoma microphyllum	Ovulation can occur from either ovary	Karim and Fazil, 1987; Karim and Banerjee, 1989 Anand Kumar, 1965; Ramaswami and Anand Kumar, 1966; Badwaik and Gopalakrishna, 1990
Ovulation can occur from either ovary, implantation can occur in either uterine cornu		
Family Vespertilionidae		
Nycticeius schlieffenii	Ovulations usually occur from both ovaries, but more take place on the right side; species is polytocous; conceptuses are usually present in both cornua, but the right cornu often carries more than the left at least early in pregnancy	van der Merwe and Rautenbach, 1987
Pipistrellus dormeri	35% of bats carried conceptus in left cornu, 30% in right cornu, and 35% in both cornua; corpora lutea in ipsilateral ovaries	Gopalakrishna *et al*., 1979; Badwaik and Gopalakrishna, 1990
Pipistrellus pipistrellus	Ovulation can occur from either or both ovaries; 70% of bats carried conceptus in right cornu, 25% in left cornu, and 5% in both cornua	Deanesly and Warwick, 1939
	In another study, 61% of ovulations were from the right ovary and 39% from the left ovary; 38 implantations occurred in the cornu ipsilateral to the corpora lutea (CL) while 13 occurred in the cornu contralateral to the CL; there was a 25% incidence of migration of the preimplantation embryo from one cornu to the other; 76% of all implantations occurred in the right cornu and 24% in the left	Potts and Racey, 1971
Both ovaries capable of functioning but usually monovular; implantation always in right uterine cornu		
Family Vespertilionidae		
Eptesicus regulus, Myotis formosus *Myotis lucifugus, Plecotus townsendii*	No observations on implantation site for *M. formosus*, but the right uterine cornu in this species was often larger than the left	Guthrie and Jeffers, 1938; Wimsatt, 1944; Pearson *et al*., 1952; Kitchener and Halse, 1978; Buchanan and Younglai, 1986; Son *et al*., 1987

Table 5.2 (continued)

Pattern observed/ Family and species of bat	Comments	References
Both ovaries release eggs during each cycle; usually at least one conceptus is carried in each uterine cornu		
Family Vespertilionidae *Chalinolobus gouldii, Eptesicus fuscus, Myotis austroriparius, Pipistrellus ceylonicus, Pipistrellus hesperus, Pipistrellus mimus, Pipistrellus subflavus, Scotophilus heathi, Scotophilus kuhli, Tylonycteris pachypus, Tylonycteris robustula*	Both ovaries usually function during each cycle, and each uterine cornu usually carries one conceptus; however, in most species, an asymmetrical distribution of corpora lutea and/or conceptuses is sometimes seen; in *E. fuscus* more blastocysts implant in the right cornu than the left, but only one survives in that cornu to term; in some populations of *E. fuscus*, the litter size is usually one; both ovaries are assumed to release eggs in *M. austroriparius* but this requires confirmation; when *M. austroriparius* carries only one conceptus, this is usually in the right cornu	Sherman, 1930; Wimsatt, 1942, 1945; Gopalakrishna, 1947; Medway, 1972; Kunz, 1974; Gopalakrishna *et al.*, 1975, 1979, 1988; Kitchener, 1975; Kitchener and Halse, 1978; Krishna and Dominic, 1981, 1982b; Son *et al.*, 1988; Badwaik and Gopalakrishna, 1990
Partial or complete dominance of the left ovary, implantation site ipsilateral to ovary with new CL		
Family Emballonuridae *Peropteryx kappleri*	Ovulation can occur from either ovary, but recently ruptured follicles or new corpora lutea (CLs) were seen in the left ovary of 43 bats (63%) and the right ovary of 25 bats (37%); alternation of ovulations between the ovaries had occurred in 75% of bats with two successive CL; unilateral oviductal and endometrial reactions; implantation in the uterine cornu ipsilateral to the new CL	Rasweiler, 1982
Family Megadermatidae *Megaderma lyra*	Complete sinistral dominance was observed in one large series; however, occasional corpora lutea and conceptuses were observed on the right side in others; also, two cases have been recorded of females carrying foetuses of different sex and size in the left and right uterine cornua at the same time	Gopalakrishna, 1950; Ramakrishna, 1951; Ramaswamy, 1961; Ramaswami and Anand Kumar, 1963; Gopalakrishna *et al.*, 1974b, 1979; Badwaik and Gopalakrishna, 1990

Family Mormoopidae		
Mormoops megalophylla	In all six pregnant animals examined, ovulation had occurred from the left ovary and the discoidal placenta was on the left side of the partially bicornuate uterus	J.J. Rasweiler and N.K. Badwaik, unpublished observations
Family Natalidae		
Natalus tumidirostris	In a large series of pregnant females, corpora lutea were observed only in the left ovaries	L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations
Family Rhinolophidae		
Hipposideros ater, Hipposideros fulvus	70% of the conceptuses were carried in the left cornu and 30% in the right; corpus luteum was in the ipsilateral ovary	Karim, 1973a, b, 1974; Gopalakrishna *et al.*, 1979; Badwaik and Gopalakrishna, 1990
Hipposideros caffer	Corpora lutea seen only in left ovary and conceptuses only in left uterine cornu	Mutere, 1970; Menzies, 1973; Bernard and Meester, 1982
Hipposideros lankadiva	Most conceptuses (85–98% depending upon study) were carried in the left cornu and the remainder in the right; corpus luteum was in the ipsilateral ovary	Bhiwgade, 1979; Gopalakrishna *et al.*, 1979; Sapkal and Bhandarkar, 1984; Badwaik and Gopalakrishna, 1990
Hipposideros speoris	95% of conceptuses were carried in the left cornu and 5% in the right; distribution of corpora lutea not specified	Gopalakrishna *et al.*, 1979, 1991a; Gopalakrishna and Bhatia, 1983; Badwaik and Gopalakrishna, 1990
Dominance of the right ovary and right uterine cornu		
Family Emballonuridae		
Taphozous melanopogon	Ovulation from right ovary and implantation in right uterine cornu with rare exceptions (see text)	Khaparde, 1976; Gopalakrishna *et al.*, 1979; Sapkal and Khamre, 1984; Badwaik and Gopalakrishna, 1990
Family Molossidae		
Chaerephon plicata, Chaerephon pumila, Molossus ater, Molossus fortis, Tadarida aegyptiaca, Tadarida brasiliensis, Tadarida melanopogon	Ovulation from right ovary and implantation in right uterine cornu; however, in a series of 301 pregnant *T. brasiliensis*, the conceptus was in the left cornu of 1 and the right cornu of 300	Sherman, 1937; Stephens, 1962; Davis *et al.*, 1962; Jerrett, 1979; Krutzsch and Crichton, 1985; van der Merwe *et al.*, 1986; Rasweiler, 1988, 1990; Badwaik and Gopalakrishna, 1990; Gopalakrishna *et al.*, 1991b

Table 5.2 (continued)

Pattern observed/ Family and species of bat	Comments	References
Family Mormoopidae		
Pteronotus parnellii	Conceptus in right uterine cornu in 15/15 females; corpus luteum in right ovary in 7/7 of those females examined histologically	Badwaik and Rasweiler, 1998, unpublished observations
Family Phyllostomidae		
Macrotus californicus	Ovulation only from right ovary and implantation always in right uterine cornu	Bradshaw, 1962; Bleier, 1975; Bleier and Ehteshami, 1981; Crichton and Krutzsch, 1985
Family Rhinolophidae		
Hipposideros bicolor	All corpora lutea in right ovary and conceptuses in right uterine cornu in 24 pregnant females	Gopalakrishna and Moghe, 1960
Rhinolophus ferrumequinum, *Rhinolophus hipposideros,* *Rhinolophus megaphyllus*	Only right ovary is functional and right uterine cornu is usually larger in diameter than the left; however, an embryo was found in the left cornu of one *R. ferrumequinum*	Matthews, 1937; Gaisler, 1965; Almatov, 1968; Oh *et al.*, 1985
Rhinolophus rouxi	The corpus luteum (CL) was in the right ovary and the conceptus was in the right uterine cornu in 284/285 pregnant specimens examined; the other animal had a single CL in each ovary and single embryos at different stages of development in the right and left cornua	Gopalakrishna and Rao, 1977; Gopalakrishna *et al.*, 1991b Gopalakrishna and Ramakrishna, 1977; Gopalakrishna *et al.*, 1979
Dominance of left ovary and implantation in right uterine cornu		
Family Vespertilionidae		
Miniopterus schreibersii	Ovulation almost always occurs from left ovary; embryo then undergoes transuterine migration and implants in the right uterine cornu; in 4/285 pregnant bats examined in one study, ovulation had occurred instead from the right ovary	Matthews, 1941; Richardson, 1977; Gopalakrishna *et al.*, 1981; Badwaik and Gopalakrishna, 1990
Miniopterus australis, *Miniopterus fraterculus*	Ovulation takes place from the left ovary and implantation then occurs in the right uterine cornu	Medway, 1971; Richardson, 1977; Bernard, 1980

attributable to differences in the content of gonadotrophin receptors between the two ovaries. Obviously, important questions also remain as to the nature of the mechanisms that establish the differences in ovarian structure and/or responsiveness in the first place. This seems likely to involve differential gene expression (Levin *et al.*, 1995).

Following experimental removal of the dominant right ovary in *M. californicus*, ovulation occurred from the left ovary (Bleier and Ehteshami, 1981; Crichton and Krutzsch, 1985). A similar response was not observed in *Molossus* sp. after removal of the dominant right ovary (Wimsatt, 1979). The details of the latter experiments were not published, however, and it is unclear if the husbandry procedures utilized were conducive to the occurrence of normal ovulations in control and sham-operated animals. Following removal of the dominant right ovary from another molossid *T. brasiliensis*, Jerrett (1978) noted some increase in weight of the left ovary in control and gonadotrophin-treated animals. No advanced follicular development or ovulations were observed on the left side of these animals, but the experiments were of relatively short duration.

As noted in Table 5.2, several species that exhibit a nearly complete dominance of one ovary will sometimes ovulate from the opposite ovary. The rhinolophid *R. rouxi* normally ovulates from the right ovary and implantation then occurs in the right uterine horn. However, in one exceptional specimen, a new CL and conceptus were present on each side of the reproductive tract (Gopalakrishna and Ramakrishna, 1977). Although the embryo in the right horn was more advanced in development, the authors reported that the CLs were identical in size and histology, thereby indicating that ovulation had probably occurred at about the same time from both ovaries. They went on to suggest that the differences in embryonic development may have been due to inadequate preparation of the left horn which in normal cycles (i.e. with ovulation on the right side) does not exhibit a progestational reaction.

While this is a reasonable hypothesis, it has not been clearly established whether the corpora lutea normally associated with embryos at these stages of development in different mothers are the same or differ significantly in histological appearance. Furthermore, it seems possible that (1) the ovulation on the left side of this animal could have occurred well after that on the right, despite the similarities of the CLs and (2) the CLs may have assumed a similar appearance because, being in the same tract, they were subjected to similar gonadotrophic stimulation. In the phyllostomid bat *C. perspicillata*, the establishment of a pregnancy can result in the rescue of lutein cells in both the CL of pregnancy and, to a variable extent (i.e. not all cells are rescued), the CL of the previous nonpregnant cycle (J.J. Rasweiler and N.K. Badwaik, unpublished observations). Thus, it remains conceivable that the presence of a dominant follicle or CL in the right ovary of this *R. rouxi* might have inhibited further folliculogenesis in the same ovary and driven the subsequent ovulation to the normally nonfunctional left ovary.

Cases apparently similar to that noted in *R. rouxi* have also been observed twice in the megadermatid *M. lyra*, which normally exhibits ovulation of a single egg from the left ovary and the implantation of one blastocyst in the left uterine horn. In each of the exceptional animals, one foetus was borne in the left horn and the other, less developed, in the right horn. As the CLs on both sides had involuted

(Ramaswami and Anand Kumar, 1963; Gopalakrishna *et al.*, 1974b), these could not provide any insights into the possible ages of the embryos.

In the emballonurid *T. melanopogon*, the conceptus was observed in the right uterine horn in 87/89 females and the CL in the ipsilateral ovary. The two other females instead carried their conceptuses in the left uterine horn. In both cases, the females appeared to be out of reproductive synchrony with most other females in the population. One of the females carried a conceptus that was significantly smaller than all of the others obtained on the same collection date. The second carried a near-term foetus, while other females collected on the same date had already delivered their young and had involuted uteri (Sapkal and Khamre, 1984). Presumably these two exceptional females had either ovulated late in the season or, more likely, suffered a reproductive failure (e.g. a failure of fertilization or normal development of the first embryo) and then bred again. If the latter was the case, the prior presence of folliculogenesis or a CL in the right ovary may again have driven subsequent folliculogenesis to the normally nonfunctional left ovary.

Preferential stimulation of the reproductive ducts on one side

A number of bats have now been found to exhibit preferential stimulation of the oviduct and/or uterine horn ipsilateral to the ovary containing the preovulatory follicle and new CL. This is of interest because it raises important questions about the mechanisms by which such local stimulation is accomplished and why such a pattern of stimulation should have evolved. It has generally been believed by reproductive physiologists working with the more commonly studied mammalian species that most steroids leave the ovaries via their venous and, to a lesser extent, lymphatic vessels. These hormones then enter the systemic circulation and should be delivered to the reproductive ducts on either side at equal levels. Where unilateral stimulation occurs, there must be either a local route for the delivery of these hormones and/or differential responsiveness on the part of the ducts on either side.

When local stimulation of the uterus was initially observed in several pteropodid and emballonurid bats, it was suggested that this might take place by way of blood vessels, possibly even of a portal nature, running from the ovary to the ipsilateral uterine horn (Marshall, 1953; Gopalakrishna and Murthy, 1960; Gopalakrishna and Karim, 1971). Convincing evidence for the existence of such a vascular arrangement, that might function efficiently enough to account for the local effects, has never been presented.

When successful methods were developed for breeding the phyllostomids *G. soricina* and *Carollia* sp. in captivity and readily obtaining periovulatory animals, it was discovered that these bats exhibit preferential stimulation of the oviduct on the side of ovulation (Rasweiler, 1972, 1993; Bonilla and Rasweiler, 1974). Although the response is somewhat variable between animals, it generally includes greater hypertrophy of the ciliated and secretory cells, and greater vacuolation of the secretory cells (due for the most part to the deposition of large amounts of glycogen), in that oviduct both prior to and immediately after ovulation (Figure 5.8). Similar observations were subsequently made in field-collected *N. albiventris*, which is closely related to the phyllostomids (Rasweiler, 1978, 1993; Badwaik and Rasweiler, this volume). Immediately following ovulation, the ampulla of the

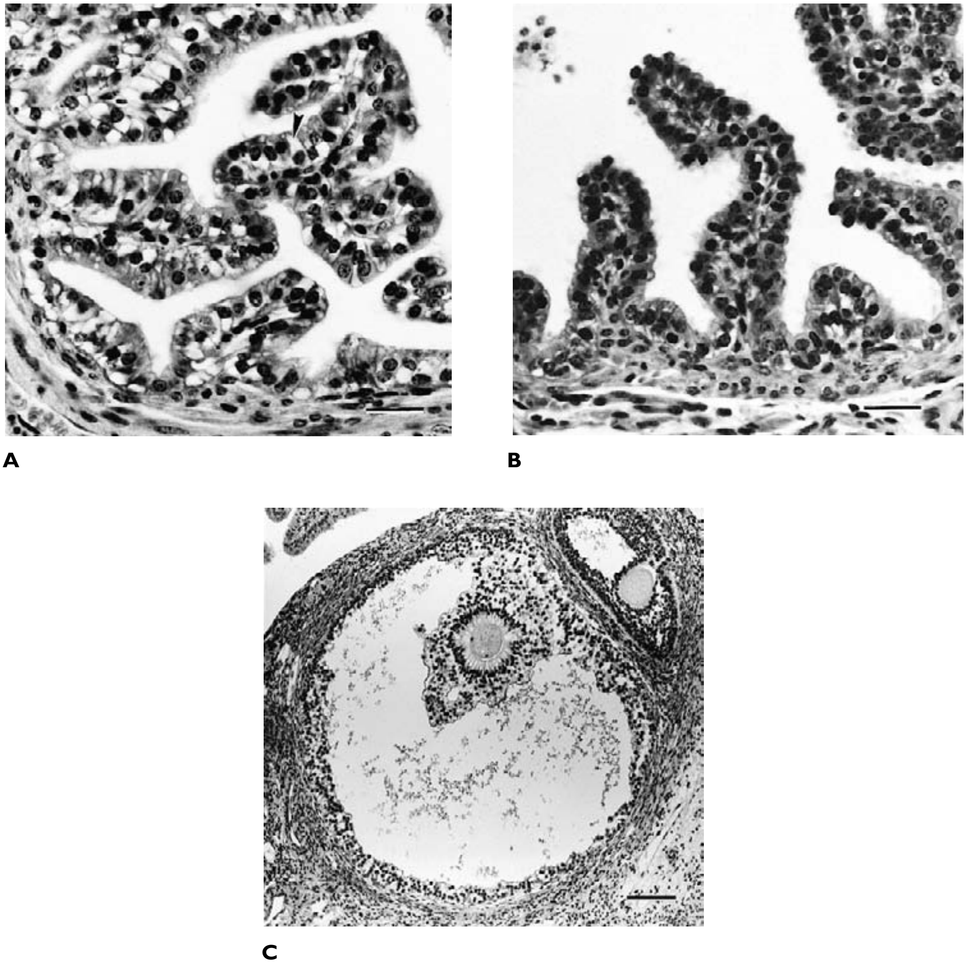

Figure 5.8 Sections of the oviductal ampullae and ovary containing a large preovulatory follicle obtained from a *Carollia perspicillata* on the morning of day 1 *postcoitum*. This animal was also menstruating (see Figure 5.13). (A) Ampulla ipsilateral to the ovary containing the follicle. (B) Ampulla contralateral to the ovary containing the follicle. The epithelium in the ipsilateral oviduct generally appeared more stimulated, as indicated by the greater hypertrophy of its epithelial cells and the more pronounced vacuolation of its secretory cells (e.g., at arrowhead). (C) Preovulatory follicle in which the oocyte was just completing its first meiotic division. Bars (A and B) = 25 μm. Bar (C) = 100 μm.

oviduct in *G. soricina* containing the egg was markedly dilated with fluid, while the contralateral one was not. Differential dilatation of the oviduct with fluid was also observed in many *Carollia* immediately after ovulation and in *N. albiventris* both before and after ovulation. No unilateral reactions have been observed in the simplex uteri of *G. soricina* and *Carollia* sp. Unilateral endometrial reactions have been noted, however, in the partially bicornuate uterus of *N. albiventris* both before and after ovulation (Rasweiler, 1978).

Several hypotheses were proposed to explain how preferential stimulation of the ipsilateral oviduct and/or uterine horn might be accomplished in these animals (Bonilla and Rasweiler, 1974; Rasweiler, 1978). The most likely of these would seem to include the following:

1. There may be a local countercurrent exchange of hormones between the ovarian venous and/or lymphatic drainage and the arterial supply of the ipsilateral oviduct and uterine horn. This could result in the delivery of higher levels of hormones to the ipsilateral ducts.
2. Hormones diffusing out of the ovarian venous and/or lymphatic vessels may have a local vasodilatory effect upon arteries supplying these ducts.
3. Hormones in the follicular fluid released at the time of ovulation may have a local effect upon the ipsilateral ducts, especially the oviduct.

Obviously, these need not be mutually exclusive, and the observed unilateral effects could depend to some degree upon all three. Finally, in order for unilateral effects to develop, systemic levels of the trophic hormones must be low, or stimulation of the contralateral ducts would be seen. At least among the microchiropterans exhibiting such effects, a variable degree of stimulation of the contralateral ducts is often seen.

In both *Carollia perspicillata* and *N. albiventris*, branches of the uteroovarian artery and vein on either side of the tract vascularize portions of the ipsilateral ducts that exhibit preferential stimulation (Rasweiler and Badwaik, 1999a). Wherever the uteroovarian artery and its branches are closely associated with the venous and lymphatic drainage of the ovary (Figure 5.9), it would seem that a countercurrent transfer of hormones might occur. This could lead, in turn, to the delivery of higher levels of steroids to the ipsilateral ducts via the arterial branches.

Work with some of the pteropodid bats (*Rousettus aegyptiacus* and three Australian species of *Pteropus*) has provided evidence that a counter- or crosscurrent transfer of steroids between the ovarian venous and lymphatic drainage and the arterial supply to the uterus also probably plays a major role in bringing about the unilateral endometrial reactions observed in those species (Pow and Martin, 1987, 1994, 1995; Bernard, 1988; Pow, 1992). These studies have established that (1) a branch of the ovarian artery vascularizes the cranial end of the ipsilateral uterine horn in these bats, i.e., the region demonstrating the most pronounced preferential stimulation, (2) the anatomical relationships between the ovarian venous and arterial vessels are unusually intimate in the presumed region of steroid transfer, and (3) ^{3}H-estradiol microinjected into the ovary of one species (*Pteropus poliocephalus*) was preferentially transported to the ipsilateral uterine horn. The elegant studies of Pow and Martin have shown particularly well, by means of both vascular injections and histological sectioning, that in the presumed region of transfer the ovarian artery is, in part, tightly coiled and surrounded by a large sinus that transports venous blood from the ovary. The arterial coiling would significantly increase the area available for hormonal transfer between the venous and arterial blood streams. Ovarian lymphatic vessels are also present in the adventitiae of both the artery and the venous sinus and would provide an additional source of steroids for transfer. The ovarian artery then continues on caudally as the major vessel supplying the cranial tip of the uterus. Here it

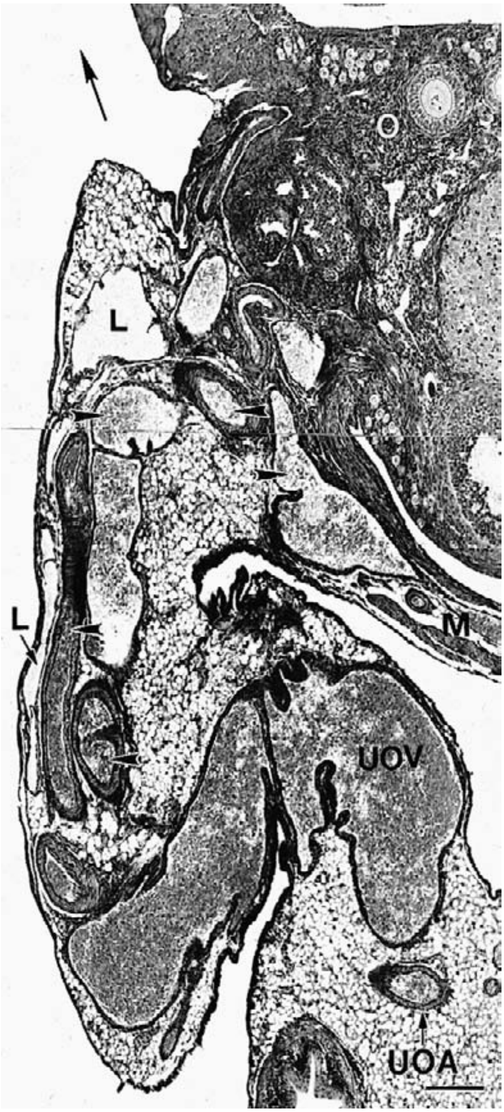

Figure 5.9 Frontal section through the broad ligament containing portions of the major blood and lymphatic vessels that serve one ovary (O), the ipsilateral oviduct and part of the uterus in a *Carollia perspicillata*. The uteroovarian artery (UOA) gives rise to branches (◄) that serve all three organs. These run adjacent to branches (►) of the uteroovarian vein (UOV), and lymphatic vessels (L) serving the ovary and oviduct. Steroids transported away from the ovary in its venous and lymphatic vessels may be transferred locally to the uteroovarian artery, as well as its branches, and then carried into the ipsilateral oviduct, to preferentially stimulate the latter. M = a portion of the mesentery of the oviduct (the mesosalpinx) which contains oviductal branches of the arteries, veins and lymphatics. The uterine branches of the uteroovarian artery and vein pass on to the uterus via a continuation of the broad ligament, which runs in the general direction shown by the arrow and is located out of the plane of this section. Weigert's resorcin fuchsin for elastin followed by Masson's trichrome stain. Bar = 100 μm.

anastomoses with the smaller uterine artery. During pregnancy in *Pteropus scapulatus* and *Pteropus poliocephalus* the ovarian artery provides the major maternal arterial supply to the discoidal placenta which is centered on the uterotubal junction (Pow and Martin, 1994). In view of the important role that this artery plays in normal uterine function, it might be more appropriate to refer to it as the 'uteroovarian' artery.

The relationship between the venous drainage of the ovary and the arterial supply to the cranial portion of the uterus in pteropodids appears far more intimate than anything that has been observed in the presumed region of steroid transfer in the microchiropteran bats *C. perspicillata* or *N. albiventris* (Figure 5.9) (Rasweiler and Badwaik, unpublished observations). It remains to be determined how an efficient enough transfer of hormones can be achieved to account for the unilateral reactions observed in these microchiropterans. Possibly this is accomplished in part by the elevation of ovarian steroids in the surrounding adipose tissue of the broad ligament and their transfer into the arterial vessels serving the ipsilateral ducts.

The possible role of steroids released with the follicular fluid (produced by cells of the cumulus oophorus released at ovulation, or diffusing out of the ovary and passing down the ipsilateral oviduct) in bringing about some of the local effects is also difficult to fully assess. Marshall (1949) considered it unlikely that follicular fluid released at ovulation could pass via the oviduct to directly prime the ipsilateral endometrium. It might prove unwise, however, to completely dismiss these possibilities without adequate study. In at least some *M. ater* (family Molossidae), cells of the cumulus oophorus released at ovulation pass into the uterus and become luteinized there (Figure 5.10). This has been observed in a number of animals, and there is no question as to the origin of the cells because of their luminal location and distinctive nuclear morphology. In nonpregnant *M. ater* the right uterine horn is often larger, with a better developed endometrium, than the left (Rasweiler, 1991a). The extent to which this reflects preferential stimulation, as opposed to greater responsiveness, of the right horn has not been determined. It should be emphasized that such overt luteinization of the cells of the cumulus oophorus released at ovulation has not been observed histologically in the species exhibiting unilateral reactions studied by the senior author (*G. soricina*, *Carollia* sp., *N. albiventris* and *P. kappleri*), but the length of time that these cells remain capable of secreting steroids is unknown. There is ultrastructural evidence that the cumulus cells surrounding oviductal oocytes collected from women may be capable of steroidogenesis; however, its physiological significance remains unclear (Motta *et al.*, 1997). In *N. albiventris*, granulosa cells released with the egg at ovulation could be positively identified in the oviduct or ovarian bursa only until the embryo had reached the 90 cell oviductal morula stage, and at that point many of the cells were clearly degenerate (Rasweiler, 1977). In *P. kappleri*, a few cells of the corona radiata were often still adherent to the zona pellucida surrounding oviductal blastocysts, but had not luteinized like the granulosa lutein cells in the CL. Granulosa cells in the Graafian follicles of this species exhibit significant preovulatory hypertrophy, however, and this could be associated with preovulatory luteinization that might also affect cells of the cumulus oophorus to some extent (Rasweiler, 1982). What happens to the

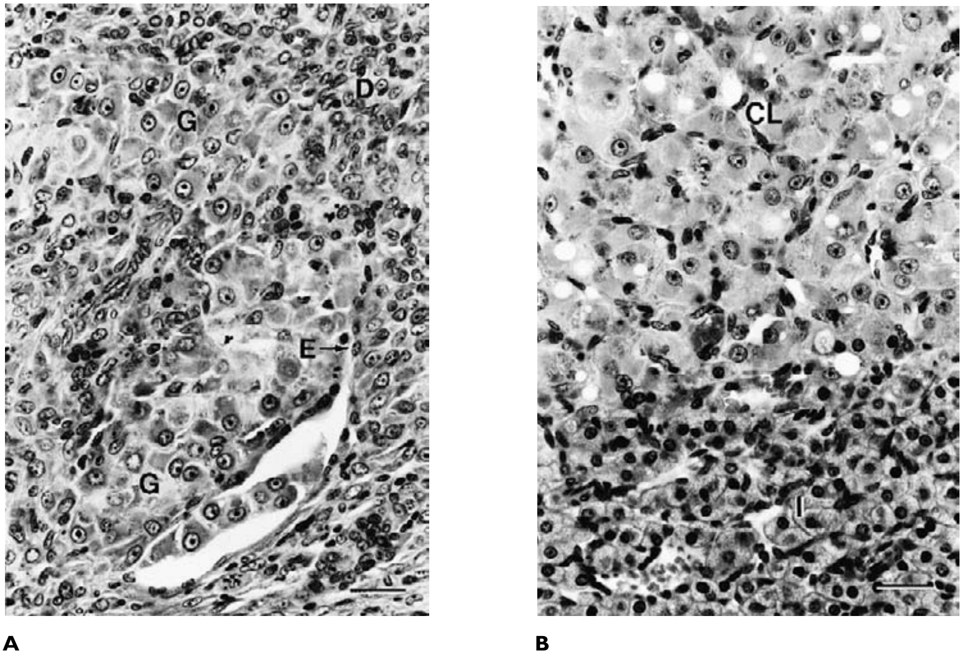

Figure 5.10 (A) Section of the spontaneously decidualized uterus of a nonpregnant *Molossus ater* which contains a mass of luteinized granulosa cells (G). These cells, which have nuclei with very prominent nucleoli, were present in a portion of the closed-down uterine lumen and a couple of uterine glands adjacent to the remnants of an unfertilized ovum. The latter and better views of the decidual reaction are depicted elsewhere (see Figures 12 and 13 in Rasweiler, 1991a). The lutein cells did not appear to be separated from the decidua (D) by a continuous layer of luminal or glandular epithelial cells (E), probably because many of the latter undergo apoptosis in concert with the decidual reaction. (B) Section of ovary from the same animal showing a portion of the most recent corpus luteum (CL) and some interstitial gland tissue (I) which is very abundant in the ovaries of *M. ater*. The granulosa lutein cells of the corpus luteum contain distinctive nucleoli like those in the uterus. Masson's trichrome stain. Bars = 30 µm.

granulosa cells released at ovulation in any of the pteropodid bats is simply unknown.

Immediately after ovulation in *G. soricina*, the oviductal ampulla containing the egg becomes markedly dilated with fluid, while the contralateral ampulla does not. Also, there appears to be a transient decrease at this time in the vacuolation of the secretory cells in the ampulla containing the egg. Both of these effects could be, in part, a local response to hormones passing directly into the oviduct with the follicular fluid (Rasweiler, 1972). On the other hand, unilateral oviductal and endometrial reactions (including some unilateral dilatation of the oviductal ampulla with fluid) have been observed in *N. albiventris* both prior to ovulation and in animals containing unruptured luteinizing follicles. Such follicles, which contained entrapped oocytes or their remnants (zonae pellucidae), were found in a significant number of the nonpregnant *N. albiventris* captured around the onset of their breeding season in the wild. When fully luteinized, they are very similar in appearance and apparently function to the CLs of early pregnancy. Further analysis indicated that the animals bearing such follicles were probably prepubertal members of the population and that the luteinization of unruptured follices may have reflected immaturity of their hypothalamo-pituitary-ovarian axis (Rasweiler, 1978, 1984). These observations provide evidence that the development of unilateral oviductal and endometrial reactions in *N. albiventris* does not depend heavily upon follicle rupture and the passage of the follicular contents down the ipsilateral ducts. They are not extensive enough, however, to permit the conclusion that the follicular contents play no role in the local effects seen in postovulatory animals. Furthermore, a closed bursal sac envelopes each ovary in *N. albiventris*, and that surrounding the ovary containing a new CL becomes preferentially dilated (which is evident grossly upon dissection). Much of this fluid is probably an ovarian exudate and could be steroid-rich (Rasweiler, 1978). The extent to which any of this may pass down the ipsilateral reproductive ducts is unknown.

Another possible explanation for these unilateral effects is that the oviduct and uterine horn on one side of the tract may be left in a refractory condition after a previous nonpregnant cycle or pregnancy. The available evidence suggests, however, that this is quite unlikely to be the case in *G. soricina* and *Carollia* sp. Although these species exhibit a tendency for successive ovulations to alternate between the ovaries, the effect is weak (62% alternation) in *G. soricina* and not significantly different (P>0.05) from a random distribution of successive CLs in the ovaries. In *Carollia* the effect is stronger (78% alternation) and statistically significant (P<0.01). For this reason, the preovulatory follicle or new CL may sometimes be in the same ovary with the old CL in these bats, but is more frequently in the opposite ovary. As a consequence, the preferentially stimulated oviduct is always ipsilateral to the ovary with the preovulatory follicle or new CL, but may be either ipsilateral or contralateral to the old CL (Rasweiler, 1972, 1979b; Bonilla and Rasweiler, 1974). It is difficult to reconcile these observations with the oviducts being left in some sort of refractory condition by a prior cycle or pregnancy. The same line of reasoning cannot be extended to *N. albiventris* because this species exhibits a high incidence of alternation of successive ovulations (92%; P<0.001), and preferentially stimulated ducts were only rarely observed ipsilateral to the ovary containing an old CL. The observations for this species do indicate, however, that the unilateral oviductal and uterine reactions are unlikely to be caused by one side having been left in a refractory condition by an earlier pregnancy. That is because in many *N. albiventris* exhibiting such effects, the uterus lacked signs of having previously been pregnant (Rasweiler, 1978, 1979b).

5.4 NONPREGNANT REPRODUCTIVE CYCLES

5.4.1 The Importance of Preventing and Coping with Reproductive Failures

With the exception of some of the vespertilionids (and even these are not prolific), bats have very low annual reproductive potentials (Kurta and Kunz, 1987; Gopalakrishna and Badwaik, 1993). Failures to successfully conceive, carry pregnancies to term, or rear infants are therefore potentially significant to the survival of populations and even species.

Exactly how significant this may be is somewhat difficult to assess, because it requires a thorough evaluation of the condition of all females sampled from a breeding population. Unfortunately, this often is not carried out. For example, it is not uncommon with reproductively synchronized populations to occasionally capture females carrying conceptuses that are less-developed than most. In these cases, ideally an effort needs to be made to determine whether these females had (1) ovulated just once, but late, or (2) may have ovulated, suffered a reproductive failure, and then ovulated again. Evidence for the latter can sometimes be obtained by checking the ovaries histologically for new and old CLs.

Observations have been presented on the occurrence of reproductive failures, and on infant mortality, in a variety of Indian bats (Badwaik *et al.*, 1992; Badwaik and Gopalakrishna, 1993). In some cases questions remain about the precise cause of the failures/mortalities, or when they occurred. Nevertheless, these studies are important because they indicate that such losses do occur and that their cumulative effect could be quantitatively significant.

When the reproductive condition was determined for all females in a collection of *N. albiventris* taken around the onset of a breeding season in the Cauca Valley of Colombia, 88 were found to have luteinizing/luteinized follicles or new CLs (Rasweiler, 1977, 1984). Of these, four carried eggs either in the process of being fertilized or newly-fertilized, eight carried oviductal embryos, one carried twin oviductal and uterine blastocysts, and 59 carried uterine blastocysts. The remaining 16 females were nonpregnant. In most (n = 14/16) of the nonpregnant animals, however, follicle rupture had failed to occur, and the oocyte or a collapsed zona pellucida was found within a luteinizing or luteinized follicle. As noted above, this unusual failure of the ovulation process is thought to be attributable to immaturity of the hypothalamo-pituitary-ovarian axis in prepubertal members of the population. One of the remaining nonpregnant females had only the remnants of an embryo (i.e. trophoblast) in her uterus, while no reason was apparent for the lack of an embryo in the other animal. Two of the bats carrying normal implanting blastocysts also bore the remnants of ova released at prior ovulations. For unknown reasons, these had not resulted in ongoing pregnancies.

While these observations indicate that the rate of fertilization failures and early embryonic losses can be quite low in *N. albiventris*, they provide no indication of conceptus losses later in pregnancy, or of how many young fail to reach maturity. It would seem that when all of these possible losses are added up, they could be significant with respect to population mean fitness.

Losses of this nature assume even greater importance when one considers that there are probably years in which environmental conditions may be suboptimal, or even totally unsuitable, for successful reproduction. In order for bat populations to maintain themselves, reproduction in good years must compensate for that in bad ones (or animals must move in from adjacent areas). Heideman (1995) noted that fluctuations in rainy and dry seasons seemed to affect the normal reproductive patterns of some pteropodid bats in the Philippines. Also, Gannon and Willig (1994) have described how a hurricane had a long term, detrimental effect upon the size of a population of red fig-eating bats (*Stenoderma rufum*) living on the Caribbean island of Puerto Rico.

In view of these considerations, it is not surprising that many of the most interesting and unusual reproductive specializations of bats seem designed to minimize reproductive failures or to efficiently cope with them should they occur. This is evident from what is known about reproduction in a highly successful species, *C. perspicillata*. Recent work with captive animals has established that *C. perspicillata* can produce young after 'normal' gestation periods of 113–119 days. This bat can, however, significantly prolong gestation if stressed in captivity, and it seems reasonable to suspect that this response may occasionally be of adaptive value in the wild as well. *Carollia perspicillata* also exhibits pregnancies in the wild that are apparently prolonged in response to seasonal factors (Cosson and Pascal, 1994; Rasweiler and Badwaik, 1997). If females abort non-term foetuses, many will quickly experience a postpartum oestrus and conceive again (Rasweiler and Badwaik, 1996). If adult females are prevented from breeding, they will run nonpregnant cycles terminated by true menstruation (see below). This is presumably an adaptation for efficiently recycling the uterus in the event of fertilization failures or the loss of early embryos (Rasweiler, 1991a, 1992; Rasweiler and Bonilla, 1992).

5.4.2 Patterns of Growth and Regression in the Reproductive Ducts

Paucity of studies

In the light of what has just been said, it should be obvious that the nature of nonpregnant cycles can play an important role in enabling females to achieve their maximum reproductive potential. Unfortunately, for several reasons, we still have only a fragmentary picture of what type of cycle most bats have. These include the fact that many bats seem to reproduce with a fairly high degree of success in the wild (or we have been missing periods when they do not), many studies of the female have tended to focus upon 'normal' reproductive events (e.g. particularly seasonality), and there may be a tendency to view those in the population that have suffered reproductive failures as 'abnormal'. It has long been clear, however, that in a sense 'to sometimes fail is normal' for every species when it comes to reproduction (Brambell, 1948; Hanly, 1961). Finally, relatively little work has been done with bats in captivity, where nonpregnant cycles can be studied by preventing females from breeding.

Observations have been made on the patterns of oviductal and uterine stimulation in field-collected material from two neotropical species – *P. kappleri* (an emballonurid) and *N. albiventris* (a noctilionid). Because these studies were not conducted under controlled conditions, they have been unable to tell us if these species are induced or spontaneous ovulators, whether they possess nonpregnant cycles with a functional luteal phase (i.e. whether the CLs become fully functional or not in the absence of copulations), or exactly how their uterine endometria may be recycled in the event of a fertilization failure or the loss of an early embryo. They do provide some indications, however, of how females may cope with the latter problems and the possible adaptive significance of some of the reproductive asymmetries that these bats exhibit.

Female reproductive function in Peropteryx kappleri

Studies of *P. kappleri* have established that this species is polyoestrous (Rasweiler, 1982). Many collected from a population living in the Colombian Andes had both recently given birth and were either preparing to ovulate again, or had recently ovulated. Successive ovulations in this monovular species tend to alternate between the ovaries on either side, but this is restrained by partial dominance of the left ovary. *Peropteryx kappleri* also exhibits differential stimulation of the oviducts and uterine horns.

Unilateral reactions are usually evident in the oviducts of most females immediately prior to ovulation. They were less frequently detected at this time in the uterine horns, either because one horn still contained debris from a recent parturition or because both horns were dilated with fluid. In the bats bearing large preovulatory follicles, the endometrium was generally shallow, although abundant mitotic figures were seen in both the epithelium and stroma. When a unilateral endometrial reaction was evident, the endometrium was slightly thicker, with more hypertrophied glands and a looser stroma, at the cranial end of the horn ipsilateral to the preovulatory follicle.

During the early tubal journey of the oocyte/embryo (up to the eight-cell stage), the level of mitotic activity in the uterine epithelium was variable. In some bats, mitotic figures were moderately abundant in the luminal and glandular epithelium of the horn ipsilateral to the ruptured follicle, but were less common or absent from the contralateral horn. In others, epithelial mitoses were rarely seen or absent at

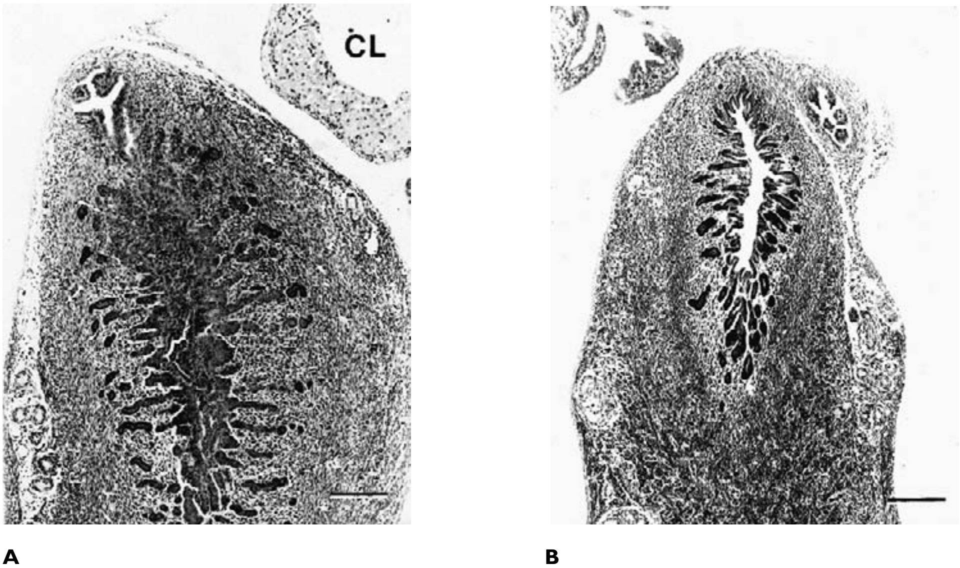

Figure 5.11 Frontal sections through the cranial ends of the uterine cornua ipsilateral (A) and contralateral (B) to the new corpus luteum (CL) in a *Peropteryx kappleri* carrying an oviductal blastocyst. Preferential stimulation of the endometrium in the ipsilateral cornu is evident. The corpus luteum in this animal was noneverted and redistended with fluid. PAS-haematoxylin. Bars = 125 µm.

this time from both horns. This was also the case in females carrying advanced tubal embryos. In contrast, proliferative activity was generally pronounced in the endometrial stroma of the cranial third of the horn ipsilateral to the ruptured follicle or new CL throughout the tubal journey of the ovum in nearly all females, but was minimal or not evident in that portion of the contralateral horn. Because the epithelial and stromal cells in the cranial third of the ipsilateral horn also exhibited preferential hypertrophy, marked differences in endometrial thickness between the cranial ends of the two horns became evident by the time embryos had reached their maximal state of development in the oviducts (i.e., to zona-encased blastocysts) (Figure 5.11). With the exception of females carrying early tubal ova, the levels of glycogen and glycoproteins were also always much greater in the glands of the cranial third of the ipsilateral horn as compared to the contralateral.

Female reproductive function in Noctilio albiventris

Similar studies have been conducted on *N. albiventris* (Rasweiler, 1977, 1978). These bats were generally found to be monovular. Ovulation could occur from either ovary (although the left was slightly favored), and embryonic development then took place in the ipsilateral oviduct and uterine horn. *Noctilio albiventris* exhibits a high incidence (>92%) of alternation of successive ovulations between the ovaries, as well as unilateral oviductal and endometrial reactions.

When a mature preovulatory follicle, a newly ruptured follicle, or a new CL was present, the more developed oviduct was consistently ipsilateral. In most respects, both uterine horns appeared quite similar in all of the bats which lacked a recent CL, possessed a mature preovulatory follicle, or carried an oocyte in the process of being fertilized. The endometrium in both horns of these animals was generally shallow, with a very dense stroma. Immediately around the time of ovulation, however, higher glycogen levels were detected in the uterine epithelium of the horn ipsilateral to the mature follicle or newly ruptured follicle. Mitotic activity was observed in the endometrial epithelial cells of many preovulatory animals, and this continued until at least the 90-cell stage. At the 24-cell morula stage, substantial mitotic activity first appeared in the endometrial stroma, and this continued through the initiation of implantation. During passage of the embryo through the oviduct, the following endometrial changes also occurred: increased epithelial cell size and glycogen levels, increased stromal cell size, and decreased stromal cell density (presumably due to changes in the composition of the extracellular matrix, e.g. its water content). In some animals, these characteristics and stromal cell mitotic activity differed between the horns and contributed to greater enlargement of the horn on the gravid side of the tract.

Female reproductive function in the phyllostomid bats

In the case of the phyllostomid bats *G. soricina*, *C. perspicillata* and *C. brevicauda* substantial work has been done with captive animals maintained under controlled conditions (Rasweiler, 1970, 1972, 1974, 1979b; Bonilla and Rasweiler, 1974; Rasweiler and Bonilla, 1992; Rasweiler and Badwaik, 1996; Badwaik *et al.*, 1997). This has established that females of these species will ovulate spontaneously. Corpora lutea were regularly observed in the ovaries of (1) *G. soricina* that had been maintained in sexual isolation, and (2) newly mated females of all three species that had been housed with males for only a brief period of time. In these latter animals, the CLs were old, and newly ruptured follicles or mature preovulatory follicles were also present. It must be cautioned that, for all of these studies, the females had been housed for extended periods in the same room with mature males although in separate cages. Thus, pheromones or other male factors could have influenced prior ovarian activity. Some evidence was, in fact, obtained that the introduction of males can have an oestrus-synchronizing effect upon female *G. soricina* (Rasweiler, 1975).

Glossophaga soricina and *Carollia* sp. have nonpregnant cycles that are terminated by true menstruation. This involves necrosis and sloughing of the superficial lamina functionalis of the endometrium with associated bleeding (Figures 5.12 and 5.14). It should be noted that Figures 5.13 and 5.14 were obtained from recent studies involving captive-bred *C. perspicillata*. Although substantial endometrial breakdown can occur in *G. soricina*, no external signs of menstruation have been noted in work with this bat. That is because most of the menstrual debris undergoes dissolution (which can actually be seen histologically) within its uterine cavity (Figure 5.12). In the case of *Carollia perspicillata*, however, it was not uncommon for vaginal aspirates obtained at the time of menstruation to be sufficiently tinged with blood so that this could be recognized with the naked eye (Rasweiler and Badwaik, 1996). This is perhaps not surprising, as substantial amounts of blood have been observed in the uterine cavity in some females (Figure 5.13).

Figure 5.12 Section of the menstrual uterus obtained from a *Glossophaga soricina* on day 1 *postcoitum*. This shows the extensive dissolution of the desquamated endometrial tissue that can occur within the uterine cavity in this species. Masson's trichrome stain. Bar = 50 µm.

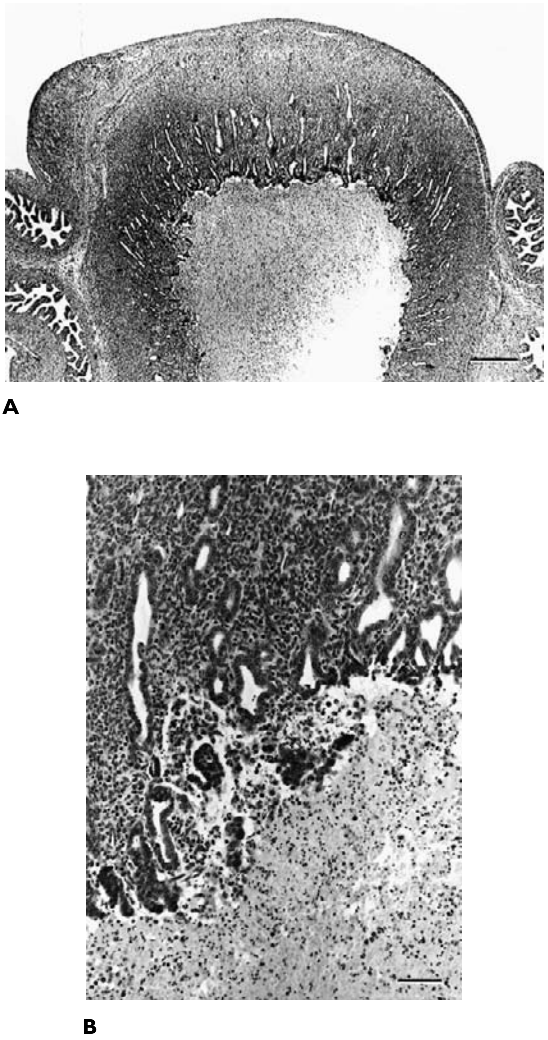

Figure 5.13 Frontal sections of the menstrual uterus from a *Carollia perspicillata* obtained on day 1 *postcoitum*. The uterine lumen contains abundant blood, many spermatozoa, and some desquamated endometrial tissue. This animal carried the preovulatory follicle shown in Figure 5.8. Haematoxylin and eosin. Bar (A) = 300 μm. Bar (B) = 50 μm.

Although the endocrine control of their uterine cycles has not been worked out for *G. soricina* and *Carollia* sp., regressing CLs are usually (but not always) present at the time of menstruation in their ovaries. This suggests that menstruation in these bats is a hormone-withdrawal phenomenon, as in catarrhine primates (e.g. the human and the rhesus monkey).

Menstruation in the phyllostomid bats does differ from that in primates in several respects. One is in its timing. Whereas menstruation in the human and

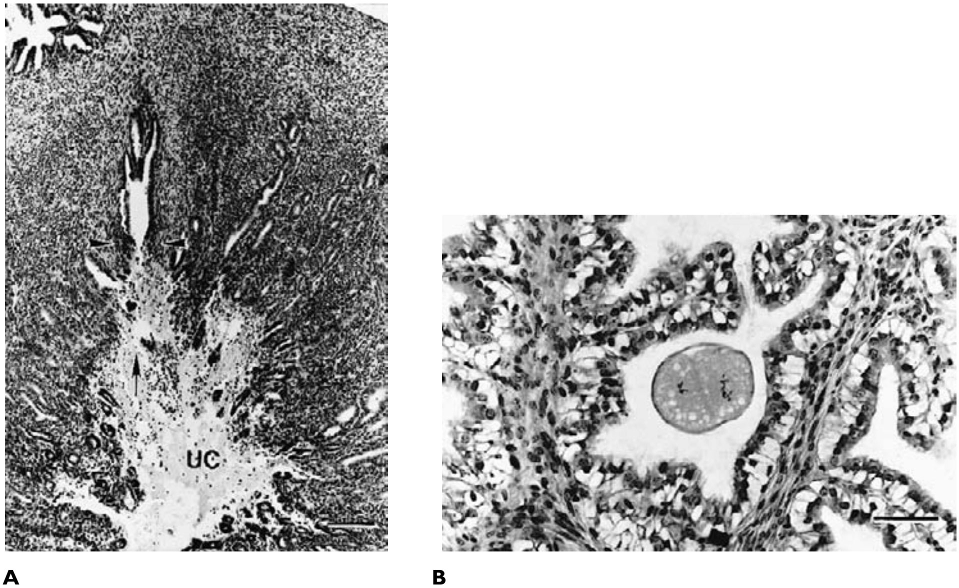

Figure 5.14 (A) Frontal section of the menstrual uterus obtained from a *Carollia perspicillata* on the evening of day 3 *postcoitum*. The menstrual process has resulted in the breakdown of much of the superficial endometrium at the usual implantation site, which is normally in the intramural uterine cornu on the side of ovulation or sometimes in the immediately adjacent portion of the main uterine cavity (UC). One of the intramural uterine cornua was probably located approximately between the arrow and the uterotubal junction (paired arrowheads). (B) Two-cell embryo in the oviductal ampulla of the same female. Haematoxylin and eosin. Bar (A) = 100 µm. Bar (B) = 50 µm.

rhesus monkey normally begins about 14 days after ovulation, in these bats the process is usually periovulatory. *Glossophaga soricina* has a cycle that ranges from about 22–26 days in length (mean 24.0±0.1 days). When 12 bats were examined on days 22–24 of their cycle (with day 1 representing the time at which newly ovulated oocytes would first be found in the oviducts), 10 had large healthy preovulatory follicles in their ovaries, and all 12 exhibited preovulatory stimulation of their oviducts. The uterine condition of these animals based upon histological assessment was as follows: one was premenstrual, seven were menstrual, and four were proliferative (postmenstrual). The uterine condition of 13 bats examined on days 1–3 of the cycle or pregnancy was as follows: one was premenstrual, 11 were menstrual and one was proliferative (Rasweiler, 1979b).

In humans and rhesus monkeys, most endometrial mitotic activity occurs during the follicular (preovulatory) phase of the menstrual cycle and drops off relatively soon after ovulation. In *G. soricina*, because of the peculiar timing of menstruation, regenerative changes in the endometrium are usually relatively limited prior to ovulation. Low levels of mitotic activity were evident in the glands and stroma on days 20–22 of the nonpregnant cycle, but did become abundant in those uterine components by days 23 and 24 (i.e. immediately before ovulation). In *G. soricina*, however, substantial mitotic activity continues in the endometrial glands until about day 13 of the nonpregnant cycle and in the endometrial stroma until about day 15 (Rasweiler, 1970, 1979b). Implantation in this species does not commence until about days 12–14. Thus, in contrast to higher primates (Noyes *et al.*, 1950; Bartlemez *et al.*, 1951; Bensley, 1951; Ferenczy *et al.*, 1979; Tabibzadeh, 1990), most endometrial growth in this bat is postovulatory. This is associated with prolonged retention of the embryo in the oviduct and development to an unusually advanced stage there, the zona pellucida-free blastocyst (Rasweiler, 1972, 1974).

Although attempts have not been made to examine the nonpregnant cycle of *Carollia* sp. *per se*, the reproductive tracts from a sizeable series of captive-bred animals killed on days 1–18 *postcoitum* (*p.c.*) have been studied histologically (Bonilla and Rasweiler, 1974; Rasweiler and Bonilla, 1992). Of those examined on day 1 *p.c.* (= the first day of a sperm-positive vaginal smear), four had newly-released oocytes in the oviduct, while five had large preovulatory follicles in their ovaries. All six examined on day 2 *p.c.* had ovulated. The uteri of the bats examined on days 1–3 *p.c.* varied considerably depending upon whether regressing CLs were present in the ovaries or not. Some of these females lacked such CLs and presumably were either pubertal or coming out of an anoestrus period. In these bats the endometrium was shallow, but considerable mitotic activity was present in its glands and stroma. In most females having regressing CLs, the endometrium was much thicker. Portions of the superficial lamina functionalis in these animals were frequently necrotic and in the process of being sloughed off with associated bleeding, i.e. in the form of a true menstrual process. Since many of these animals were examined shortly after the initiation of copulations, the regressing CLs must have been formed as a result of earlier spontaneous ovulations. Furthermore, the thickness of the endometrium in many suggests that functional CLs had been formed in the absence of mating activity. Menstruation had been completed in one of two females with regressing CLs examined on day 3 *p.c.*, but the final stages of menstrual breakdown of the endometrium were still evident in another specimen examined on day 5 *p.c.* It was common until day 8 *p.c.* to still see some menstrual debris in the uterine lumen. This periovulatory timing of menstruation has been reconfirmed in our recent work with captive-bred *C. perspicillata* (Figures 5.13 and 5.14). Most *Carollia* sp. killed between days 6 and 10 *p.c.* had proliferative endometria with mitotic activity evident in the glandular epithelial cells and stroma of the lamina functionalis. After day 10 *p.c.*, mitotic activity in the glandular epithelium dropped off; however substantial levels continued to be seen in the stroma through the initiation of implantation. Implantation commences in *Carollia* sp. on days 13–16 *p.c.* Thus, as in *G. soricina*, most endometrial growth in *Carollia* in preparation for implantation is postovulatory.

The common vampire bat, *Desmodus rotundus*, also menstruates at the end of nonpregnant cycles. As in *G. soricina* and *Carollia* sp., this commences during the periovulatory period in females with regressing CLs. In *D. rotundus* degenerative areas continued to be seen in the superficial lamina functionalis of the endometrium for somewhat longer after ovulation (until day 13 *p.c.*), although regenerative changes increasingly became apparent in underlying portions of the lamina functionalis. These included hypertrophy and hyperplasia of the stromal and glandular elements and a gradual decrease in stromal cell density. When no regressing CL was present, the periovulatory endometrium appeared shallow and proliferative. Although *D. rotundus* exhibits a unilateral oviductal reaction, differential stimulation of its rather short uterine horns has not been noted (Quintero and Rasweiler, 1973, 1974, unpublished observations).

The unusual temporal relationship between menstruation and ovulation in at least some of the phyllostomid bats brings to mind the prooestrous bleeding of dogs ('pseudomenstruation') and the postoestrous (metoestrous) bleeding of cattle. In dogs and cattle, the bleeding occurs from capillaries in an otherwise intact endometrium. In the phyllostomid bats, however, menstruation is clearly the result of a degenerative process that culminates in substantial endometrial breakdown (Figures 5.12–5.14). Furthermore, this is usually (but not always) associated in these bats with the presence of a regressing CL (Rasweiler, 1970; Rasweiler and Bonilla, 1992).

Vascularization of the endometrium and its role in the menstrual process

There is also a difference between the phyllostomid bats that have been well-studied and catarrhine primates in the vascularization of the endometrium. Only the endometria of the latter are vascularized by spiral arterioles (Kaiser, 1947a; Rasweiler, 1970, 1979b; Rasweiler and Bonilla, 1992). Increased coiling of these arterioles in catarrhine primates, along with vasoconstriction, has been thought by many to be responsible for circulatory deprivation of the lamina functionalis late in the cycle, leading to cell death, tissue degeneration and desquamation with associated bleeding (i.e. menstruation) (Shaw and Roche, 1980). Much of this was based upon the studies of Markee (1940, 1950), who followed microscopically the cyclic changes exhibited by living pieces of isogenic endometrium transplanted to the anterior chamber of the eyes of rhesus monkeys. While the elegance of these studies cannot be disputed, other authorities have minimized the possible importance of arteriolar coiling in bringing about menstruation (Bartelmez, 1956; Hisaw and Hisaw, 1961).

At least some platyrrhine monkeys menstruate, but these animals lack spiral arterioles (Goodman and Wislocki, 1935; Dempsey, 1939; Hamlett, 1939; Kaiser, 1947b). Because endometrial tissue loss and bleeding have generally been found to be less in the platyrrhines than in catarrhines, there is at least the implication that the absence of spiral arterioles is responsible for the difference. It must be noted that relatively few platyrrhine uteri have been examined histologically at the time of menstruation, and the process may not have been observed at its peak.

Observations on menstruation in *G. soricina* and *Carollia* sp. provide additional reason to be concerned about the dearth of thorough histological studies in the platyrrhine monkeys. In these bats there clearly is some intraspecies variability in the timing of the process. Furthermore, studies on bats indicate that external observations, or even the microscopic examination of vaginal aspirates, may provide a very inaccurate indication of the extent of endometrial breakdown and bleeding, because of the dissolution of menstrual debris within the uterine cavity. This must also be kept in mind when considering whether menstruation may or may not occur in other mammals.

Finally, vascular differences between the phyllostomid bats and catarrhine primates call into further question the suggested central role of spiral arterioles in the mechanism of menstruation. Although endometrial breakdown in *G. soricina* can be as extensive as anything ever observed in primates (Figure 5.12) (Rasweiler, 1979b), this bat has an endometrium in which the lamina functionalis is vascularized only by capillaries. This probably reflects in large part the much smaller size of *G. soricina* (9–12 grams). Arterioles containing a single layer of smooth muscle fibers have been observed in the deep lamina basalis of *Carollia* sp. The degree of coiling of these vessels has not been examined carefully during different phases of the nonpregnant cycle, but they certainly do not form prominent arteriolar fields (i.e. multiple cross sections of the same spiral arteriole) in premenstrual uteri, such as is the case in the uteri of catarrhine primates (Kaiser, 1947a).

Despite the lack of typical endometrial spiral arterioles in *G. soricina* and *Carollia* sp., there is evidence that similar physiological mechanisms may be at least partially responsible for the endometrial involution that culminates in menstruation in these bats and catarrhine primates. The density of cells in the endometrial stroma increases late in the cycle in all of these species, presumably due to a decrease in the water content of the extracellular matrix. This is probably caused by reduced capillary hydrostatic pressure which may, in turn, reflect circulatory deprivation due to changes in the contractility of smooth muscle fibers in the uterine arterioles. The latter may be brought about by the withdrawal of ovarian steroids (Bartelmez, 1937; Markee, 1940; Bartelmez *et al.*, 1951; Prill, 1963; Rasweiler, 1970; Rasweiler and Bonilla, 1992).

Although the most likely cause of such hormonal withdrawal would be luteal regression, menstruation has also been observed in several *Carollia* that lacked regressing CLs. This simply suggests that the phenomenon may occur under more than one set of circumstances. It is well known that anovulatory menstruation can take place in primates (Corner, 1923; Hartman, 1932; Bartelmez, 1937; Bartelmez *et al.*, 1951; Rasweiler and Bonilla, 1992).

Finally, it must be pointed out that while vascular deprivation and ischemic damage probably play an important role in the events leading to menstruation, recent evidence indicates that other factors are also involved. Endometrial epithelial apoptosis increases during the secretory phase of the human menstrual cycle and reaches a peak during menstruation. The latter could be a response to both the withdrawal of steroid hormonal support for the endometrium and ischemic injury (Tabibzadeh, 1996; Vinatier *et al.*, 1996). Furthermore, at least some critical aspects of the menstrual process can be mimicked *in vitro* by subjecting human endometrial explants or stromal cells to the withdrawal of sex steroids. This leads to dissolution of the extracellular matrix and dissociation of the stromal cells. This can also be selectively and reversibly blocked by inhibitors of the enzymes (matrix metalloproteinases) responsible for at least some of the matrix breakdown (Irwin *et al.*, 1996; Marbaix *et al.*, 1996).

Female reproductive function in Molossus ater

Menstruation occurs as well in the molossid bat *M. ater*. This was discovered during the course of work with a captive colony, in which females were housed initially for many months in the same room, but in separate cages from the males. To obtain specimens for the study of implantation and placentation, the sexes were then placed together, and females killed at various intervals after the onset of breeding activity.

Because female *M. ater* do not exhibit a limited period of oestrus, it proved difficult to accurately time ovulations and conception from the first appearance of spermatozoa in daily vaginal aspirates. Although many of these females did successfully conceive, a substantial number of others proved to be nonpregnant when examined histologically. These latter animals had tracts that seemed in every respect to be normal; however, they simply did not appear to have been inseminated at the appropriate time for conception to occur. In addition, a small group of mature females were killed, after having been housed with a stud male only for 24 hours.

Many of the females that had been housed with males only for a short period of time (including just for 24 hours) had well-developed CLs, sometimes of two or three different ages. Thus, *M. ater* must be a spontaneous ovulator. Furthermore, their CLs must become functional in the absence of mating activity, because many of these females also had uteri that had apparently been subjected to luteal stimulation. Indeed, the endometrial lining of some of these animals exhibited a very advanced degree of stimulation. Many of the fibroblasts in the stroma of the lamina functionalis had been converted into plump, more tightly-packed decidual cells, and in some individuals, these had even become multinucleated decidual giant cells. This is highly unusual. In most mammals which develop decidual reactions (all species do not), the stromal fibroblasts undergo this reaction only upon invasion of the endometrium by trophoblast cells of the embryos or following experimental manipulation of the uterus. The elephant shrew, *Elephantulus myurus*, is one exceptional species, since decidual cells develop spontaneously in its uterus during the nonpregnant cycle (van der Horst and Gillman, 1941, 1942). Late in the menstrual cycle of the human and chimpanzee, many endometrial stromal cells begin to differentiate into decidual cells, i.e. the so-called 'predecidual reaction' (Noyes *et al.*, 1950; Witt, 1963; Graham, 1973; Wynn, 1977). Less pronounced morphological changes of a similar nature have also been observed at this time in the baboon and rhesus monkey (Dollar *et al.*, 1982; Brenner and Maslar, 1988), although it has been questioned whether the minor stromal cell changes noted in the baboon are sufficient enough to be considered a predecidual reaction (Enders, 1991).

The postovulatory phase of the cycle in *M. ater* is also marked by the development of an unusual vascular tuft in the endometrium surrounding the cranial end of each uterine horn and by pronounced hypertrophy (and presumably differentiation) of the endothelial cells lining many of the tuft vessels. Many of the endothelial cells in the venules serving the tuft become almost 'glandular' in appearance (i.e. cuboidal to columnar in shape) and accumulate glycoprotein-rich granules. This tuft is formed anew during each ovulatory cycle and is, without question, one of the most pronounced examples of normal (nonpathogenic) angiogenesis thus far observed in adult mammals (cf. Torry and Rongish, 1992). The density of capillaries or endothelial tubules in the tuft seems far greater than is necessary to simply vascularize that part of the endometrium and, during early pregnancy, it is uncertain how much blood actually flows through the tuft. The main function of the finest vessels in this tuft actually appears to be a morphogenetic one during the development of the definitive discoidal placenta (Rasweiler, 1991b; Badwaik and Rasweiler, this volume). The tuft arterioles and venules are, in the event of pregnancy, eventually converted to the maternal arteries and veins that serve the definitive discoidal placenta.

At the end of nonpregnant cycles in *M. ater*, the decidualized endometrial tissue (including the vascular tuft) becomes necrotic and is sloughed off with bleeding from the endometrial blood vessels. This was always associated with the presence of at least one CL in the right ovary, and it fits the definition of menstruation as offered by Bartelmez (1937). Although decidualization progresses further in the nonpregnant cycle of *M. ater* than in anthropoid primates, the development of a pronounced decidual reaction does not appear to be a prerequisite for menstruation to occur. In one female, extensive sloughing of what had probably been a well-decidualized endometrium was evident in the right horn. Decidualization was quite modest in the left horn; yet menstruation (albeit less pronounced) was still taking place. Despite the fact that endometrial degeneration, sloughing and bleeding can be extensive in *M. ater*, most of the desquamated tissue appears to undergo dissolution within the uterus, and relatively little recognizable cellular debris passes into the vagina (Rasweiler, 1991a).

In contrast to the phyllostomid bats, *M. ater* does have an endometrium vascularized by spiral arterioles, but their precise role in bringing about menstruation remains to be elucidated. There is not an obvious increase in coiling of these vessels as the cycle progresses; however, involution of the endometrium culminating in menstruation could still be caused by decreased blood flow through these vessels due to increased contractility of their smooth muscle fibers. It deserves to be noted that these spiral arterioles clearly do have another important role in *M. ater* which is to vascularize the diffuse endotheliochorial placenta that is prominent in mid-gestation. As pregnancy progresses, these vessels enlarge and their coils appear to be payed out as the gravid right horn enlarges. They then apparently recoil during postpartum involution of the uterus. The problem here is not that the spiral arterioles may have multiple functions in *M. ater*, but rather that some primate biologists have attached such special significance to them in the mechanism of menstruation. As noted above, observations for the phyllostomid bats now indicate that menstruation occurs and can be extensive in species that completely lack such vessels.

The pattern of endometrial growth also differs between *M. ater* and the phyllostomid bats. Whereas menstruation is periovulatory and most endometrial growth is postovulatory in *G. soricina* and *Carollia* sp., in 5/9 *M. ater* with both CLs from a previous cycle and one or more large, preovulatory follicles, menstruation had been completed and the endometrium was in a proliferative state. The remaining four animals also had proliferative endometria, but one was in the final stages of menstruation while the other three still had menstrual debris in their uterine lumina. This suggests that while the endometrial cycle of *M. ater* does have a preovulatory proliferative phase, it may be shorter than in the human or rhesus monkey. In any case, endometrial regeneration is sufficiently advanced by ovulation in *M. ater* so that the uterus is apparently prepared to receive an embryo much sooner than is the case in the phyllostomids (Badwaik and Rasweiler, this volume). Two *M. ater* examined on days 5 or 7 after being placed with a male carried, respectively, a uterine morula or a free uterine blastocyst.

Although both the molossid and phyllostomid bats menstruate, these generally are not considered to be closely related microchiropterans. Furthermore, there are significant temporal differences in the nature of their menstrual cycles and early pregnancy. These considerations raise the possibility that menstruation may be more widespread in the microchiropterans than presently realized and/or may have arisen more than once in this group by means of parallel evolution. It should be possible to determine if the former is the case by (1) looking at the reproductive cycles of captive females that are prevented from breeding (when a species can be maintained in captivity), or (2) more thoroughly examining adult females in reproductively-synchronized wild populations. When many females in such a population are carrying recently implanted embryos, some others should be present that are menstrual or perimenstrual, if the species is capable of menstruating. That is because it is unlikely that all females (even reproductively normal ones) will breed or successfully establish ongoing pregnancies.

5.5 ADAPTIVE SIGNIFICANCE OF REPRODUCTIVE ASYMMETRIES AND MENSTRUATION

5.5.1 Complete Dominance of One Ovary and/or Uterine Horn

Bats are clearly unusual among mammals in the frequency with which they exhibit the dominance of one ovary and/or uterine horn. The possible selective advantage that is conveyed by such asymmetrical functioning of the reproductive organs has long been a puzzle. Everything considered, it would seem that this might be related in particular to the fact that (1) bats are flying mammals, which must place a limit upon the total weight that they can carry during pregnancy and (2) most normally bear only a single infant which is large relative to the weight of the mother at birth (Kurta and Kunz, 1987; Gopalakrishna and Badwaik, 1993; Rasweiler and Badwaik, 1999a). This must, in turn, direct attention at the issue of why giving birth to large young should be advantageous to bats.

Kurta and Kunz (1987) have struggled with the question of whether bats are altricial or precocial but ultimately concluded that, at least with respect to their tenacious clinging ability, bats are precocial. In the case of species such as *C. perspicillata*, babies must have considerable muscular strength and motor skills from the time of birth, because almost immediately each must attach to one of their mother's teats and cling firmly to her while she either hangs in the roost or flies about. The babies are carried extensively by their mothers during their first couple of weeks of neonatal life, but it is unclear exactly when the mothers begin to forage alone (Fleming, 1988; Rasweiler and Badwaik, 1999a). Kurta and Kunz (1987) noted that since bats do not provide insulating nests for their offspring, the relatively large size and resulting thermal inertia of neonates might also ameliorate drops in body temperature when their mothers leave to forage. This presumably promotes the maintenance of coordination and motor function and, in combination with their relatively greater neuromuscular maturity at birth, may enable baby bats to remain close to where they have been left by their mothers. This is probably critically important to their survival, because many bats occupy diurnal roosts where a fall could be either directly injurious to the young or put them at significantly greater risk of predation (Rasweiler and Badwaik, 1999a; Badwaik and Rasweiler, this volume).

With the exception of some of the vespertilionids, which have litters of two to four young, bats are normally monotocous and would probably find it difficult (if not impossible) to successfully carry more than a single conceptus to term. If two young should be born by a normally monovular/monotocous species (e.g. has been reported for one *Myotis lucifugus*; Barbour and Davis, 1969), the question then becomes whether the mother can successfully raise both. In order to avoid litter sizes larger than one, most bat species must therefore pursue one of the following options: (1) restrict ovulation during each cycle to but one follicle; (2) if more than one egg is ovulated, restrict implantation to a single embryo; or (3) if multiple eggs are ovulated, fertilized and the embryos implant, permit only a single conceptus to be carried to term. There are many bats that practise the first option, releasing only a single egg. Depending upon the species, this single ovulation may be from either ovary, predominantly from just one ovary, or consistently from just one ovary (Table 5.2). It would seem that the dominance of one ovary may have evolved as a method of precluding the ovulation and implantation of eggs on both sides of the reproductive tract.

The extent to which the second or third options may be pursued by some species is unclear. *Myotis lucifugus* will occasionally ovulate two eggs, and in rare cases two embryos can implant and even be carried to term (Wimsatt, 1945; Barbour and Davis, 1969). Observations of such multiple pregnancies are too few, however, to get a sense of their usual fate. In captive *M. ater*, 11/72 females (15%) with CLs had two or more CLs of the same age (Rasweiler, 1988, 1992). The precise fate of extra eggs, should more than one be fertilized, was not determined. It is also unclear whether the occasional ovulation of more than a single follicle by *M. ater* is a normal phenomenon or possibly a response to the availability of unlimited food in captivity. The plane of nutrition will affect ovulation rates in sheep (Sadlier, 1969). This could conceivably be advantageous to *M. ater*, because it provides a second egg or embryo in case one egg remains unfertilized or the embryo dies. Since *M. ater* only carries conceptuses in the right horn and has a preferred implantation site in that horn, it seems quite unlikely that two conceptuses could successfully be carried to term. In the vespertilionids *E. fuscus* and *P. subflavus*, the number of ova shed outnumber the conceptuses that survive until parturition. In these bats, most of the embryos succeed in implanting, but those situated less advantageously in the uterus are subsequently resorbed (Wimsatt, 1945). The number that survive is also apparently influenced by other genetic and/or environmental factors, because some populations of *E. fuscus* usually give birth to one young instead of two (Kunz, 1974). Similar observations have been made for *Pipistrellus pipistrellus* (Wimsatt, 1945).

In *Miniopterus schreibersii* ovulation almost always occurs from the left ovary and implantation of the embryo then consistently takes place in the right horn (Table 5.2). This is an unusual pattern of function for the ovaries and uterus; however, it also may have evolved as another means of ensuring that normally only a single conceptus is carried to term.

5.5.2 Alternation of Successive Ovulations and Local Control of the Reproductive Ducts

Many monovular bats with two functional ovaries also exhibit the ability to alternate successive ovulations between those ovaries (Table 5.2). This would appear to be advantageous in two different situations:

1. When these species have another oestrus relatively soon after parturition and bicornuate or duplex uteri, the ability to alternate ovulations may facilitate the successful establishment of the new pregnancy. In the emballonurid *P. kappleri* approximately 75% of successive ovulations alternate between the ovaries. In one recently postpartum female, however, the new ovulation had occurred on the same side that had carried the prior pregnancy. Spermatozoa had been able to ascend into the oviduct contralateral to the ruptured follicle, but not into the ipsilateral one because the postpartum uterine cornu was still blocked by debris. This had prevented fertilization of the egg. In three other bats, the remnants of a degenerate ovum (an empty zona pellucida) were observed in the oviduct ipsilateral to a recently parturient horn, possibly for the same reason (Rasweiler, 1982, 1993).
2. All of the species that alternate successive ovulations also exhibit local stimulation of the ipsilateral oviduct and/or uterine horn. In the case of polyoestrous bats with bicornuate or duplex uteri (e.g. *P. kappleri* and some of the pteropodids), the ability to alternate ovulations and to stimulate the reproductive ducts via local pathways may decrease the time that must pass between some common types of reproductive failures and efforts to establish a new pregnancy. Since endometrial development on the side of the uterus contralateral to the CL is minimal during early pregnancy (and presumably during the luteal phase of any nonpregnant cycle) in many of these species, it seems likely that this horn might be prepared to accommodate first sperm and then a conceptus with minimal delay in the event of a fertilization failure or the loss of an early embryo. A well-developed, but aging/deteriorating endometrium of pseudopregnancy would not first have to be eliminated, because this would frequently or always be limited to the uterine horn ipsilateral to the CL. This might permit these animals to complete the new pregnancy and to successfully rear young within the same reproductive season. This should be of considerable adaptive value to species that have low reproductive potentials.

In the case of *P. kappleri*, a number of females that were collected from a reproductively synchronized population living in the Colombian Andes showed evidence of having suffered reproductive failures, e.g. the remnants of degenerate ova or embryos in their oviducts. Furthermore, several females carried pregnancies that had probably been established well after those borne by the bulk of the population. It seems reasonable to suspect that these exceptional females may have experienced reproductive failures and then ovulated again (Rasweiler, 1982, 1993).

In most *P. kappleri* (n = 7/10) carrying early uterine embryos, both oviducts appeared involuted relative to the periovulatory condition, and mitotic activity was rare in the uterine horn contralateral to the CL. Interestingly, however, in the remaining three bats 'reverse' unilateral oviductal and/or uterine reactions were evident. The epithelial cells of the oviduct contralateral to the CL in all three animals appeared more hypertrophied and, in two of these, many of the cells contained higher levels of glycogen as well. Furthermore, in two of the females mitotic figures had become abundant in the epithelium of the nonpregnant uterine horn. In all three bats, the nonluteal ovary also contained growing vesicular follicles, although the largest always displayed early signs of atresia. Stimulation of the nongravid side of the tract in these animals may simply reflect a failure of pregnancy to completely suppress follicular development there. On the other hand though, it may indicate that the nongravid side is poised to produce a new embryo quickly should the existing pregnancy fail (Rasweiler, 1982).

Because differential stimulation of the two uterine horns is not pronounced in many *N. albiventris* during early pregnancy, it seems difficult at first to envision how, in the event of a reproductive failure, this might facilitate preparation of the uterus for another attempt at establishing a pregnancy. The gravid horn does undergo a pronounced decidual reaction at the time of implantation, however, and this reaction does not extend to the nongravid horn. This suggests that, in the event of the early loss of an embryo – even an implanting or implanted one – the nongravid horn might be prepared more rapidly for another attempt at establishing a pregnancy. This would be facilitated by the high incidence of alternation of successive ovulations in *N. albiventris* (ca. 92%) (Rasweiler, 1978, 1993). Alternatively, the unilateral reactions observed in the female reproductive tracts of *N. albiventris* and many phyllostomid bats may very well be primitive retentions that are of limited, or no, adaptive value to these species.

An important element that is still missing from the story for the species that exhibit unilateral endometrial reactions is exactly how the endometrium that is preferentially stimulated is dealt with in the event of a reproductive failure. Is this gradually recycled by means of lysosomal action and the secretion of extracellular proteases, as occurs in some subprimate mammals (Padykula, 1980), or does the most highly differentiated portion of the lamina functionalis degenerate and then slough off in menstrual fashion? One could argue that the alternation of successive ovulations in conjunction with preferential stimulation of the endometrium in just one uterine horn eliminates the need for rapid/efficient recycling of that horn (as might be accomplished by a menstrual process). This question might be best addressed through studies of a pteropodid bat, some of which can be easily maintained in captivity.

5.5.3 Menstruation

Although there have been unsubstantiated assertions that menstruation may be common among mammals (Profet, 1993), there is good evidence for its occurrence only in catarrhine and platyrrhine primates, phyllostomid and molossid bats, and elephant shrews (Hamlett, 1934; van der Horst and Gillman, 1941; Eckstein and

Zuckerman, 1956; Rasweiler, 1970; 1979a,b, 1991a; Quintero and Rasweiler, 1973, 1974; Rasweiler and Bonilla, 1992). A menstrual process may also occur in some of the tree shrews, *Tupaia* sp. (Conaway and Sorenson, 1966), but that requires further study.

There has long been a question as to the adaptive significance of menstruation. It is now clear, however, that all menstruating species share common reproductive characteristics that would seem to point to the probable value of the process. The anthropoid primates and many of the phyllostomid bats possess simplex uteri, which eliminates the possibility of preparing alternate cornua for pregnancies in successive cycles. At least two menstruating species, *D. rotundus* and *M. ater*, do possess partially bicornuate uteri. In *D. rotundus*, however, the cornua are short, and no evidence of their differential stimulation has ever been noted in nonpregnant or pregnant individualts carrying preimplantation embryos. The possibility has even been raised that *D. rotundus* may have evolved from menstruating ancestors with simplex uteri, and this receives some support from the fact that the closely related *D. youngi* has a uterus with extremely short cornua (Rasweiler, 1982; Badwaik *et al.*, 1998; J.J. Rasweiler and H.F. Quintero, unpublished observations). *M. ater* has a bicornuate uterus, but only the right horn normally carries pregnancies (Table 5.2).

All of these menstruating species that have been adequately studied also exhibit advanced endometrial growth and differentiation during the nonpregnant cycle. In at least some of the catarrhine primates (the human and the chimpanzee), the elephant shrew and *M. ater*, the endometrium develops predecidual, or even true decidual cells, in the absence of an embryo. Prominent predecidual or decidual reactions have not been observed during the luteal phase of the cycle in all catarrhine primates (Brenner and Maslar, 1988; Enders, 1991) or in premenstrual or menstrual *Carollia* sp. All of these species do, however, develop thickened endometria after ovulation, and it seems reasonable to suspect that they have become highly differentiated in a functional sense, in preparation for the possible implantation of a blastocyst.

The adaptive significance of menstruation may rest in the fact that it permits females to eliminate such endometria rapidly in the event of fertilization failures or early embryo losses. This may, in turn, give the seasonal breeders another opportunity to establish a pregnancy and to bear young still at a reasonably optimal time within the same season. This would be an obvious advantage to species with the relatively low reproductive potentials characteristic of all of the menstruating species (Rasweiler, 1991a, 1992; Rasweiler and Bonilla, 1992).

It has been more difficult to see the possible advantage of menstruation to individual humans. Modern-day women do not exhibit discrete mating and birth seasons, although annual reproductive rhythms are still clearly evident in the birth statistics for large human populations (Roenneberg and Aschoff, 1990a,b). Furthermore, women now have long reproductive life-spans, and so can potentially produce many children. It has been difficult, therefore, to envisage the significance of shortening their reproductive cycle by menstruation (Finn, 1987; Rasweiler, 1991a). It should be kept in mind, however, that our own species evolved from ancestors that menstruated and were also presumably seasonal breeders. Studies of free-ranging primate populations have established that many display either birth seasons or birth peaks (Lancaster and Lee, 1965).

Two other proposals have recently been put forth to explain why some mammals may menstruate. Profet (1993) has suggested that menstruation may have evolved to protect the uterus and oviducts from pathogens transported up the female tract by spermatozoa. This has received no support from reproductive physiologists or specialists in reproductive medicine (Finn, 1994; Strassmann, 1996). Profet's thesis assumes that menstruation must occur widely among mammals, when there is little evidence for this. It makes no effort either to deal with species (such as some bats) that exhibit prolonged female receptivity to the male and/or sperm storage. Furthermore, in the event of conception, menstruation would not occur. During years when environmental conditions permit normal reproduction, nonpregnant cycles may be quite uncommon in wild mammals that menstruate. Menstruation is also a relatively rare event among women of reproductive age in populations (e.g. more primitive societies) that do not practise contraception (Short, 1976; Strassmann, 1996).

Profet attempts to get around this problem by asserting that other forms of uterine bleeding (prooestrous, periovulatory, implantational and postpartum) have a similar antipathogen function. However, this ignores the fact that female *M. ater* are receptive to males for prolonged periods and usually do not exhibit prooestrous, periovulatory or implantational bleeding. A few exceptional preovulatory females were still menstruating after having run nonpregnant cycles but, as noted above, such cycles may occur infrequently in the wild (Rasweiler, 1987, 1988, 1990, 1991a). In *Carollia* sp., significant bleeding into the uterine cavity normally occurs only at the end of nonpregnant cycles and immediately after parturition. There is no suggestion that it might occur routinely after copulations by pubertal females or those coming out of a seasonal anoestrous period (Rasweiler and Bonilla, 1992; Badwaik *et al.*, 1997; J.J. Rasweiler and N.K. Badwaik, unpublished observations).

Carollia perspicillata does exhibit an unusual, and sometimes pronounced, extravasation of blood into the endometrial stroma (not the uterine lumen) immediately prior to and during implantation. This appears to be an exaggeration of the increased capillary permeability observed at the implantation site in a variety of other mammals. In *C. perspicillata* this extravasation has been observed between days 12–16 *p.c.* and thus well after insemination (Oliveira *et al.*, 2000). It is therefore very difficult to believe that such bleeding may be somehow designed to cope with pathogens transported up the female tract by spermatozoa.

Strassmann (1996) has proposed that the uterine endometrium is shed or resorbed whenever implantation fails because cyclical regression and renewal are energetically less costly than maintaining the endometrium in the metabolically active state required for implantation. She has proposed that the cost of accomplishing the latter would be further increased by the associated hormonal conditions. These are responsible for a higher basal metabolic rate during the luteal phase of the menstrual cycle in women.

There are a number of problems with Strassman's theory:

1. In the evolution of mammalian reproductive cycles it is highly unlikely that, following the failure of embryos to implant, the endometrium could continue to

be maintained in an implantation-ready state until new blastocysts are produced. That is because the reproductive tract must also carry out a variety of other important functions (e.g. the production and release of eggs, as well as the transportation and maintenance of spermatozoa and preimplantation embryos). These generally require different endocrine conditions and responses by the female tract than those that would prevail during implantation. For example, in both *M. ater* and *C. perspicillata* (as well as a number of other mammals), implantation is preceded by, or associated with, closure of the uterine lumen (Rasweiler, 1987, 1990, 1991a; Oliveira *et al.*, 2000). Closure not only brings the blastocyst into close contact with the endometrium, but in at least some bats seems to play a critical role in positioning the blastocyst (or even unfertilized eggs) at the preferred implantation site (Rasweiler, 1991a, 1992; Oliveira *et al.*, 2000). In the case of *M. ater*, closure is also associated with the expulsion of spermatozoa that had previously colonized the uterus, and it precludes new sperm transport or movement up the tract. When *M. ater* that had developed spontaneous decidual reactions were placed with males and mated, spermatozoa were observed in their cervices but had been unable to enter their uterine cornua because of closure (Rasweiler, 1987, 1991a).

2. When one looks at the extensive endometrial changes that occur in the nonpregnant cycle of *M. ater*, as well as the mass of tissue that is desquamated during menstruation and then reformed (Rasweiler, 1991a), it is difficult to accept that this could be significantly less expensive energetically than maintaining an implantation-ready endometrium.
3. As noted previously, it is probably unusual for free-ranging wild mammals (including those which menstruate) to experience many nonpregnant cycles. During normal reproductive seasons, it is more likely that they will breed and conceive. If there are extended periods that are unsuitable for carrying pregnancies to term and rearing young, females are instead likely to enter seasonal anoestrous periods, or in some species to exhibit sperm storage or delays in development of the conceptus.
4. The energetic costs of maintaining a peri-implantational endometrium must pale in comparison to many other activities routinely conducted by female mammals (e.g. carrying pregnancies and lactation).
5. Finally, many mammals maintain both the endometrium and embryos for extended periods, at reduced levels of metabolic activity, during delayed implantation (Aitken, 1977, 1981; Mead, 1993). Although its adaptive significance has not been established for every species, delayed implantation usually occurs because of energetic and nutritional considerations, such as when the females are simultaneously lactating, or environmental conditions would not be favorable for more rapid progression of the pregnancy and/or the rearing of young. Because delayed implantation has apparently evolved independently on multiple occasions in mammals (Sandell, 1990), the question arises as to why some mammals also have not developed the ability to continue to maintain endometria in a peri-implantational, but less active metabolic state, after fertilization failures or the loss of early embryos (i.e. until new embryos could be produced). This would seem to be a logical alternative to menstruation, if Strassmann's theory is valid. The likely explanation is that the reproductive

> tract must first be recycled, so that it can carry out the other important functions outlined above.

Neither Profet nor Strassmann display a sufficient appreciation that cyclic endometrial differentiation may also progress too far to be compatible with the initiation of a new implantation following a fertilization failure or early embryonic loss. *Molossus ater* exhibits profound stromal decidualization and endometrial vascular changes late in its nonpregnant cycle. This creates an endometrium which is really quite different from the one existing at the initiation of implantation (Rasweiler, 1990, 1991a). It is well known, from embryo transfer experiments, that proper synchrony must exist between the postovulatory state of the embryo and the endometrium in order for implantation to occur (Psychoyos, 1973; Hodgen and Itskovitz, 1988; Tabibzadeh and Babaknia, 1995; Beier, 1997). Menstruation provides an efficient means of dispensing with such a highly differentiated endometrium. Finally, it is incorrect for Profet and Strassmann to state that the antipathogen hypothesis is the first attempt to explain the function of menstruation (see Finn, 1987; Rasweiler, 1982, 1991a, 1992; Rasweiler and Bonilla, 1992).

This leads to the question of why menstruating species should exhibit relatively advanced endometrial differentiation during their cycles. Finn (1987) made the reasonable suggestion that this may relate to problems associated with an invasive form of implantation, as exemplified by the interstitially-implanting human blastocyst. Implantation in menstruating species (anthropoid primates, molossid and phyllostomid bats, and elephant shrews) actually varies from central to interstitial (Luckett, 1974; Wimsatt, 1975; Rasweiler, 1979a, 1990). All of these animals do, however, possess invasive trophoblast and eventually form endotheliochorial and/or haemochorial placentas (Badwaik *et al.*, 1998; Rasweiler and Badwaik, 1999b). This suggests that some of the endometrial differentiation evident during their reproductive cycles is related to the development of mechanisms for controlling and/or limiting trophoblastic growth in the event of pregnancy. Although this is generally thought to be one of the functions of decidual cells (Bell, 1985; Loke and King, 1995), evidence has not yet been obtained for the formation of decidual or even pre-decidual cells during the nonpregnant cycle in all of these species. Dollar *et al.* (1982) concluded that the endometrium of the baboon exhibits a pre-decidual reaction, but Enders (1991) did not find their observation of relatively small numbers of rotund stromal cells very convincing. Also, with an unusual exception (see below), clear examples of decidual or pre-decidual cells have not been noted in premenstrual or menstrual uteri of *C. perspicillata* (J.J. Rasweiler, H. Bonilla and N.K. Badwaik, unpublished observations). This may simply reflect the failure to examine the endometria of these animals late in the cycle with sensitive enough techniques (e.g. electron microscopy). On the other hand, functional differentiation of the endometrium in these species, in preparation for possible trophoblastic invasion, could be significant even in the absence of such overt morphological changes in the stromal cells.

Endometrial differentiation in *M. ater* is not limited to the stromal cells, but also extends to the endothelial cells in some of the endometrial vessels. Some of these cells hypertrophy and develop more prominent basal laminae both late in the menstrual cycle and during early pregnancy. Substantial evidence has been obtained to suggest that maternal endothelial cells may be playing major roles in controlling trophoblastic growth and differentiation in *M. ater* and possibly many other species (Rasweiler, 1991b, 1992, 1993; Rasweiler and Badwaik, 1999b; Badwaik and Rasweiler, this volume).

Clearly, in order to fully understand the significance of menstruation, it will be important to identify in a variety of menstruating animals (not just higher primates) (1) how the endometrium differentiates in a biochemical and functional sense during the cycle, and (2) why in these species the endometrium must be recycled by a process that includes substantial cell death, extracellular matrix breakdown and sloughing, rather than by dedifferentiation or phenotypic modulation of its cellular components, less frequent cell death, and more modest changes in the extracellular matrix.

In *C. perspicillata*, it was not unusual to see decidual giant cells in the isthmus of the uterus of periovulatory, or early pregnant, parous females. Such cells were abundant there on the day of birth and during succeeding weeks, but a few may be able to persist for prolonged periods or possibly even to develop there anew in nonpregnant cycles. Decidual giant cells were noted in the isthmus of one preovulatory female examined on day 1 *p.c.* – 176 days after that female had last given birth. In this species, periodic menstrual sloughing does not appear to extend to the endometrial lining of the isthmus (J.J. Rasweiler and N.K. Badwaik, unpublished observations).

Finally, it deserves to be noted that while all menstruating animals have invasive trophoblast, some populations of trophoblast are unusually invasive. In the human, the extravillous or ‘intermediate’ trophoblast can normally penetrate as far as the inner myometrium by migration into the spiral arterioles that supply the placenta (the endovascular route) and through the connective tissues of the uterus (the interstitial route) (Pijnenborg *et al.*, 1981; Pijnenborg, 1996). The extravillous trophoblast of macaques is also quite invasive, as indicated by its migration relatively soon after the initiation of implantation into the walls of some of the endometrial spiral arterioles, and possibly the adjacent endometrial stroma, at some distance from the developing placenta (Blankenship *et al.*, 1993a,b; Enders *et al.*, 1996; Blankenship and Enders, 1997). In comparison, the trophoblast of cricetid and murid rodents (hamsters, mice and rats) invades the endometrium during pregnancy, but some continues on into the myometrium only via an endovascular route along the uterine arteries (Carpenter, 1982; Pijnenborg *et al.*, 1981).

A variety of bats, including at least two phyllostomids (*C. perspicillata* and *D. youngi*) and a molossid (*M. ater*) also develop some trophoblast that is extraordinarily invasive (Badwaik and Rasweiler, this volume; Badwaik *et al.*, 1998; Rasweiler and Badwaik, 1999b). In the case of one *D. youngi* that carried an early implanted blastocyst, some trophoblast had penetrated the myometrium almost to the serosal surface of the uterus. This penetration had taken place solely via interstitial migration along the maternal blood vessels. In *C. perspicillata*, widespread trophoblastic penetration of the myometrium occurs via the same route at the primitive streak stage, but apparently only in delayed pregnancies. In all three species, the most highly invasive trophoblast can migrate at least as far as the mesenteries of the reproductive tract and, to a limited extent, the ovaries. Even neglecting these specialized populations, the trophoblast in bats is quite invasive, progressing as far as the endometrial-myometrial border and establishing endotheliochorial or haemochorial placentae. Thus, it would seem that in addition to nourishing the embryo, some of the endometrial development and differentiation observed in these animals during early pregnancy must be directed at controlling trophoblastic growth and probably differentiation should implantation occur. In the event of a fertilization failure or early embryonic death, menstruation provides an efficient mechanism for recycling an endometrium that has undergone such changes.

Whatever the merits of these arguments, it seems clear that further studies on bats should be able to add significantly to our understanding of the mechanisms involved in, and the adaptive significance of, menstruation.

ACKNOWLEDGMENTS

Original research reported in this chapter was supported by the Department of Obstetrics and Gynecology, Cornell University, and by National Institutes of Health Grants AI-10050, HD-17739 and HD-28592. Manuscript preparation was also supported by Cornell and NIH Grant HD-28592.

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6

Pregnancy

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6.1 INTRODUCTION

In view of the remarkable adaptive radiation that has occurred during the evolution of the Chiroptera, it is perhaps not surprising that members of this order exhibit both some common, and many different, reproductive characteristics relating to pregnancy. In general, bats have low reproductive potentials, producing only one to several young in each pregnancy. Furthermore, in comparison to many other small mammals, bats give birth to young that are large relative to the mass of the mother and after gestation periods that are often quite long. On the other hand, however, bats exhibit unusual species' diversity with respect to the stage of embryonic development attained within the oviduct, the anatomy of the uterus,

Reproductive Biology of Bats
0-12-195670-9

positioning of the blastocyst within the uterus at the time of implantation, development of their foetal membranes, location and morphogenesis of their chorioallantoic placentae, nature of the interhaemal barrier in the placenta, and the temporal aspects of conceptus development.

This has created both opportunities and problems for those interested in chiropteran and comparative mammalian reproductive biology. Ideally, the study of pregnancy-related processes and characteristics should help us in efforts to sort out phylogenetic relationships within the Chiroptera and possibly with other mammalian orders. In the case of bats, however, the diversity in these processes and characteristics has often proved more confusing than helpful in this regard. This probably reflects the need for more information and analysis, rather than the existence of insurmountable obstacles to this line of inquiry. Comparative studies that attempt to explain how and why such diversity has developed within a closely related group, such as bats, can also help us to better understand similar processes or events in other mammals. When some aspect of pregnancy in a bat species closely resembles that in humans, that species is an obvious candidate for possible development as an experimental model. Finally, some bats exhibit phenomena during gestation that are clearly unusual when compared to what occurs in many other species. This includes, for example, the differential transport of living embryos and degenerating ova by the oviducts, or the occurrence of significant postimplantational delays in conceptus development. Although these are inherently fascinating, their study might also lead to the discovery of underlying processes and/or controls of broader relevance in mammalian reproduction.

Information has now become available on pregnancy in many different bat species. Because of space limitations and the desire to prepare a readable account, coverage has therefore often been restricted to the better-studied representatives of each chiropteran family. Also, when species' names have been changed, the nomenclature used by Koopman (1994) has generally been substituted for those in the original studies.

6.2 PREIMPLANTATION DEVELOPMENT

6.2.1 Oviductal Phase of Development

Embryonic development in the oviduct

Information is now available on the early embryology of representatives of most families of bats, and it is evident that there are major differences in the stage of development attained in the oviduct (Table 6.1). In bats belonging to the families Molossidae, Rhinolophidae, Rhinopomatidae and Vespertilionidae, embryos pass into the uterus during cleavage or at the morula stage. Where temporal data have been obtained from captive-maintained animals, the sojourn of the embryo in the oviduct was found to be relatively short (<4–5 days in the little brown bat, *Myotis lucifugus*, and < 5 days in the black mastiff bat, *Molossus ater*). In the little sac-winged bat, *Peropteryx kappleri* (family Emballonuridae), and some of the pteropodids, development usually progresses to the blastocyst stage in the oviduct. In the noctilionid and phyllostomid bats, embryos develop to the blastocyst stage

Table 6.1 Patterns of preimplantation development in selected species of bats

Family and species	Latest oviductal stage observed	Earliest uterine stage observed	References
Family Pteropodidae			
Ptenochirus jagori	Morula (c. 250 cells)	Blastocyst (–ZP)	Heideman and Powell, 1998
Pteropus giganteus	Morula (64 cells)	Morula (at least 128 cells)	Marshall, 1949
Rousettus amplexicaudatus	Blastocyst (–ZP)	Blastocyst (–ZP)	Kohlbrugge, 1913
Rousettus leschenaulti	Blastocyst (+ZP)	Blastocyst (+ZP)	Karim, 1972a
Family Emballonuridae			
Peropteryx kappleri	Blastocyst (+ZP)	Morula (48 cells)	Rasweiler, 1982
Family Megadermatidae			
Megaderma lyra	–	8 cells	Gopalakrishna and Khaparde, 1978a
Family Molossidae			
Chaerephon plicata	8 cells	Morula (about 32 cells)	Pendharkar and Gopalakrishna, 1983
Molossus ater	10 cells	Morula (17 cells)	Rasweiler, 1988, 1990
Tadarida aegyptiaca	2 cells	Blastocyst (free)	Gopalakrishna *et al.*, 1991
Family Mystacinidae			
Mystacina tuberculata	Blastocyst (+ZP)	–	Rasweiler, Badwaik and Daniel, unpublished observations
Family Noctilionidae			
Noctilio albiventris	Blastocyst (–ZP)	Blastocyst (–ZP)	Rasweiler, 1977
Family Phyllostomidae			
Carollia perspicillata	Blastocyst (–ZP)	Blastocyst (–ZP)	Badwaik *et al.*, 1997
Desmodus rotundus	Blastocyst (–ZP)	Implanting blastocyst	Wimsatt, 1954; Quintero and Rasweiler, 1974, unpublished observations
Glossophaga soricina	Blastocyst (–ZP)	Implanting blastocyst	Rasweiler, 1972, 1974
Macrotus californicus	Blastocyst (–ZP)	Implanting blastocyst	Bleier, 1975
Family Rhinolophidae			
Hipposideros speoris	16 cells	Morula	Jeevaji, 1979; Gopalakrishna and Bhatia, 1983
Rhinolophus blasii	5 cells	Morula (22–26 cells)	Levi, 1915
Rhinolophus euryale	3 cells	11 cells	Levi, 1915
Rhinolophus rouxi	Morula (22 cells)	3 cells	Gopalakrishna and Ramkrishna, 1983
Family Rhinopomatidae			
Rhinopoma hardwickei	Morula (48 cells)	Morula (16 cells)	Karim and Fazil, 1987
Family Vespertilionidae			
Eptesicus fuscus	about 12 cells	3–5 cells	Wimsatt, 1942, 1944b
Miniopterus schreibersii	Morula (34 cells)	Morula (38 cells)	Gopalakrishna and Chari, 1985
Myotis lucifugus	4–7 cells	4 cells	Wimsatt, 1944a; Buchanan, 1987
Myotis myotis	8 cells	8 cells+	Sluiter and Bels, 1951
Pipistrellus ceylonicus	Morula (16 cells)	Morula (18 cells)	Gopalakrishna *et al.*, 1988
Vespertilio murinus[a]	4 cells to morula	3 cells to morula	Duval, 1895a; van Benden, 1899, 1911; Levi, 1915; Redenz, 1929

[a] In some cases the bats referred to as *V. murinus* by earlier investigators may in fact have been *M. myotis* (discussed briefly by Allen, 1939).

and lose their zonae pellucidae in the oviduct. Temporal data obtained from laboratory-bred females indicate that the tubal journey of the embryo is also prolonged in some of the phyllostomid bats (12–13 days in the long-tongued bat, *Glossophaga soricina*, > 11 days in the short-tailed fruit bat, *Carollia perspicillata*, and >13 days in the common vampire bat, *Desmodus rotundus*) (Rasweiler, 1972; Bonilla and Rasweiler, 1974; Quintero and Rasweiler, 1974; Badwaik *et al.*, 1997). These are the longest tubal journeys recorded for mammals (Wimsatt, 1975). Furthermore, loss or shedding of the zona pellucida (an extracellular covering around the egg/embryo) is generally a uterine phenomenon in other mammals.

A zona-encased oviductal blastocyst has also been found in a lesser short-tailed bat, *Mystacina tuberculata* (family Mystacinidae), fortuitously preserved as a museum specimen (Rasweiler and Badwaik, this volume). Unfortunately, we still do not know whether the blastocyst of this endangered species loses its zona pellucida in the oviduct or uterus.

For much of the time that the embryo is passing through the oviduct in the noctilionid and phyllostomid bats, the oviductal secretory cells (particularly in the ampulla and isthmus) have an unusual, highly vacuolated appearance in standard histological sections. This is attributable for the most part to the presence of

Figure 6.1 Electron micrograph of the ampullary epithelium in the oviduct carrying a preimplantation embryo obtained from a *Carollia perspicillata* on day 6 *post coitum*. The epithelium is composed of ciliated (C) and secretory (S) cells. The latter contain large glycogen deposits (G) and some lipid droplets (e.g. at arrowhead). Bar = 1.58 μm.

unusually large quantities of glycogen in the secretory cells (Rasweiler, 1977, 1993), although in the case of *C. perspicillata* some lipid droplets occur as well (Figure 6.1).

In the noctilionid and phyllostomid bats, there appears to have been a significant shift in responsibility for maintenance of the preimplantation embryo from the uterus to the oviduct as compared to other mammals. Not only is embryonic development relatively advanced in the oviduct, but glycogen (which is presumably broken down to nourish the early embryos) is present in smaller amounts in the uterine glandular epithelial cells of *N. albiventris* and *C. perspicillata* than in the oviducts (J.J. Rasweiler, 1993, unpublished observations).

Although systematic biologists have largely ignored early pregnancy in assessing phylogenetic relationships among bats, some characteristics of the embryo and maternal reproductive tract during this period would seem to be of considerable value in this regard. For example, early investigators thought that the noctilionid bats were most closely related to the emballonurids, while it has been concluded more recently that the noctilionids actually possess closer systematic affinities to the phyllostomids (Hood and Pitocchelli, 1983; Forman *et al.*, 1989; Koopman, 1994). Based upon comparative studies of early pregnancy, the latter view would clearly seem to be the correct one. *Noctilio albiventris* and *Carollia* sp. share development of their embryos to the zona pellucida-free blastocyst stage in the oviduct and engorgement of their oviductal secretory cells with glycogen. There seems to be little question that these are unique, derived, homologous reproductive characters (synapomorphies). On the other hand, in *P. kappleri* (an emballonurid bat) the embryo develops to the blastocyst stage in the oviduct, but does not shed its zona pellucida until passing into the uterus, and less glycogen accumulates in the cytoplasm of the oviductal secretory cells during early pregnancy (Rasweiler, 1982, 1993).

The observation of a zona-encased blastocyst in the oviduct of a *M. tuberculata*, as well as other female reproductive characteristics of this species, provides additional evidence that the mystacinids may be more closely allied to the noctilionoid than to the vespertilionoid bats (Rasweiler and Badwaik, this volume).

Significance of advanced embryonic development in the oviduct

Development to the blastocyst stage in the oviduct occurs only in five families of bats, the Emballonuridae, Mystacinidae, Noctilionidae, Phyllostomidae and Pteropodidae. It is also relatively uncommon among other mammals, having been previously observed only in the loris (*Nycticebus*), the tree shrew (*Urogale everetti*), the short-tailed shrew (*Blarina brevicauda*), long-tailed shrews (*Sorex* sp.), the armadillo (*Dasypus novemcinctus*), the northern fur seal, and rabbits (involving only some embryos) (reviewed by Rasweiler, 1977).

In the case of bats, there would seem to be three reasons why development to the blastocyst stage in the oviduct might be advantageous:

1. Some species with this trait (e.g. *C. perspicillata*, *P. kappleri*, and *Rousettus leschenaulti*) can also experience a postpartum oestrus. In the case of *C. perspicillata*, which has a simplex uterus, it may be advantageous to hold the embryo in the oviduct for as long as possible, until postpartum endometrial

regeneration has been completed. *Peropteryx kappleri* and *R. leschenaulti* have bicornuate uteri and frequently alternate successive ovulations between the ovaries. Therefore, if conception occurs at a postpartum oestrus, the new pregnancy is often (*P. kappleri*) or usually (*R. leschenaulti*) carried in the opposite uterine cornu from the previous one. This reduces or eliminates the need for time to reestablish a receptive implantation site in the postparturient cornu. However, depending upon the endometrial growth curve for these species, prolonged retention of the embryo in the oviduct may still be required to adequately prepare the opposite cornu for implantation.

2. *Carollia* sp., *N. albiventris*, *P. kappleri* and *R. leschenaulti* all exhibit implantation within relatively small, predetermined implantation zones (see below). Transport of the embryo into the uterus at the blastocyst stage may facilitate its proper attachment within this zone.
3. Finally, all of these species exhibit unilateral stimulation of the oviducts and/or uterus around the time of ovulation (see Rasweiler and Badwaik, this volume). This presumably involves the delivery of ovarian hormones to the ducts via local pathways and suggests that the quantity of hormones being released into the systemic circulation by the ovary containing the Graafian follicle or corpus luteum must be low (or the ducts on both sides would be equally stimulated). This suggests, in turn, that either the local pathway for hormone delivery must be very efficient or it takes time to adequately stimulate the endometrium at the prospective implantation site. If the latter situation prevails, it could be advantageous to hold the embryo in the oviduct for a relatively long period.

Differential transport of living and dead eggs

Because many bats are small, it is much less of a task to serially-section and examine the female reproductive tract microscopically than would be the case with larger mammals. When this was done with captive-bred, *G. soricina* (family Phyllostomidae), for studies of the early embryology of the species, the discovery was made that its oviducts can somehow distinguish between living and dead eggs (Rasweiler, 1979a, 1993). In females carrying oviductal or early uterine embryos, retained eggs from previous nonpregnant cycles (which usually had occurred prior to the introduction of breeding males) were frequently observed in the oviducts. Furthermore, in a number of cases, a dead ovum had been retained in the same oviduct that had transported a living embryo into the uterus! The zona pellucida shed by the blastocyst was also usually retained by the oviduct when transport into the uterus occurred.

It was initially believed that unfertilized mammalian eggs or degenerating embryos are transported into the uterus much like living embryos, in response to changing ovarian hormone levels. However, unfertilized ova accumulate in the oviducts of mares, donkeys and some aged mice and hamsters, as well as in several other bats. There is also now evidence for the differential transport of fertilized and unfertilized rat ova (Villalón *et al.*, 1982). The remnants of retained ova have been observed in the oviducts of field-collected *P. kappleri* (family Emballonuridae) and *N. albiventris* (family Noctilionidae), and captive-bred *Artibeus lituratus* (family

Phyllostomidae) after transport into the uterus should have occurred (Rasweiler, 1977, 1993, unpublished observations).

The mechanisms whereby the oviducts distinguish between living embryos and dead eggs or discarded zonae pellucidae remain to be identified. Some work with horses suggests that prostaglandin E_2 secreted by their embryos might be responsible for stimulating transport out of the oviduct. It is difficult to imagine how the response to such a chemical stimulus might be localized at the level of the tunica muscularis, so as to permit living embryos to be transported past dead ova and empty zonae pellucidae in the same oviduct (Rasweiler, 1979a, 1993; Hunter, 1989). Some attention deserves to be focused upon the possibility that electric currents generated by living embryos (see Marx, 1981), working in conjunction with physical differences from the dead ova or empty zonae, might be involved.

Finally, there is the interesting question of why such differential transport occurs. In the case of bats, there would seem to be two reasonable possibilities. One might be to minimize interference by discarded zonae pellucidae with initial attachment of the blastocyst to the endometrium at the implantation site. The retention of dead ova or embryos may be incidental to that. While this could be important to *G. soricina* and *N. albiventris* (which exhibit shedding of the zona in the oviduct), it would not apply to *P. kappleri* (which exhibits shedding of the zona at the implantation site in the uterus). The other possibility might be that contractions of the oviductal musculature may be finely controlled and localized to the vicinity of living embryos in order to prevent transport of the latter beyond the preferred implantation zone (which is limited in extent).

6.2.2 Uterine Phase of Preimplantation Development

Embryo transport in the uterus

In the case of the emballonurid, phyllostomid and noctilionid bats, the site of implantation is close to the uterotubal junction, and the earliest uterine blastocysts have generally been found within the implantation zone. Unfortunately, it is difficult to study carefully the initial stages of blastocyst attachment to the endometrium, because these are so easily disrupted by the shrinkage associated with standard histological processing. One gets the impression, however, that the implantation process in these species may commence relatively soon after the embryo enters the uterus.

Good temporal data on early uterine development of the embryo are not available for any of the pteropodids. However, like the emballonurid and noctilionid bats, these species exhibit preferential stimulation of the prospective implantation site (which is at the cranial end of the uterine horn on the side of ovulation). Furthermore, embryonic development within the oviduct is advanced, particularly in *Rousettus* sp. This may also prepare the blastocyst for attachment relatively soon after passing into the uterus.

The sequence of events clearly is quite different in the molossid, rhinolophid and vespertilionid bats. In these families the embryo generally is transported into the uterus during early cleavage or at the morula stage, and implantation occurs at a site further removed from the ovary that originally released the egg. In the molossid *M. ater*, ovulation always occurs from the right ovary and implantation then takes place in the middle of the right uterine horn. In the vespertilionid *M. lucifugus*, ovulation occurs with about equal frequency from either ovary, but implantation is nearly always in the middle of the right uterine horn (Wimsatt, 1944a,b, 1945a, 1975). This means that in approximately half of all pregnancies the embryo must be transported through the left horn in order to reach the right one. In another vespertilionid, *Miniopterus schreibersii*, ovulation usually takes place from the left ovary, but implantation always occurs in the middle of the right uterine horn. In the most thorough study of this, 285 pregnant specimens were examined histologically. The corpus luteum was in the left ovary of 281 and the right ovary of 4. Of the 77 specimens with secondary oocytes or preimplantation stage embryos, 21 had secondary oocytes to early morulae in the left oviduct, 26 had morulae up to the 38 cell stage in the left uterine horn, and 30 had late morulae (38 cells or more) to free blastocysts in the right uterine horn. Two other specimens had a secondary oocyte or a morula in their right oviduct and a corpus luteum in the right ovary (Gopalakrishna *et al.*, 1981; Gopalakrishna and Chari, 1985).

In those bats which exhibit delayed implantation (*Eidolon helvum, Miniopterus* sp., some *Rhinolophus rouxi*), the embryo would remain unattached in the uterine lumen for a much longer period.

6.3 IMPLANTATION OF THE BLASTOCYST

6.3.1 Site of Implantation

Implantation in bats is of special interest because this process generally seems to occur at predetermined, highly localized sites. Depending upon the species, these may be found near the cranial end, middle, or even towards the caudal end of the uterus (Table 6.2). This contrasts with the situation in the more commonly studied litter-bearing species, where virtually the entire length of the uterus is competent for implantation purposes (although mechanisms do operate that result in spacing of the embryos) (Wimsatt, 1975).

This raises the question of how, in bats, embryos may be directed to the correct implantation sites. Presumably contractile activity of the oviductal and uterine musculatures are involved in the transport process, but one must suspect that other factors may also play roles in directing implantation to the preferred region. These might include closure of the uterine lumen and regional variations in the ability of uterine epithelial elements to affect, or interact with, the embryo. In the emballonurid *P. kappleri*, transport of the embryo beyond the normal implantation site at the cranial end of the uterine horn seems to be prevented by such closure. Also, the most stimulated uterine luminal and glandular epithelial cells are found in this region. In the molossid *M. ater*, ovulation occurs only from the right ovary, and implantation always seems to take place in the middle of the right uterine horn. During implantation, the uterine lumen was observed progressively closing around implanting blastocysts in histological sections (Rasweiler, 1990). This probably commences even earlier than recognizable, because some shrinkage and opening up of artifactual spaces typically occurs during paraffin embedding.

Table 6.2 Location of blastocyst implantation site along the longitudinal axis of the uterus in selected species of bats

Site	Family	Species	References
Cranial end of uterine cornu	Pteropodidae	*Cynopterus sphinx*	Gopalakrishna and Murthy, 1960
		Haplonycteris fischeri	Heideman, 1989
		Otopteropus cartilagonodus	Heideman *et al.*, 1993
		Pteropus giganteus	Marshall, 1953
		Rousettus leschenaulti	Gopalakrishna and Karim, 1971; Gopalakrishna and Choudhari, 1977
	Emballonuridae	*Peropteryx kappleri*	Rasweiler, 1982
		Taphozous longimanus	Gopalakrishna and Murthy, 1960
	Molossidae	*Tadarida aegyptiaca*	Gopalakrishna *et al.*, 1991
		Chaerephon plicata	Pendharkar and Gopalakrishna, 1983
	Noctilionidae	*Noctilio albiventris*	Rasweiler, 1979b, 1993; Rasweiler and Badwaik, 1999a
	Rhinolophidae	*Hipposideros fulvus, Hipposideros lankadiva*	Gopalakrishna and Karim, 1973; Sapkal *et al.*, 1988
		Rhinolophus rouxi	Bhiwgade, 1976
	Rhinopomatidae	*Rhinopoma hardwickei*	Karim and Fazil, 1987
Fundic (simplex uterus)	Phyllostomidae	*Carollia brevicauda, Carollia perspicillata*	Rasweiler, 1979b; Badwaik *et al.*, 1997; Rasweiler and Badwaik, 1999a
		Glossophaga soricina	Rasweiler, 1974
Middle of the uterine cornu	Megadermatidae	*Megaderma lyra*	Gopalakrishna, 1971; Gopalakrishna and Khaparde, 1978a
	Molossidae	*Molossus ater*[a]	Rasweiler, 1988, 1990
	Phyllostomidae	*Desmodus rotundus*	Wimsatt, 1954
		Diaemus youngi[b]	Badwaik *et al.*, 1998
	Vespertilionidae	*Miniopterus schreibersii*	Gopalakrishna and Chari, 1985
		Myotis lucifugus	Wimsatt, 1944
		Scotophilus heathi	Sastry and Madhavan, 1984
		Vespertilio murinus	Duval, 1895b; Nolf, 1896
Caudal end of uterine cornu	Vespertilionidae	*Scotophilus kuhli*	Gopalakrishna, 1971
Corpus of partially bicornuate uterus	Natalidae	*Natalus tumidirostris*	L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations

[a] In *M. ater*, implantation is initiated slightly to the cranial side of the middle of the cornu.
[b] *D. youngi* has a partially bicornuate uterus with extremely short cornua.

Closure in *M. ater* seems to be caused by a combination of endometrial growth, the expulsion of luminal fluid into the cervix, and probably the endocytotic uptake of luminal fluid by the uterine luminal epithelium (Rasweiler, 1987, 1990, 1992).

Evidence was also obtained that the luminal epithelium at the implantation site in both *M. ater* and *Molossus bondae* may have distinctive functional capabilities. In both species, zonae pellucidae surrounding or associated with embryos at the implantation site became markedly swollen. They also exhibited preferential erosion where exposed to the uterine lumen or epithelial cells, but this did not occur where they were in contact with the trophoblast. Similar changes were not observed, or were much less pronounced, when the zona had been shed by the embryo and transported elsewhere in the uterus. Swelling and dissolution of zonae at the implantation site might be attributable to the action of an as yet unidentified lytic factor or complex secreted by uterine epithelial cells in that region. This could conceivably make the zona sticky, thereby promoting adherence of the embryo to the preferred implantation site. The lytic factor or complex might also be involved in early phases of the implantation process (Rasweiler, 1990).

The basis for implantation being restricted to a particular spot in just the right horn in *M. lucifugus* and *M. schreibersii* is an interesting puzzle that remains to be solved. Approximately half of the ovulations in *M. lucifugus*, and almost all of those in *M. schreibersii*, occur from the left ovary. Thus, transport of the embryo through the left horn of those bats to the implantation site in the right horn must play an important role in this. Transperitoneal migration of ova would not be possible in either species, because there is a complete bursa (sac) around each ovary (Mossman and Duke, 1973; Gopalakrishna *et al.*, 1981). In the case of *M. lucifugus*, the right uterine horn is generally larger in nonpregnant parous females, while the horns of nulliparous uteri are virtually symmetrical (even when mature ovarian follicles are present) (Wimsatt, 1944b; Buchanan, 1987). In *Miniopterus* sp., the right horn of juvenile animals is larger than the left, but it is unclear when this difference develops. The right horn is significantly larger than the left in nonpregnant, parous adult animals. There is no information on the relative size of the horns in nonparous adults (Bernard, 1980; Gopalakrishna and Chari, 1985; Gopalakrishna *et al.*, 1985). Histological changes in the endometrium of the two horns are similar in pregnant *M. schreibersii* until the morula enters the left horn. After this, changes in the right horn become progressively augmented, while the endometrium of the left horn reverts to an anoestrous condition (Gopalakrishna *et al.*, 1985).

In several of the bats exhibiting implantation near the cranial end of the uterus, the anatomy of the implantation site is unusual and deserving of special comment. In the phyllostomid *G. soricina*, the blastocyst normally does not pass into the main uterine cavity. Rather, it lodges and implants in what initially appears to be the uterine end of the oviduct on the side of ovulation. This narrow tubular segment is surrounded by typical endometrial tissue, however, and much of the segment is lost when menstruation occurs in this species (Rasweiler, 1974). In the closely related *C. perspicillata* implantation occurs in a similar region (Figure 6.2), which differs only in being somewhat wider and possessing uterine glands, or in an adjacent region of the main uterine cavity (Rasweiler, 1979b; Rasweiler and Badwaik, 1999a). Because these tubal segments in *G. soricina* and *C. perspicillata* are clearly uterine, it has been suggested that they are homologous to the cranial ends of the horns in a bicornuate uterus, and they have been termed 'intramural uterine cornua'.

This assessment is supported by comparative observations. Although the vampire bats, *D. rotundus* and *Diaemus youngi*, are also phyllostomids, they possess partially bicornuate uteri with short horns – extremely short in the case of *D. youngi*. Implantation occurs in the middle of these horns (Wimsatt, 1954; Badwaik *et al.*, 1998). In *N. albiventris* (family Noctilionidae), which also has a partially bicornuate uterus but with longer horns, implantation occurs at the cranial end of the horn on the side of ovulation (Rasweiler, 1979b). Finally, in the emballonurid *P. kappleri*, which has a bicornuate uterus, implantation occurs in a narrow tubular region that is interposed between the end of the oviduct and the main cavity of the uterine horn on the side of ovulation. This intramural segment is also lined by endometrium and therefore clearly uterine (Rasweiler, 1982).

In most bats exhibiting implantation at the cranial end of one of their uterine horns, that horn generally appears preferentially stimulated during early pregnancy, particularly at the prospective site of implantation. Several theories have been proposed to explain how this might be accomplished (see Rasweiler and Badwaik, this volume); however, the most likely possibility would appear to be that hormones produced by the Graafian follicle and new corpus luteum leave the ovary via the ovarian vein and lymphatics. They may then be transferred locally via counter- or crosscurrent exchange to closely associated arterial vessels supplying the ipsilateral uterine horn. It is quite clear that the cranial end of the ipsilateral horn is vascularized by a branch of the uteroovarian or ovarian artery in both a microchiropteran (*N. albiventris*) and several megachiropterans (*Rousettus aegyptiacus* and *Pteropus* sp.) (Rasweiler, 1978; Bernard, 1988; Pow and Martin, 1994; Rasweiler and Badwaik, 1999a). Furthermore, the thorough studies of Pow and Martin (1994, 1995) have established that, at least in *Pteropus* sp., the arteries and veins (and to a lesser extent, also the lymphatics) are closely associated in the probable area of exchange.

The phyllostomids *G. soricina* and *C. perspicillata* have simplex uteri and do not demonstrate obvious unilateral stimulation of the uterus. However, these bats are closely related to a species that does (*N. albiventris*), they exhibit unilateral oviductal reactions that probably have a similar physiological basis, and the fundic region of the uterus in *C. perspicillata* (which is the usual site of implantation) is vascularized by the uterine branches of the uteroovarian arteries (Figure 6.2) (Rasweiler and Badwaik, 1999a).

The rhinolophid bat *Hipposideros fulvus* may be an exception to this association between localization of the implantation site at the cranial end of the uterus and the occurrence of unilateral oviductal and/or uterine reactions. Implantation occurs at the cranial end of the left uterine horn in *H. fulvus*, but there are no reports of unilateral uterine stimulation in this or other rhinolophid bats. Cranial positioning of the implantation site in *H. fulvus* is presumably related in large part to the fact that the caudal half of each uterine horn in this species is lined by a stratified squamous epithelium, and only short cranial segments are lined by a typical endometrium (Karim, 1973, 1974). These observations also raise intriguing questions about how the functions normally associated with the cervix are

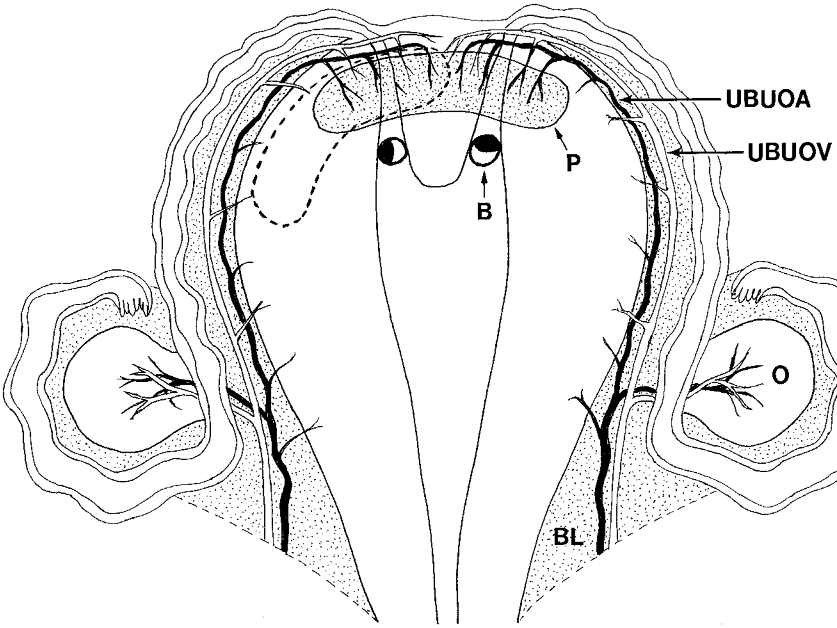

Figure 6.2 Diagrammatic representation of the uterus, oviducts and ovaries of *Carollia* in frontal section. The blastocyst (B) usually implants within the intramural uterine cornu on the side of ovulation, with its inner cell mass oriented towards the uterotubal junction. The discoidal placenta (P) then develops so that it is centered on the fundus of the uterus and vascularized equally on both sides by branches of the uteroovarian artery (UBUOA) and vein (UBUOV). The hypothetical, mesometrial orientation of the inner cell mass in the blastocyst on the left might be more conducive to abnormal positioning (as shown by the dotted lines) and vascularization of the placenta. BL = broad ligament. O = ovary. (Modified with permission from Rasweiler and Badwaik, 1999a.)

performed in *H. fulvus*. Possibly some of the stratified epithelium in the female tract of this bat at times undergoes mucification.

The vascular anatomy of the female reproductive tract has not been so carefully examined in any of the bats having a bicornuate uterus and exhibiting implantation at a mid-horn location; however, none of these species have been reported to demonstrate unilateral endometrial reactions which appear to be associated with vascularization of the implantation site by a branch of the ovarian or uteroovarian artery. It may be that the site of implantation in these bats is vascularized primarily by branches of the uterine artery. Observations presented by Nolf (1896) indicate that this is apparently the case in *Vespertilio murinus* (family Vespertilionidae). Comparative studies on laboratory and domesticated mammals have established that the extent to which the uterus is vascularized by the ovarian and uterine arteries varies considerably between species (see Ginther, 1976).

6.3.2 Other Features of Implantation

When implantation is characterized, major features examined usually include the portion or pole of the blastocyst that initially attaches to the endometrium, the region of endometrium (relative to the uterine mesenteries) to which the blastocyst attaches, and the depth of implantation (Mossman, 1937, 1971). Although these characteristics have generally been quite highly conserved within most major taxonomic groups of eutherian mammals, the bats are a notable exception (Table 6.3). Implantation characteristics vary considerably between species, sometimes even when other criteria clearly indicate that particular species are closely related. We are just beginning to understand why some of these substantial differences may exist.

In most eutherian mammals, at the time of first attachment of the blastocyst to the endometrium, its inner cell mass has an almost constantly specific orientation to the uterus. Usually, the mass faces towards the uterine mesentery (i.e. in a mesometrial direction), away from the mesentery (antimesometrial), or in an intermediate direction (lateral or orthomesometrial). This positioning of the inner cell mass is usually correlated with the site of initial trophoblastic attachment to the endometrium, as well as with subsequent development of the foetal membranes and placental structures. For example, in many mammals the first chorioallantoic placentation is established dorsal to the embryo (i.e. on the side of the uterus towards which the inner cell mass or embryonic disc initially faces), and the various types of yolk sac placentae develop to the ventral side, or somewhat lateral, to the disc (Mossman, 1937, 1971).

When one looks at the orientation of the inner cell mass upon first attachment of the blastocyst in bats, the picture seems terribly confusing. Not only are there species that exhibit mesometrial, antimesometrial or lateral orientations, but in several species the orientation appears to be quite variable, or to change as pregnancy progresses. Furthermore, this characteristic has traditionally been examined microscopically in transverse sections of the uterus because the positions of the mesenteries and the blastocysts could thereby be readily compared. When implantation was examined in captive-bred *G. soricina*, however, the female reproductive tracts were routinely sectioned in a frontal plane because of other experimental considerations. This led to the discovery that the inner cell mass has an unusual orientation at the time of initial blastocyst attachment, i.e. facing the uterotubal junction (Rasweiler, 1974). A similar orientation has since been observed in several other bats (Table 6.3).

While this uterotubal orientation of the inner cell mass would seem to be unique, more careful analysis of the situation in *C. perspicillata* suggests that it may actually be a variant of the mesometrial orientation observed in a number of other bats (Rasweiler and Badwaik, 1999a). When *C. perspicillata* uteri are examined in advanced pregnancy, it is readily evident that the placenta is centered on the fundus and equally vascularized by the uterine branches of the uteroovarian artery and vein on either side of the tract (Figure 6.2). In histologically sectioned tracts from periovulatory and early pregnant animals, these same vessels can be seen running along the side of the uterus in the broad ligament. Finally, at the cranial end of that mesentery on each side, the vessels

Table 6.3 Implantation characteristics of selected species of bats

Family and species	Site of initial trophoblastic attachment	Orientation of ICM upon initial attachment	Depth of implantation	References
Family Pteropodidae				
Cynopterus sphinx, *Cynopterus sp.*	Circumferential	Mesometrial	Partially interstitial	Keibel, 1922; Moghe, 1956b
Pteropus giganteus, *Pteropus vampyrus*	Mesometrial	Mesometrial	Partially interstitial	Göhre, 1892a; Moghe, 1951
Family Emballonuridae				
Peropteryx kappleri	Circumferential	Towards uterotubal junction	Superficial	Rasweiler, 1982
Taphozous longimanus	Circumferential	Mesometrial changing to lateral	Superficial	Gopalakrishna, 1958
Taphozous melanopogon	Circumferential	Variable	Superficial	Sapkal, 1981
Family Megadermatidae				
Megaderma lyra	Mesometrial	Variable	Superficial	Gopalakrishna and Khaparde, 1972
Family Molossidae				
Chaerephon plicata	Circumferential	Lateral (early); antimesometrial (late)	Superficial	Pendharkar and Gopalakrishna, 1983
Molossus ater, *Molossus molossus*	Lateral and antimesometrial; soon becomes circumferential	Lateral (early); antimesometrial to lateral (late)	Superficial	Sansom, 1932; Rasweiler, 1990
Tadarida aegyptiaca	Circumferential	Lateral (early); antimesometrial (late)	Superficial	Gopalakrishna *et al.*, 1991
Tadarida brasiliensis	Circumferential	Antimesometrial	Superficial	Mossman, 1937; Stephens, 1962
Family Noctilionidae				
Noctilio albiventris	Bipolar	Becomes antimesometrial to lateral	Partially interstitial	Anderson and Wimsatt, 1963; Rasweiler, 1979a; Rasweiler and Badwaik, 1996b, 1999a
Family Phyllostomidae				
Carollia perspicillata	Circumferential in IUC[a] or at cranial end of main uterine cavity	Towards uterotubal junction	Secondarily interstitial	Rasweiler, 1979b; Badwaik *et al.*, 1997; Rasweiler and Badwaik, 1999a

Desmodus rotundus	Antimesometrial	Antimesometrial	Interstitial	Wimsatt, 1954
Glossophaga soricina	Circumferential in IUC	Towards uterotubal junction	Secondarily interstitial	Rasweiler, 1974
Macrotus californicus	Circumferential	Towards uterotubal junction	Superficial	Bodley, 1974; Bleier, 1975; Luckett, 1980
Family Rhinolophidae				
Hipposideros bicolor	Circumferential	–	Superficial	Gopalakrishna and Moghe, 1960
Hipposideros fulvus	Antimesometrial to lateral	Lateral to antimesometrial (early)	Superficial	Karim, 1974; Bhiwgade, 1979
Hipposideros lankadiva, *Hipposideros speoris*, *Hipposideros ater*	Circumferential	Lateral to antimesometrial (early); disc later becomes mesometrial[b]	Superficial	Bhiwgade, 1979; Jeevaji, 1979; Sapkal *et al.*, 1988
Rhinolophus ferrumequinum	Circumferential	Mesometrial	Superficial	Almatov, 1968
Rhinolophus rouxi	Circumferential	Lateral (early) to antimesometrial (later)	Superficial	Bhiwgade, 1976
Family Rhinopomatidae				
Rhinopoma hardwickei	Circumferential	Mesometrial	Superficial	Karim and Fazil, 1987
Rhinopoma kinneari	Circumferential	Lateral (early), lateral to mesometrial (later)	Superficial	Srivastava, 1952; Gopalakrishna, 1958
Family Vespertilionidae				
Miniopterus schreibersii	Antimesometrial	Antimesometrial	Superficial	Celestino da Costa, 1920, 1922; Gopalakrishna and Chari, 1985
Myotis lucifugus	Antimesometrial	Antimesometrial	Superficial	Wimsatt, 1944b
Pipistrellus pipistrellus, *Pipistrellus ceylonicus*	Antimesometrial	Antimesometrial	Superficial	Potts and Racey, 1971; Gopalakrishna *et al.*, 1989
Scotophilus kuhli	Antimesometrial	Antimesometrial	Superficial	Gopalakrishna, 1949
Vespertilio murinus[c]	Antimesometrial	Antimesometrial	Superficial	Duval, 1895b; Nolf, 1896; van Beneden, 1899

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[a] IUC = intramural uterine cornu.

[b] Sapkal *et al.* (1988) indicated that the orientation of the ICM upon initial attachment in *H. lankadiva* is mesometrial or mesometrial to lateral.

[c] In some cases the bats referred to as *V. murinus* by earlier investigators may in fact have been *M. myotis* (discussed briefly by Allen, 1939).

begin to penetrate the uterine wall and to ramify in the area surrounding the intramural portion of the oviduct.

When the blastocyst begins to implant in *C. perspicillata*, its inner cell mass becomes oriented towards three landmarks – the uterotubal junction through which it passed, the maternal vessels radiating into the uterus immediately around its external junction with the oviduct, and the cranial end of the broad ligament. The inner cell mass appears to be least centered upon the mesentery. This makes some sense because, if the discoidal placenta developed dorsal to a mesometrially oriented blastocyst, it might be inappropriately oriented relative to the major uterine vessels (Figure 6.2). Instead, the inner cell mass becomes oriented towards the uterotubal junction. Interactions between its highly invasive trophoblast and the decidualized endometrium lead initially to the development of a horseshoe-shaped preplacenta around the interstitially implanted blastocyst. As the conceptus enlarges, however, the following major changes occur: the preplacenta extends across the fundic region of the uterus; the preplacenta begins to tap blood vessels arising from the uterine branches of the uteroovarian artery and vein on the contralateral side of the uterus, as well as those supplying the original implantation site; the inner cell mass becomes shifted towards the midsagittal plane of the uterus; the chorioallantoic placenta becomes progressively more discoidal in shape and centered on the fundus of the uterus; and the placenta develops a dual maternal vascular supply. This may permit *C. perspicillata* to produce large and relatively precocious young (see section 6.4.5, under Form and Location of the Definitive Placentae).

Several lines of evidence would seem to support the suggestion that the uterotubal orientation of the inner cell mass in *C. perspicillata* may be a variant of the mesometrial orientation observed in some other bats (Table 6.3). First, the inner cell mass is partially directed towards the cranial-most portion of the ipsilateral mesentery (the broad ligament). Second, both the position of the implantation site and the orientation of the blastocyst seem to be targeting major maternal vessels that enter the uterus from the broad ligament and will provide a major part of the blood supply for the placenta. Finally, the position and vascularization of the implantation site in *C. perspicillata* are very similar to what have been observed in some of the pteropodid bats.

Implantation in the pteropodids occurs at a homologous site, at the extreme cranial end of the uterine horn ipsilateral to the new corpus luteum (Keibel, 1922; Gopalakrishna and Murthy, 1960; Marshall, 1953). Orientation of the ICM during implantation has been described as being mesometrial in *Cynopterus* sp. and *Pteropus* sp. (Göhre, 1892a; Keibel, 1922; Moghe, 1951, 1956b) and uterotubal in *R. leschenaulti* (Karim, 1975a). This merits reexamination in the first two genera, however, as their uteri were transversely sectioned. The uterotubal orientation is most readily recognized in frontally sectioned uteri.

In the pteropodids, the uterine horn on the side of ovulation is also preferentially stimulated (particularly at the prospective implantation site at its cranial end), apparently by ovarian hormones delivered via a local route (Marshall, 1949, 1953; Gopalakrishna and Karim, 1971; Bernard, 1988; Pow and Martin, 1994, 1995). This is thought to involve a counter- and crosscurrent transfer of hormones between the ovarian venous and lymphatic drainage and the arterial supply to the ipsilateral uterine horn. The uterine branches of the ovarian artery and vein approach the cranial end of the horn in the broad ligament and enter the uterus close to its external junction with the oviduct. Although unilateral stimulation has not been observed in the simplex uterus of *C. perspicillata*, vascularization of the female tract is very similar. Furthermore, the oviduct on the side of ovulation is preferentially stimulated in *Carollia* sp., and this may involve comparable local pathways for the delivery of ovarian hormones (Bonilla and Rasweiler, 1974; Rasweiler and Bonilla, 1992; Rasweiler and Badwaik, this volume).

The emballonurid *P. kappleri* also exhibits preferential stimulation of the endometrium at the prospective implantation site (at the cranial end of the uterine horn ipsilateral to the new corpus luteum) and orientation of the inner cell mass towards the uterotubal junction during implantation (Rasweiler, 1982). The final position of the chorioallantoic placenta in *P. kappleri* has not been established; however, it is lateral to mesometrial in several other emballonurids (Wimsatt and Gopalakrishna, 1958).

Early blastocysts of *N. albiventris* (family Noctilionidae) are unusual in lacking a typical inner cell mass. Instead, cells inside of the trophoblast in unimplanted and early implanting blastocysts are dispersed as a monolayer. In slightly more advanced implanting blastocysts, clusters of cells had developed in several regions. While these appeared to represent efforts to form an inner cell mass, they still did not exhibit any particular orientation relative to the uterus. It was only as implantation continued that a typical inner cell mass formed and became properly oriented. These observations indicate that orientation of the inner cell mass in *N. albiventris* blastocysts is accomplished by preferential proliferation of the interior cells at the presumptive embryonic pole and/or their migration toward that pole from the ‘improperly positioned’ early clusters of cells (Rasweiler, 1979b; Rasweiler and Badwaik, 1996b). The latter was also suggested as a mechanism for inner cell mass orientation in mice (Kirby *et al.*, 1967; Kirby, 1971), but subsequently disproven for that species by the experiments of Gardner (1975, 1990). These involved labelling some trophoblast cells with melanin granules *in vitro* and then transplanting the blastocysts to the uteri of pseudopregnant mice. When the blastocysts were examined histologically after they had become oriented, no changes in the relative positions of the marked trophoblast cells and the inner cell mass could be detected. Obviously, the results of these experiments do not negate cell migration as a possible mechanism for inner cell mass orientation for *N. albiventris*.

In contrast to *G. soricina* and *Carollia* sp. which have simplex uteri, *D. rotundus* and *N. albiventris* possess partially bicornuate uteri with specialized endometrial ridges that run along the antimesometrial (*D. rotundus*), or antimesometrial to lateral (*N. albiventris*), sides of each uterine horn. In *D. rotundus* and *N. albiventris*, the inner cell mass of the blastocyst becomes oriented towards the ridge at the time of implantation, and the chorioallantoic placenta subsequently forms there as well (Wimsatt, 1954; Anderson and Wimsatt, 1963; Rasweiler, 1979b). In these bats, the presence of the ridge clearly plays a central role in directing the inner cell mass to assume a different orientation, and the chorioallantoic placenta to initially become positioned differently, than in the closely related *G. soricina* and *Carollia* sp. (Rasweiler, 1993; Rasweiler and Badwaik, 1998).

Although Anderson and Wimsatt (1963) reported that the definitive chorioallantoic placenta in *N. albiventris* is antimesometrial to lateral, further studies have called this into question (Rasweiler and Badwaik, 1999a). By the onset of gastrulation in *N. albiventris*, the developing placenta and the epiblastic vesicle (= presumptive embryonic shield and amniotic ectoderm) had clearly shifted to a lateral to mesometrial location in some uteri, and may have been in the process of doing the same in the remainder. Remarkably, the full shift must involve a rotation of the blastocysts of about 180° from their orientation in the uterus early during implantation. By midgestation in *Noctilio leporinus* (foetal C–R length: 17–19 mm), the placenta was mesometrial to lateral in all three specimens examined. As in *C. perspicillata*, this positioning of the placenta facilitates its vascularization by two sets of major maternal vessels. In the case of *N. albiventris*, these are the uterine branches of the uteroovarian artery and vein, as well as the uterine artery and vein, serving the gravid cornu. This may be a critical factor that permits *Noctilio* sp. to produce large and more precocious young than might otherwise be the case (see section 6.4.5, under Form and Location of the Definitive Placentae).

Because *N. albiventris* are born lacking fur and with eyes barely open, Brown *et al.* (1983) considered the young of this species to be altricial. However, the babies are relatively large at birth and in some respects do not appear altricial. Brown *et al.* reported that six neonatal *N. albiventris* weighed 6.0–9.0 grams (mean: 7.3 ± 1.0 grams), while 30 mature, periovulatory or early pregnant females captured with little or no food in their stomachs weighed 23.9–30.0 grams (mean: 26.8 ± 1.6 grams) (Rasweiler, 1978, unpublished observation). Thus, neonatal *N. albiventris* weigh approximately 22–34% of the mean mass of adult females around the start of pregnancy. Furthermore, the neonates must be well-developed enough to cling to (and remain at) safe roosting sites while their mothers forage. This would seem to require considerable behavioral, neuromuscular and possibly thermoregulatory maturity.

In the molossid and natalid bats, orientation of the inner cell mass during implantation is lateral to antimesometrial, depending upon species, and significantly different from the mesometrial position of the definitive discoidal placenta (Tables 6.3 and 6.5 (page 252)). These bats are unusual, however, in having two different and successive types of chorioallantoic placentae. During midpregnancy a diffuse endotheliodichorial placenta lines much of the uterine interior and is presumably the principle site of physiological exchange between conceptus and mother. The diffuse placenta then involutes and is replaced by one (*Chaerephon, Molossus, Tadarida*) or two (*Natalus*) discoidal chorioallantoic placentae as the principle site(s) of foetomaternal exchange during late pregnancy (Stephens, 1969; Stephens and Cabral, 1972; Rasweiler, 1990, 1991b; Pendharkar and Gopalakrishna, 1983; Gopalakrishna *et al.*, 1989b; L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations). Although the inner cell mass of the blastocyst is oriented towards a portion of the uterus where the diffuse placenta will form, this is different from the more limited region involved in formation of the discoidal placenta(e).

Several species of bats are unusual in exhibiting a variable orientation of the inner cell mass at initial attachment of the blastocyst. In a small series of pregnant *Megaderma lyra* (family Megadermatidae), the orientation of the mass during implantation was as follows: mesometrial (five females), lateral (eight females),

Table 6.4 Types of interhaemal barriers found in the chorioallantoic placentae of bats

Type of barrier	Typical elements of the minimal barrier
Endotheliomonochorial	Hypertrophied maternal endothelium lining placental labyrinth Thick basal lamina (interstitial membrane) Cytotrophoblast Basal laminae of trophoblast and foetal endothelium (probably fused) Foetal endothelium
Endotheliodichorial	Hypertrophied maternal endothelium lining placental labyrinth Thick basal lamina (interstitial membrane) Syncytiotrophoblast Cytotrophoblast Basal lamina of trophoblast and foetal endothelium (probably fused) Foetal endothelium
Haemomonochorial (cytotrophoblastic)	Cytotrophoblast (containing a discontinuous intracytotrophoblastic lamina in some species) lining placental labyrinth Basal laminae of cytotrophoblast and foetal endothelium (probably fused) Foetal endothelium
Haemomonochorial (syncytiotrophoblastic)	Syncytiotrophoblast (containing a discontinuous intrasyncytial lamina) lining placental labyrinth [Discontinuous cytotrophoblastic layer] Basal laminae of trophoblast and foetal endothelium (probably fused) Foetal endothelium
Haemodichorial	Syncytiotrophoblast (containing a discontinous intrasyncytial lamina) lining placental labyrinth Cytotrophoblast (often highly attenuated) Basal laminae of cytotrophoblast and foetal endothelium (probably fused) Foetal endothelium

mesometrial to lateral (three females), and antimesometrial (one female). By the embryonic shield stage, when the blastocyst was bigger, the shield had become oriented towards the mesometrium. This is also the side of the uterus where the discoidal chorioallantoic placenta subsequently formed (Gopalakrishna and Khaparde, 1972). The authors suggested that this might result from migration of the mass inside of the blastocyst. Although this cannot be dismissed, there would also seem to be other possible explanations. At the time of blastocyst orientation, the uterine endometrium at the implantation site in *M. lyra* appears to be composed of many leaf-like folds or projections with a high ratio of epithelial to stromal components (Gopalakrishna and Khaparde, 1972, 1978a). This raises the possibility that, for most blastocysts, proper orientation might be accomplished by preferential expansion of the embryos in one direction and modest deformation of these thin endometrial projections. It is harder to accept that this might also work for implanting blastocysts in which the inner cell mass initially has an antimesometrial orientation (i.e. 180° from the eventual orientation of the embryonic disc); however, only one such embryo was observed, and there is no way of knowing its potential for future development. Inversely oriented blastocysts occasionally occur in rabbits (Denker, 1974). Finally, it would seem that a mesometrial position of the embryonic shield might be attained in some *M. lyra* (i.e. those with a mesometrial to lateral inner cell mass at earlier stages) by preferential growth of the placenta in that direction as happens in *N. albiventris* (Rasweiler and Badwaik, 1999a).

In the rhinolophid *H. fulvus*, the inner cell mass changes from an antimesometrial orientation in early implanting blastocysts to a lateral one in more advanced blastocysts (Gopalakrishna and Karim, 1973; Karim, 1974), and the chorioallantoic placenta is eventually formed on the mesometrial side of the uterus. The authors suggested that this early shift might involve migration of the inner cell mass. While this may be the case, it also seems conceivable that the blastocyst could be rotated 90° as it becomes more deeply embedded in the endometrium, and then further as placental development continues. This is what takes place in *N. albiventris* and possibly in *P. giganteus* as well (Rasweiler and Badwaik, 1999a; Moghe, 1951).

In the emballonurid *Taphozous melanopogon*, orientation of the embryonic mass of implanting blastocysts was reported to vary from being slightly lateral to antimesometrial in one, to lateral in another, to between lateral and mesometrial in a third. In slightly more advanced blastocysts (during early amniogenesis), the mass was antimesometrial in one and lateral to antimesometrial in another. In all of these cases, the orientation of the mass was not markedly different from the eventual, lateral location of the discoidal chorioallantoic placenta (Sapkal, 1981; also see Wimsatt and Gopalakrishna, 1958) and might be accomplished by one of the mechanisms discussed above for other species. Similarly, in the rhinolophid *Rhinolophus rouxi* the inner cell mass of implanting blastocysts has a lateral to mesometrial orientation, which is not much different from the mesometrial location of the discoidal chorioallantoic placenta (Bhiwgade, 1976).

Before leaving this subject, it deserves to be noted that much of the information on orientation of the inner cell mass during implantation in bats has been obtained from transversely-sectioned uteri. While this is the traditional approach (because the position of the uterine mesenteries can be readily assessed), it is now clear that the examination of frontal sections can provide additional, essential information on spatial relationships between the conceptus and uterus both during and after implantation (Rasweiler and Badwaik, 1999a). Furthermore, these characteristics might be more effectively utilized in assessing the phylogenetic relationships of bats, if they can be augmented with observations on the vascularization of the implantation and placentation sites.

Implantation is also commonly characterized by the degree of penetration of the uterine mucosa by the blastocyst. Implantation is considered to be ‘central’ or ‘superficial’ if the blastocyst expands to fill a significant portion or all of the uterus by the end of the process. It is characterized as being ‘partially interstitial’ if the blastocyst instead becomes partially embedded in the endometrium, and 'interstitial' if the blastocyst becomes completely embedded within the endometrium. Finally, implantation is termed 'eccentric' if the blastocyst implants off to one side of the uterus in a small portion of the uterine lumen.

While these terms are appropriately descriptive, the form and final depth of implantation is determined by a variety of processes and factors. These include the rate of expansion of the early uterine blastocyst, invasiveness of its trophoblast, nature of the endometrial (decidual) response, and positioning of the implantation site. Misleading conclusions can be drawn about the nature of implantation in bats, and possible phylogenetic relationships between bat species, if the process is characterized only by the commonly used terms. For example, implantation in most bats is central, while that in most phyllostomid bats is interstitial (Table 6.3). However, blastocysts of the phyllostomid bats *G. soricina* and *Carollia* sp. initially attach circumferentially within intramural uterine cornua, which are homologous to the cranial ends of the horns in bats with bicornuate uteri. This is probably best considered to be a modified central form of implantation. Implantation then becomes secondarily interstitial as the blastocyst becomes buried within the fundic endometrium. This condition arises as the lumen of the intramural uterine cornu is obliterated, and decidua fills in around the expanding blastocyst. Implantation in another phyllostomid bat, *Macrotus californicus*, which has a partially bicornuate uterus, is central (Bleier, 1975).

Implantation in *N. albiventris* is partially interstitial and occurs near the cranial end of one of the uterine cornua. Initial attachment of the blastocyst in this species is bipolar, however, and is again probably best viewed as a modified central form of implantation. The early implanting blastocyst of *N. albiventris* is small, while the uterine lumen is relatively large and crescent-shaped (because of the presence of the specialized endometrial ridge). This precludes the more extensive contact between the blastocyst and endometrium that results in circumferential attachment in *G. soricina* and *Carollia* sp. and central implantation in most other bats. Although decidual reactions occur in the endometrial stroma underlying both attachment sites in *N. albiventris*, the decidua at the abembryonic poles subsequently deteriorates, freeing that pole of the blastocyst. The decidual reaction occurring in the endometrial ridge is, on the other hand, much more pronouced and almost completely engulfs the blastocyst. Continued growth of the yolk sac prevents the decidua from completely covering the abembryonic pole of the blastocyst (as occurs in *G. soricina* and *Carollia* sp.), and implantation is therefore ultimately only partially interstitial in *N. albiventris* (Anderson and Wimsatt, 1963; Rasweiler, 1974, 1979b, 1993; Badwaik *et al.*, 1997).

In general, *N. albiventris* and these two phyllostomids exhibit many similarities in early embryology, placement of the implantation site, mode of initial trophoblastic attachment to the endometrium, and depth of implantation. As some of these, such as advanced embryonic development within the oviduct, appear to be shared, unique, derived characteristics (synapomorphies), these bats are clearly closely related and deserve placement in the same taxonomic grouping (the superfamily Noctilionoidea). Superficial comparisons of uterine anatomy and implantation characteristics in these animals might lead some to mistakenly conclude otherwise.

Implantation in the common vampire bat, *D. rotundus* (family Phyllostomidae) is also interstitial in an endometrial ridge running along the antimesometrial side of the uterine cornu (Wimsatt, 1954). Unfortunately, Wimsatt did not have any *D. rotundus* tracts containing blastocysts in the initial phases of attachment to the endometrium. In the closely related white-winged vampire bat, *D. youngi*, however, initial attachment is probably bipolar or circumferential. We have examined a specimen in which a blastocyst had recently implanted on the antimesometrial side of one of the short uterine horns. Although a preplacenta had been formed in this region, the abembryonic pole of the blastocyst was also attached more superficially to a broad expanse of the opposing wall of the horn. The decidua in that region was degenerating in a very similar fashion to that described for *N. albiventris* (J.J. Rasweiler, N.K. Badwaik and F. Muradali, unpublished observations; Rasweiler, 1979b).

Implantation has not yet been studied in any of the thyropterid bats; however, Wimsatt and Enders (1980) observed that all of the presomite stage embryos of *T. tricolor* which they examined were completely enclosed by endometrial tissue, i.e. the chorionic sac was sealed off from the uterine lumen by a decidua capsularis. This led them to infer that implantation in *T. tricolor* is interstitial as well. Although completely interstitial implantation has been observed in several of the phyllostomid bats, it had not previously been reported for any member of the superfamily Vespertilionoidea, the grouping to which the Thyropteridae has generally been assigned by chiropteran systematists. Further studies are needed to clarify how implantation in *T. tricolor* compares to that in other vespertilionoid bats.

6.4 DEVELOPMENT OF PLACENTAL ORGANS AND FOETAL MEMBRANES

6.4.1 Introduction

The foetal membranes of bats include the amnion, yolk sac, allantois and chorion. These participate in the formation of placentae and protective coverings for the conceptus in the uterus. Placentae are intimate associations of maternal and embryonic/foetal tissues that develop during pregnancy to facilitate physiological exchange between mother and conceptus.

6.4.2 Amnion

All bats examined to date have been found to exhibit the development of a primordial amniotic cavity (or cavities) within the embryonic cell mass during implantation. Further development then varies in different species (Figure 6.3). In many, the epiblastic roof of the primordial cavity becomes attenuated and is finally disrupted, thereby creating a new trophoepiblastic cavity between the embryonic disc and the trophoblast. The definitive amnion is then formed by upfolding of the epiblast and mesoderm from the margins of the disc.

In the noctilionid *N. albiventris,* and the phyllostomid *C. perspicillata,* the primordial cavity is formed by a cavitation process that involves considerable cell death. This is attributable to apoptosis, because the affected epiblast cells generally

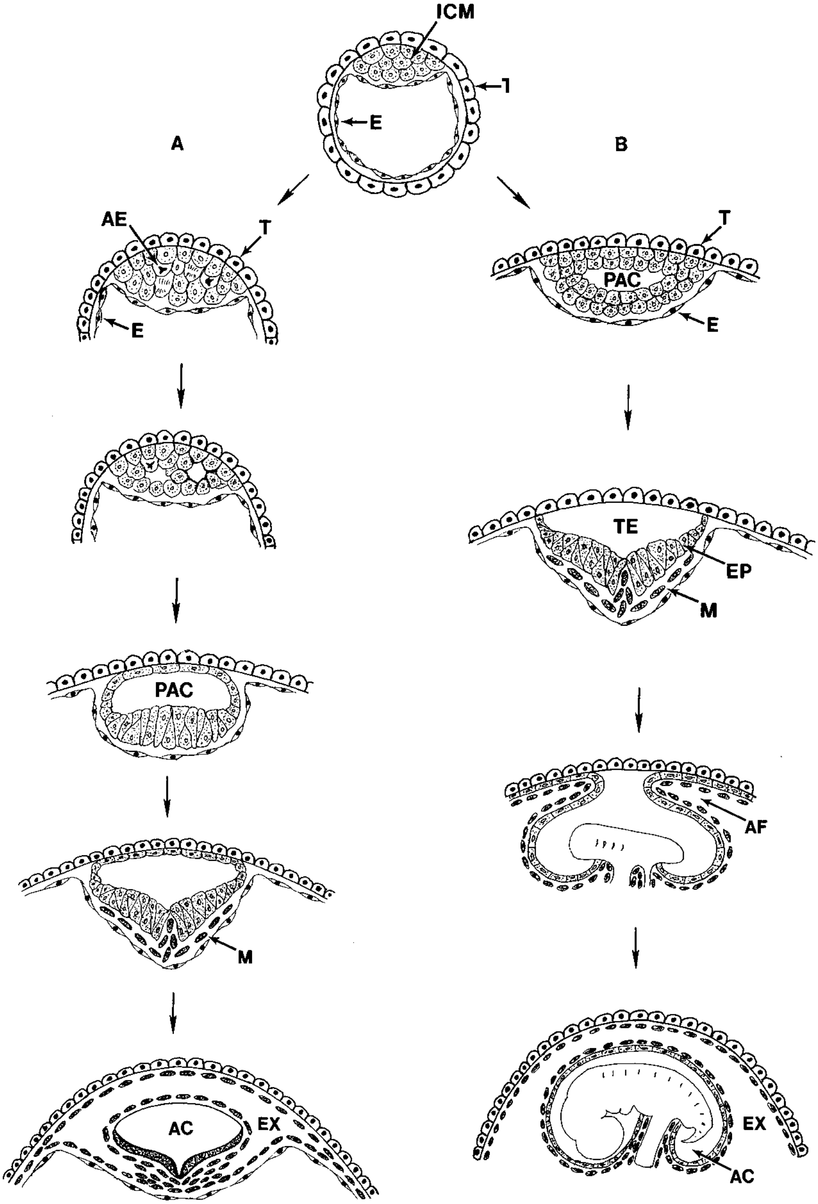


Figure 6.3 Diagram depicting the principle modes of amniogenesis in bats. In some species, this occurs by cavitation (Pathway A), which initially involves considerable apoptosis within the inner cell mass (ICM). This is followed by the coalescence and expansion of spaces within the mass to form a large, primordial amniotic cavity (PAC). The latter is then converted into the definitive amniotic cavity (AC). In other species, amniogenesis occurs by folding (Pathway B). This also involves the formation of one or more primordial amniotic cavities. The epiblastic roof of each cavity is then disrupted, creating a temporary trophoepiblastic cavity (TE). Finally, the definitive amnion is formed by upfolding of the margins of the embryonic shield. AE = apoptotic epiblast cells. AF = amniotic fold. E = endoderm. EP = epiblast. EX = exocoelom. M = mesoderm. T = trophoblast.

exhibit cytoplasmic shrinkage, nuclear condensation and then fragmentation. Many of the epiblast cells bordering on the small cavities that develop initially in the embryonic mass have a radial orientation and have clearly become polarized. These then coalesce and enlarge to form a single large cavity, with the epiblast differentiating into a shield and amniotic ectoderm (Anderson and Wimsatt, 1963; Rasweiler and Badwaik, 1996b; Badwaik *et al.*, 1997). The process appears to be similar in other phyllostomids, e.g. *G. soricina* and probably *D. rotundus* (Hamlett, 1935; Wimsatt, 1954; Rasweiler, 1974), *T. tricolor* (Wimsatt and Enders, 1980), and several megachiropterans (Göhre, 1892a; Kohlbrugge, 1913; Keibel, 1922; Moghe, 1951, 1956b; Heideman, 1989; Heideman and Powell, 1998).

Early amniogenesis is unusual in at least some of the megachiropterans (*C. sphinx, Otopteropus cartilagonodus*, and *Ptenochirus jagori*), because two primordial amniotic cavities form initially in the inner cell mass, one above the other. The partition between the two then breaks down to form what soon becomes the definitive amniotic cavity (Moghe, 1956b; Heideman *et al.*, 1993; Heideman and Powell, 1998). The significance of this sequence of events is not completely clear, but it may be involved in the differentation of the epiblast surrounding much of the lower cavity into shield epiblast and of that surrounding the upper cavity into amniotic ectoderm.

Although the molossid *M. ater* and the vespertilionid *M. schreibersii* exhibit amniogenesis by folding, the formation of primordial amniotic cavities in both species appears to involve the apoptosis of some epiblast cells, as well as a radial arrangement and polarization of epiblast cells in the inner cell mass as seen in *N. albiventris* and *C. perspicillata* (Celestino da Costa, 1920; Sansom, 1932; Rasweiler, 1990; Badwaik *et al.*, 1997). There are reports that the trophoblastic roof of the trophoepiblastic cavity in some *M. schreibersii* is disrupted, exposing the embryonic shield to uterine lumen lined by an apposing layer of endometrial stroma (Gopalakrishna and Karim, 1980; Gopalakrishna and Chari, 1983; Chari and Gopalakrishna, 1984). This represents a novel arrangement for a vespertilionid bat and therefore needs to be confirmed.

6.4.3 Development of Endoderm and Formation of the Yolk Sac

In most bats, as in nearly all other mammals, primitive endoderm (hypoblast) develops initially on the basal surface of the inner cell mass and then spreads peripherally to line the interior of the blastocyst cavity. This creates the yolk sac. In the phyllostomid bats *G. soricina* and *C. perspicillata*, the primitive endoderm also differentiates on the surface of the inner cell mass, but then proliferates to create an unusual meshwork of mesenchyme-like cells in the blastocystic cavity. This multilocular yolk sac is soon converted into a unilocular one lined by an endodermal monolayer (Rasweiler, 1974; Badwaik *et al.*, 1997). The development of an endodermal meshwork is quite rare among mammals, having been previously noted only in a few primates including the human (Luckett, 1974).

Comparative studies suggest that an endodermal meshwork may develop in *G. soricina* and *C. perspicillata*, in large part, because implantation in these bats usually takes place in a relatively constricted site (i.e. one of the intramural uterine cornua interposed between the end of each oviduct and the main cavity of the uterus). Furthermore, during the early phases of implantation in *C. perspicillata*, blastocyst expansion appears to be constrained by closure of the uterine lumen, growth of the decidua over the abembryonic pole of the blastocyst, and a temporary retardation of trophoblastic invasion of the endometrial stroma by the basal laminae of uterine luminal and glandular epithelial cells (Badwaik *et al.*, 1997). Early uterine blastocysts in the closely related *N. albiventris* do not develop a similar endodermal meshwork. In *N. albiventris*, however, implantation occurs at the cranial end of one of the uterine horns, and the yolk sac can expand into a more spacious lumen (Anderson and Wimsatt, 1963; Rasweiler, 1979b; Rasweiler and Badwaik, 1996b, 1999a). Early proliferation of the endoderm may also be important in *G. soricina* and *C. perspicillata* because it creates a cellullar reservoir that can be used for subsequent rapid growth of the yolk sac. The large yolk sacs that develop in all three species presumably play an important role in early physiological exchange between the embryo and mother, and provide space for further growth of the conceptus (Badwaik *et al.*, 1997).

Early embryonic development in *G. soricina* and *C. perspicillata* is also unusual because endoderm surrounds much of the inner cell mass and Reichert's membrane (a thickened basal lamina) continues over the dorsal side of the mass. A similar envelopment of the inner cell mass by what appears to be endoderm has been reported for a variety of megachiropterans (Göhre, 1892a; Kohlbrugge, 1913; Keibel, 1922; Moghe, 1956b; Heideman, 1989; Heideman *et al.*, 1993; Heideman and Powell, 1998). It may also occur in the vampire bat *D. rotundus*, where it was identified as precociously-formed extraembryonic mesoderm (Wimsatt, 1954), the disc-winged bat *T. tricolor* (Wimsatt and Enders, 1980), the armadillo (*Dasypus*; Patterson, 1913), the elephant shrews (*Elephantulus* sp.; van der Horst, 1942, 1944; Lange, 1949), the tenrec (*Hemicentetes*; Goetz, 1937) and the gliding lemur (*Cynocephalus volans*; Hubrecht, 1919).

In rats and mice, Reichert's membrane occurs between the parietal endoderm and mural trophoblast of blastocysts and is generally considered to be secreted by the endoderm, although recent evidence indicates that the mural trophoblast may be involved in its initial formation as well (reviewed by Badwaik *et al.*, 1997). Ultrastructural observations indicate that both endoderm and cytotrophoblast probably contribute to the formation of Reichert's membrane in *C. perspicillata*.

Reichert's membrane has also been noted dorsal and lateral to the inner cell mass in other phyllostomid bats (*G. soricina* and *M. californicus*), a thyropterid bat (*T. tricolor*), and a noctilionid bat (*N. albiventris*). Interestingly, during the initial formation of Reichert's membrane dorsal to the inner cell mass in *N. albiventris*, no endoderm is evident in that region. Therefore, the supraembryonic portion of Reichert's membrane in *N. albiventris* must be a product of the epiblast and/or cytotrophoblast, the only cell types present there (Rasweiler and Badwaik, 1996b).

Reichert's membrane is linked to a meshwork of basal lamina-like material at the endoderm–epiblast interface and extending into both cell layers in *C. perspicillata* and *N. albiventris*, the species in which it has been most thoroughly examined. It has been suggested that Reichert's membrane may play an important mechanical role in tethering the inner cell mass and embryonic shield to the preplacenta (chorionic placenta) in these bats. This may be important because, at least in comparison to primates like the human and rhesus monkey, these components of the embryo become unusually large in *C. perspicillata* and *N. albiventris* before mesoderm appears and contributes to the early development of a connecting stalk (Rasweiler and Badwaik, 1996b; Badwaik *et al.*, 1997).

The peripheral spread of extraembryonic mesoderm between the trophoblast and yolk sac endoderm, and the development of blood vessels within this mesoderm, has been found to lead to the formation of a transient choriovitelline placenta in all bats that have been adequately studied. The fate of the yolk sac then follows several different patterns (Wimsatt, 1954; Moghe, 1956a; Gopalakrishna and Karim, 1980; Luckett, 1980).

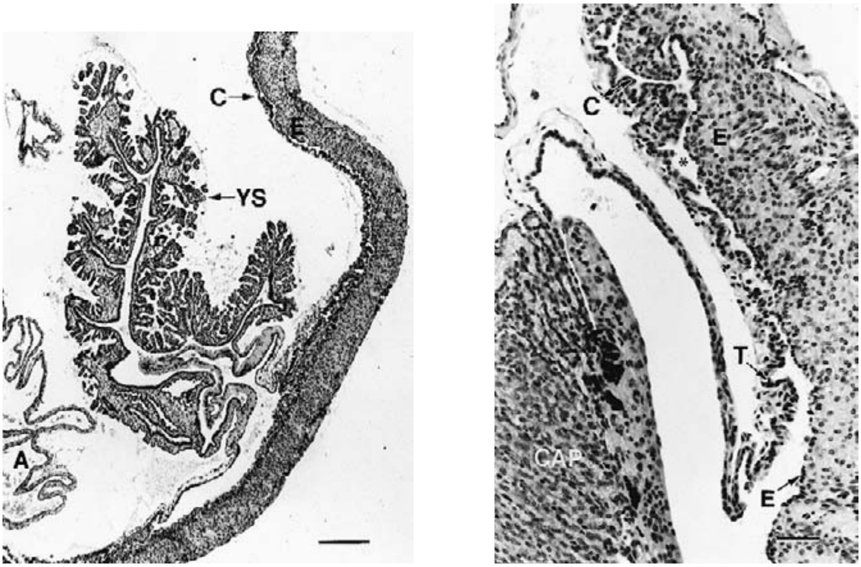
In the emballonurid, rhinolophid and rhinopomatid bats, the entire yolk sac wall becomes vascularized and is then invaded by the exocoelom. This is followed by the collapse of the sac, infolding of its wall, and hypertrophy of its endodermal and sometimes mesothelial cells (Srivastava, 1952; Moghe, 1956a; Gopalakrishna, 1958; Gopalakrishna and Moghe, 1960; Gopalakrishna and Karim, 1975; Khaparde, 1976; Bhiwgade, 1977; Jeevaji, 1982; Sapkal *et al.*, 1988). In the pteropodid and molossid bats, the process is similar; however, hypertrophy and, in at least some cases, hyperplasia of the epithelial elements is more pronounced. This gives rise to a glandular body that persists until parturition. The endoderm cells of the collapsed sac exhibit considerable mitotic activity in the molossid *M. ater*, so that its wall becomes much thicker and liver-like in appearance. In the pteropodids *R. leschenaulti* and *P. giganteus*, the endoderm cells become grouped together in acinus-like structures (Karim, 1972b; Karim *et al.*, 1979; Gopalakrishna and Karim, 1980), while this is not obviously the case in *M. ater* (Rasweiler, 1990). In the pteropodids and the molossid *Tadarida brasiliensis*, the yolk sac cavity is apparently obliterated completely, while in *M. ater, Tadarida aegyptiaca* and *Chaerephon plicata* small remnants persist (Stephens, 1962; Stephens and Easterbrook, 1971; Gopalakrishna and Karim, 1979, 1980; Karim *et al.*, 1979; Gopalakrishna *et al.*, 1989b).

In the phyllostomids and noctilionids, the peripheral spread of vascularized extraembryonic mesoderm is restricted to the embryonic half of the yolk sac, and the abembryonic portion of the yolk sac remains bilaminar. The endodermal cells of the vascularized portion become hypertrophied, while those of the bilaminar part (the bilaminar omphalopleure) remain attenuated. Growth of the embryo and the amnion cause the invagination of the vascularized embryonic portion of the yolk sac into the abembryonic portion, and reduction of the yolk sac lumen to a thin slit (Wimsatt, 1954; Anderson and Wimsatt, 1963).

Finally, in the vespertilionid and megadermatid bats, extraembryonic mesoderm spreads all around the yolk sac, but expansion of the exocoelom is incomplete. This results in the persistence of a small area of trilaminar omphalopleure at the abembryonic pole. In these species, the endodermal and mesothelial cells of the yolk sac splanchnopleure also hypertrophy (Nolf, 1896; Wimsatt, 1945b; Karim, 1977; Gopalakrishna and Khaparde, 1978b; Gopalakrishna and Karim, 1979; Chari and Gopalakrishna, 1984; Gopalakrishna *et al.*, 1989a). Experimental studies have established that this portion of the yolk sac in the little brown bat, *M. lucifugus*, is involved in the absorption of proteins from the exocoelom (Enders *et al.*, 1976).

Wimsatt and Enders (1980) reported that the extension of the exocoelom in the thyropterid bat, *T. tricolor*, is limited and the abembryonic two-thirds of the chorionic sac persists to term as an extensive avascular trilaminar omphalopleure. Furthermore, the latter supposedly remains closely apposed to the parietal decidua in the form of an avascular choriovitelline placenta. They noted that the persistence of such an extensive trilaminar omphalopleure in *T. tricolor* was unique among the bats, but seemed most similar to the persisting bilaminar omphalopleure of phyllostomid bats. This, the absence of a specialized paraplacental structure involving the extraplacental chorioallantoic membrane, and several other reproductive characteristics led them to suggest that the thyropterids might have closer phylogenetic affinities to the superfamily Phyllostomatoidea (now considered to be the superfamily Noctilionoidea) than to the Vespertilionoidea, where they continue to be placed (Koopman, 1994).

Our observations on the gravid reproductive tract from a *T. tricolor* carrying a 12 mm (crown–rump length) embryo add significantly to those reported previously by Wimsatt and Enders (1980). This animal was collected on the western slope of the Western Cordillera of the Colombian Andes, and voucher specimens from this area have been deposited with both the United States National Museum and the American Museum of Natural History. In this tract, the yolk sac (which had been



6.4 6.5

Figures 6.4 and 6.5 Sections of the gravid uterus from a *Thyroptera tricolor* carrying a 12 mm embryo. In Figure 6.4, the collapsed yolk sac (YS) may be seen attached to the abembryonic wall of the chorionic sac. With the exception of the discoidal chorioallantoic placenta (CAP), some of which is shown in Figure 6.5, the chorion (C) is avascular and closely associated with the endometrial lining (E) of the uterus, forming an extensive chorionic placenta. The trophoblast (T) of this portion of the chorion appears to be noninvasive, and the adjacent uterine epithelium (E) is still largely, if not completely, intact. A = amnion which collapsed following removal of the embryo. * = probable artifactual space between the chorion and endometrium. Bar (Figure 6.4) = 200 µm. Bar (Figure 6.5) = 50 µm.

serially sectioned) is completely separated from the chorion except for a localized region of trilaminar omphalopleure at the abembryonic pole of the chorionic sac (Figure 6.4). This faces a small area of persisting uterine lumen. With the exception of the region that has participated in formation of the discoidal chorioallantoic placenta, much of the remainder of the sac consists of avascular chorion closely apposed to the endometrial lining of the uterine corpus (Figure 6.5). The trophoblast belonging to this portion of the sac appeared to be noninvasive, and much of the adjacent uterine epithelium was still intact. This created an extensive chorionic placenta and not an avascular choriovitelline placenta as postulated to exist late in pregnancy by Wimsatt and Enders (1980). Thus, with respect to the fate of its yolk sac, *T. tricolor* appears more similar to other vespertilionoid bats than to the noctilionoid bats. As the yolk sac separates from the chorion in the molossid bats and *Natalus tumidirostris*, it is replaced by vascularized allantoic mesoderm creating a diffuse endotheliodichorial placenta (Stephens, 1962; Stephens and Cabral, 1972; Gopalakrishna *et al.*, 1989b; Rasweiler, 1990; L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations). There is no suggestion that this occurs in *T. tricolor*.

The pteropodid and molossid bats are unique among mammals in exhibiting the conversion of the yolk sac into a solid, glandular body lying in the exocoelom. This and other embryological similarities between these two groups have even prompted the suggestion that they might be more closely related than generally believed by taxonomists (Gopalakrishna and Badwaik, 1990, 1992a). The fact that the yolk sac becomes so profoundly modified in these bats as pregnancy progresses indicates, however, that this characteristic may be of considerable adaptive significance and could have evolved independently in the two families.

The precise functional significance of these glandular yolk sacs remains to be established. Towards the end of pregnancy in the molossid *M. ater*, there was a marked reduction in the glycogen content of the yolk sac endoderm cells (Rasweiler, 1990), and a similar pattern has been reported for both glycogen and lipids in the yolk sac of another molossid, *T. brasiliensis* (Stephens and Easterbrook, 1968, 1969, 1971). It was suggested these metabolic stores might be mobilized in both species in anticipation of unusual energy demands immediately before and during parturition. Like other bats (Kurta and Kunz, 1987), the molossids give birth to relatively large young. For example, Rasweiler (1990) removed single foetuses that weighed about 5.5 grams from female *M. ater* that weighed 31.3 and 33.1 grams with the foetuses still *in utero*. For this reason foraging would probably be energetically most demanding for female molossids late in pregnancy and possibly less successful due to reduced maneuverability in flight. Experimental studies with the vespertilionid *Myotis yumanensis* have established that maneuverability decreases with increasing flight load (Aldridge and Brigham, 1988).

It also seems conceivable that female molossids might reduce or alter their foraging activities during advanced pregnancy to minimize encounters with predators, which they would presumably have greater difficulty evading. *Molossus ater* begin to forage when there is still significant light, and they are therefore at considerable risk of being attacked by raptors. This is also apparently the case with *T. brasiliensis* which is preyed upon by kites, falcons and hawks (Barbour and Davis, 1969). Falcons have been observed circling over a *M. ater* roost on the Aripo Savannah of Trinidad late in the day, prior to the departure of any bats. Efforts were then made by the falcons to catch bats as they flew away from the roost. These were unsuccessful, however, because the bats were able to avoid capture at the last minute by means of evasive maneuvers (Rasweiler, unpublished observations).

6.4.4 Allantois

The allantois is a sac-like outgrowth of the embryonic hindgut that is lined by endoderm and covered by vascularized mesoderm. In eutherian mammals, the allantois varies from being very large in some groups (Artiodactyla, Carnivora, Cetacea, Hydracoidea, Perissodactyla, Proboscidea, Tubulidentata, and strepsirhine primates) to small or rudimentary in others (haplorhine primates, Lagomorpha, myomorph and hystricomorph rodents) (Luckett, 1974; Mossman, 1987). In Chiroptera, the allantoic vesicle is moderately large in the megadermatids (Gopalakrishna and Khaparde, 1978b), pteropodids (Göhre, 1892b; Moghe, 1951), rhinopomatids (Srivastava, 1952) and rhinolophids (Gopalakrishna, 1958; Gopalakrishna and Moghe, 1960; Gopalakrishna and Bhiwgade, 1974; Gopalakrishna and Karim, 1975; Bhiwgade, 1977; Jeevaji, 1982; Sapkal *et al.*, 1988). It is somewhat smaller in the molossids (Mossman, 1937; Stephens, 1962; Gopalakrishna *et al.*, 1989b; Gopalakrishna and Badwaik, 1990; Rasweiler, 1990), natalids (Wimsatt cited in Mossman, 1987), thyropterids (Wimsatt and Enders, 1980) and vespertilionids (Gerard, 1928; Ramaswami, 1933; Wimsatt, 1945b; Gopalakrishna, 1950; Gopalakrishna *et al.*, 1989a). In all of these bats, the allantoic vesicle regresses and disappears during the second half of pregnancy, although a remnant of the allantoic duct frequently persists in the umbilical cord. In the phyllostomids and noctilionids the allantois never becomes vesicular, but instead remains tubular and rudimentary throughout pregnancy (Hamlett, 1935; Wislocki and Fawcett, 1941; Wimsatt, 1954; Anderson and Wimsatt, 1963).

In all of these bats, as in other eutherian mammals, the allantois performs a very important function in vascularizing the embryonic side of the chorioallantoic placentae and accessory placental areas when they exist. In some families (the molossid and natalid bats), the spread of vascularized allantoic mesoderm beyond the developing discoidal chorioallantoic placenta(ae) is significant and results in the formation of extensive, diffuse chorioallantoic placentae (see next section). Unfortunately, studies of most other species simply have failed to note the extent to which the smooth chorion becomes vascularized by vessels of allantoic origin.

6.4.5 Chorionic and Chorioallantoic Placentae

Chorionic and diffuse chorioallantoic placentae

Bats belonging to at least two families – the Molossidae and the Natalidae – are unusual in developing two different types of chorioallantoic placentae that function for the most part in succession. The first is a diffuse chorioallantoic placenta that appears to be the principal site of physiological exchange between mother and conceptus during midpregnancy. This then involutes and is replaced by a single discoidal chorioallantoic placenta in the molossids (*Tadarida, Molossus* and *Chaerephon*) or by a pair of discoidal chorioallantoic placentae in the natalids. In the case of the molossids the diffuse placenta lines all of the right uterine horn, except for the portion occupied by the developing discoidal placenta, and is endotheliodichorial (Stephens, 1962, 1969; Stephens and Cabral, 1972; Gopalakrishna *et al.*, 1989b; Rasweiler, 1990, 1991b). In the case of the natalid *N. tumidirostris*, the diffuse placenta lines all of the uterus except for the portions at the cranial ends of the uterine horns occupied by the developing discoidal placentae and is also endotheliodichorial (L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations).

The early placental arrangements just described would not appear to differ greatly from those exhibited by many other bats. The pteropodids, emballonurids, rhinopomatids, rhinolophids, megadermatids and phyllostomids all have extensive horseshoe-shaped preplacentae (chorionic placentae). In the emballonurid *Taphozous longimanus*, rhinopomatid *Rhinopoma kinneari*, and several rhinolophids (*Hipposideros bicolor, Hipposideros lankadiva*, and *R. rouxi*), these are then converted to extensive chorioallantoic placentae with the spread of allantoic mesoderm and finally to discoidal chorioallantoic placentae (Gopalakrishna, 1958; Gopalakrishna and Moghe, 1960; Bhiwgade, 1977; Sapkal *et al.*, 1988).

Upon examining a *T. tricolor* (family Thyropteridae) carrying a 12 mm embryo, we observed both an extensive chorionic placenta and a discoidal chorioallantoic placenta (Figures 6.4 and 6.5). The chorionic placenta resembled the diffuse placentae that develop in some other bats (the vespertilionoid, molossid and natalid) in extent, but differed in lacking an associated investment of vascularized allantoic mesoderm and by not invading the endometrium. It would appear from our observations, and those of Wimsatt and Enders (1980) on earlier stages of development, that allantoic mesoderm simply does not spread beyond the margins of the developing placental disc in *T. tricolor*. Although the chorionic placenta in *T. tricolor* lacks an embryonic blood supply, it may still be a site of significant physiological exchange, because it is so intimately associated with the endometrium over such a large area. Material transported across this placenta and into the exocoelom could conceivably be taken up by the rich vascular supply of the yolk sac splanchnopleure, by allantoic vessels on the interior of the discoidal placenta, and/or cross into the amniotic fluid for uptake by the embryo.

In the vespertilionid *M. lucifugus*, some vascularized allantoic mesoderm does spread beyond the placental disc. In the area adjacent to the disc, the chorioallantois takes the form of villous folds which are covered by an absorptive and phagocytic cytotrophoblast (see Accessory Placental Structures below) and then continues as a smooth membrane apparently still vascularized by some allantoic vessels (Wimsatt, 1945b). With the seemingly minor exception that the *T. tricolor* conceptus examined by us had a smooth chorion (rather than a smooth chorioallantois), the general arrangement of its placenta and fetal membranes was very similar to that illustrated in Wimsatt's figure 6 for *M. lucifugus* at about the same stage of pregnancy and quite different from what has been described for phyllostomid bats (particularly with respect to the relationships between the yolk sac and the chorion). Based upon foetal membrane and other reproductive characteristics (see Rasweiler and Badwaik, this volume), the Thyropteridae would appear to be more closely allied to the Vespertilionoidea, where they are now classified, than to the Phyllostomatoidea (now the Noctilionoidea) as suggested by Wimsatt and Enders (1980).

Spread of the allantoic mesoderm beyond the chorioallantoic placenta to create a smooth chorioallantois may also occur in the emballonurid *T. longimanus* (see figure 1 in Wimsatt and Gopalakrishna, 1958), but this requires further study. As noted by Mossman (1987), many investigators have failed to note whether embryonic blood vessels are present or not in the 'smooth chorion' and the trilaminar omphalopleure when characterizing the foetal membranes of a species. This, in turn, makes it difficult to ascertain the homology of these membranes in different taxonomic groups. The vascularity, or lack of it, is also certainly of functional significance when characterizing a foetal membrane.

Form and location of the definitive placentae

The definitive chorioallantoic placentae that are the principal sites of physiological exchange between mother and foetus in late pregnancy are generally discoidal in shape in bats and, in most cases, only a single placenta serves each foetus. There are, however, exceptions. In the funnel-eared bats, *Natalus stramineus* and *N. tumidirostris*, the single foetus is served by a pair of discoidal placentae that are centered over the uterotubal junction on each side of the uterus. At least in *N. tumidirostris*, this is also the region where major maternal vessels supplying the placenta enter via the mesometrium (Wimsatt, cited in Mossman, 1987; L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations). In the long-fingered bat, *M. schreibersii*, a single discoidal placenta (the main or primary placenta) develops initially, but this atrophies as gestation progresses and is replaced by a pair of discoidal placentae which form on either side of the primary placenta (Malassiné, 1970; Chari and Gopalakrishna, 1984; Kimura and Uchida, 1984). As gestation progresses in the hipposiderine bats, a cleft at the center of the chorioallantoic placenta becomes wider and deeper. This eventually divides the organ into two separate, but adjacent, discs (Gopalakrishna, 1958, personal communication; Gopalakrishna and Moghe, 1960; Gopalakrishna and Karim, 1975; Bhiwgade, 1977; Sapkal *et al.*, 1988).

The definitive chorioallantoic placentae of bats always develop at species-specific locations within their uteri. As with most other mammals, these sites are usually characterized by their positions relative to the uterine mesentaries. Bats have for some time presented a problem in this regard because, in contrast to other orders of mammals, they exhibit what appears to be unusual diversity in the positioning of their placentae (Table 6.5). To some extent, this reflects the existence of real differences between some closely related species. It may also indicate, however, a need for further study and possibly even a different approach to characterizing the positions of the placentae.

This problem is exemplified well by comparing the position of the

Table 6.5 Characteristics of the chorioallantoic placentae in selected species of bats

Family and species	Form and location of placentae	Fine structure of interhaemal barrier (nature of evidence[a])	References
Pteropodidae			
Pteropus giganteus	Discoidal, mesometrial	Haemodichorial (probably not advanced pregnancy) (EM)	Moghe, 1951; Karim and Bhatnagar, 1996
Rousettus leschenaulti	Discoidal, mesometrial	Haemomonochorial (syncytial type) with discontinuous intrasyncytial lamina (EM)	Karim, 1972a,b; Bhiwgade, 1990
Emballonuridae			
Taphozous melanopogon	Discoidal, lateral to mesometrial with haematoma attached to mesometrial margin	Endotheliomonochorial (EM)	Khaparde, 1976; Bhiwgade, 1990
Furipteridae			
Furipterus horrens	Discoidal	Larger vascular channels – haemodichorial with widely scattered maternal endothelial cells; smaller vascular channels – endotheliodichorial (somite stage embryo) (LM)	Present authors
Megadermatidae			
Megaderma lyra	Discoidal, mesometrial	Haemodichorial with scattered maternal endothelial cells (EM)	Gopalakrishna and Khaparde, 1978b; Bhiwgade, 1990
Molossidae			
Molossus ater	I. Midpregnancy – diffuse II. Late pregnancy – discoidal, centered on uterotubal junction (mesometrial)	I. Diffuse – endotheliodichorial (LM) II. Discoidal – haemomonochorial (cytotrophoblastic type) (EM)	Rasweiler, 1991b

Tadarida brasiliensis	I. Midpregnancy – diffuse II. Late pregnancy – discoidal, centered on uterotubal junction (mesometrial)	I. Diffuse – endotheliodichorial (EM) II. Discoidal – haemomonochorial (cytotrophoblastic type) with discontinuous intracytotrophoblastic lamina (EM)	Stephens, 1962, 1969; Stephens and Cabral, 1972
Mormoopidae			
Pteronotus parnellii	Discoidal, lateral to mesometrial	Haemodichorial	Badwaik and Rasweiler, 1998
Natalidae			
Natalus tumidirostris	I. Midpregnancy – diffuse II. Late pregnancy – bidiscoidal – one disc in each horn centered on uterotubal junction (mesometrial)	I. Diffuse – endotheliodichorial (LM) II. Discoidal – endotheliomonochorial (cytotrophoblastic type) (LM); confirmed at EM level for placentae serving a 15.2 mm (C–R length) foetus	L. Rampaul-Persad, J.J. Rasweiler, I. Omah-Maharaj and Badwaik, unpublished observations
Noctilionidae			
Noctilio leporinus	Discoidal, mesometrial to lateral	Haemodichorial but with abundant persisting maternal endothelial cells (mid-pregnancy) (EM)	Present authors
Phyllostomidae			
Carollia perspicillata	Discoidal, fundic (simplex uterus)	Haemodichorial (EM)	Rasweiler and Badwaik, 1999a,b
Desmodus rotundus	Discoidal, antimesometrial (partially bicornuate uterus)	Haemodichorial (EM)	Wimsatt, 1954; Björkman and Wimsatt, 1968
Macrotus californicus	–	Haemodichorial (EM)	Bodley, 1974
Rhinolophidae			
Hipposideros fulvus	Discoidal changing to bidiscoidal, mesometrial	Haemodichorial (EM)	Gopalakrishna and Karim, 1975; Bhiwgade, 1990; Gopalakrishna, personal communication
Rhinolophus rouxi	Discoidal, mesometrial	Endotheliodichorial (EM)	Bhiwgade, 1977, 1990

Table 6.5 (continued)

Family and species	Form and location of placentae	Fine structure of interhaemal barrier (nature of evidence[a])	References
Rhinopomatidae			
Rhinopoma hardwickei	Discoidal, mesometrial	Endotheliomonochorial (EM)	Karim and Fazil, 1986; Gopalakrishna and Badwaik, 1987; Bhiwgade, 1990
Thyropteridae			
Thyroptera tricolor	Discoidal, antimesometrial	Haemodichorial (EM)	Wimsatt and Enders, 1980
Vespertilionidae			
Miniopterus schreibersii	Main (1°) – discoidal, antimesometrial Accessory (2°) – bidiscoidal, antimesometrial Tertiary – below accessory (2°); therefore bidiscoidal and antimesometrial	Main (1°) – haemodichorial at midpregnancy prior to involution (EM[bc]) Accessory (2°) – haemomonochorial (EM[b]) or endotheliomonochorial to haemomonochorial in some regions (EM[c]) (see text) Tertiary – haemodichorial (EM[c])	Malassiné, 1970; Chari and Gopalakrishna, 1984; Kimura and Uchida, 1984; Bhiwgade *et al.*, 1992
Myotis lucifugus	Discoidal, antimesometrial	Haemodichorial (EM)	Wimsatt, 1945b; Enders and Wimsatt, 1968; Cukierski, 1987

[a] LM = light microscopic; EM = electron microscopic.

[b] Kimura and Uchida (1984).

[c] Bhiwade *et al.* (1992).

chorioallantoic placenta in the closely related families Phyllostomidae and Noctilionidae (both of which belong to the superfamily Noctilionoidea) with that of many other bats. The phyllostomid bats *G. soricina, C. perspicillata*, and *Artibeus jamaicensis* all have simplex uteri and exhibit a fundic positioning of their discoidal placenta (Hamlett, 1935; Wislocki and Fawcett, 1941; Rasweiler and Badwaik, 1999a). Although this placenta begins to develop dorsal to the inner cell mass on one side of the uterus in at least the first two species, it will ultimately span the fundus. This is presumably facilitated by the simplex (single-chambered) nature of the uterus, the uterotubal orientation of the inner cell mass during implantation (rather than full mesometrial; see Figure 6.2), and the invasiveness of the trophoblast in these bats. This, in turn, permits the placenta to become vascularized by the uterine branches of the uteroovarian artery and vein on both sides of the uterus. This is probably one of the essential adaptations that allow *C. perspicillata* to produce large and precocious young. At birth the infant weighs about 27–37% of the mass of newly-mated adult females, has open eyes, and a well-furred dorsal surface. Furthermore, the baby must possess considerable muscular strength and motor skills from the time of birth, because almost immediately it has to attach to its mother's teats and adhere firmly to her while she either hangs in the roost (suspended by her feet) or flies about. While the baby is carried extensively during its first days of neonatal life, it is unclear exactly when the mother begins to forage alone (Kleiman and Davis, 1979; Porter, 1979). Continued growth must soon make it necessary, however, for the infant to hang by itself while its mother forages. Unlike many other small mammals, bats do not build nests that would serve to contain and protect altricial young (Rasweiler and Badwaik, 1999a).

Implantation and placental development are somewhat different in the closely related bats *D. rotundus* (family Phyllostomidae) and *N. albiventris* (family Noctilionidae), both of which have partially bicornuate uteri. Implantation in *D. rotundus* occurs in an endometrial ridge or implantation pad running along the antimesometrial side of the one of the uterine horns, and the definitive placenta develops in an antimesometrial position (Wimsatt, 1954). Implantation in *N. albiventris* occurs in a ridge running along the lateral to antimesometrial side of one of the uterine horns, and the chorioallantoic placenta then begins to develop in an antimesometrial to lateral position (Anderson and Wimsatt, 1963; Rasweiler, 1979b, 1993). While the chorionic sac could conceivably expand into the opposite horn to take advantage of the maternal blood supply on that side (as occurs in some ungulates), that does not happen, and the placenta instead forms along the ridge in just one horn. Elongation of the developing placenta along the ridge may be the critical factor that permits the caudal (lateral) half of the placenta to be vascularized by branches of the uterine artery and vein and the cranial (mesometrial) half to be vascularized to a similar extent by branches of the uteroovarian artery and vein. This dual vascular supply may permit *N. albiventris* (like *C. perspicillata*) to produce larger, more precocious young than might the case with just one set of major maternal vessels vascularizing the placenta (Rasweiler and Badwaik, 1999a).

Although Anderson and Wimsatt (1963) reported that the definitive discoidal placenta of *N. albiventris* is antimesometrial to lateral, this requires re-examination because the developing placenta had assumed a lateral to mesometrial position in some of our specimens by early gastrulation (later stages were not available for study). At mid-gestation in *N. leporinus*, the placenta had a mesometrial to lateral position at the cranial end of one of the uterine horns (Rasweiler and Badwaik, 1999a). This is similar to the mesometrial, or mesometrial to lateral, location of the placental disc observed in the pteropodid (Göhre, 1892a; Moghe, 1951; Karim, 1972a), emballonurid, megadermatid, molossid, rhinolophid and rhinopomatid bats (Gopalakrishna and Karim, 1979, 1980). The position (relative to the broad ligament and oviduct) and vascularization of the mesometrial half of the placenta in *N. leporinus* also looks very similar to that of each half of the placenta in *C. perspicillata* (Rasweiler and Badwaik, 1999a). This supports the suggestion that both the implantation and placental sites in *C. perspicillata* are variants of the mesometrial ones in some other bats.

It seems likely that the vascularization of the uterus also probably plays a major role in positioning of the definitive placenta in the pteropodid bats. In *P. giganteus*, the placenta is mesometrial (Moghe, 1951). In Australian *Pteropus* sp., the prospective implantation and placentation site is vascularized by branches of both the ovarian (uteroovarian) and uterine arteries; however, that region exhibits a unilateral endometrial reaction during early pregnancy. This is apparently due to the preferential delivery of ovarian hormones via a local route that involves their counter- or crosscurrent transfer from the ovarian vein and probably the ovarian lymphatics to the ovarian artery. A branch of the latter vessel then supplies the usual implantation site (Pow and Martin, 1994, 1995).

The vespertilionoids exhibit considerable diversity in the form and positioning of their chorioallantoic placentae. In the thyropterid (Wimsatt and Enders, 1980) and most of the vespertilionid bats (Nolf, 1896; Ramaswami, 1933; Wimsatt, 1945b; Gopalakrishna, 1950; Gopalakrishna and Karim, 1972; Karim, 1977; Ramakrishna and Madhavan, 1977; van der Merwe and Rautenbach, 1988; Gopalakrishna *et al.*, 1989a), each conceptus is served by a single discoidal placenta which is located on the antimesometrial side of the uterus. Many vespertilionids normally carry only a single young, but many others can and sometimes do carry two or more conceptuses (Kurta and Kunz, 1987). The frequency with which the latter occurs can vary geographically in different populations of the same species (Kunz, 1974). Generally, when vespertilionids carry two or more conceptuses, at least one occupies each uterine horn (Sherman, 1930; Deanesly and Warwick, 1939; Wimsatt, 1945a, 1979; Kunz, 1974; Krutzsch, 1975; Gopalakrishna *et al.*, 1979; Badwaik and Gopalakrishna, 1990). Furthermore, in *Pipistrellus subflavus* and *Eptesicus fuscus*, the number of embryos implanting generally exceeds the number of conceptuses surviving until birth (Wimsatt, 1945a; Kunz, 1974). In the case of *P. subflavus*, when a cornu contains two embryos, that situated nearest to the uterine corpus is most likely to be lost. Although the physiological basis for this has not yet been worked out, one possibility would seem to be that the maternal blood supply is unable to support the normal development of placentae in the latter locations. *Lasiurus* sp. frequently have been found to carry as many as three to four conceptuses, although there is one report of an exceptional *Lasiurus borealis* bearing five suckling young (Cockrum, 1955; Hamilton and

Stalling, 1972; Webster *et al.*, 1980; Shump and Shump, 1982a, b). Nothing has been published about placentation in these polytocous North American vespertilionids.

Considerable information is available on implantation and placentation in some of the polytocous vespertilionids inhabiting the Indian subcontinent. These include *Vesperugo leisleri* (Ramaswami, 1933), *Pipistrellus ceylonicus* (Gopalakrishna *et al.*, 1988, 1989a), *Pipistrellus mimus* (Gopalakrishna and Karim, 1972; Karim, 1975b, 1977), *Scotophilus heathi* (Ramakrishna and Madhavan, 1977), and *Scotophilus kuhli* (previously identified as *Scotophilus wroughtoni*; Gopalakrishna, 1949, 1950). In general, when these bats carry two embryos, one implants in each horn, usually near the middle, and a discoidal placenta forms on the antimesometrial side of that horn (Gopalakrishna *et al.*, 1979; Badwaik and Gopalakrishna, 1990; Gopalakrishna and Badwaik, 1992b).

The molossids (*Tadarida, Molossus*, and *Chaerephon*) and the natalid *N. tumidirostris* differ from other vespertilionoid bats in first developing diffuse chorioallantoic placentae. These then involute and are replaced in late pregnancy by single (the molossids) or double (*Natalus*) discoidal chorioallantoic placentae that have a mesometrial location within the uterus. Why these animals develop successive chorioallantoic placentae is an important question. The most likely possibility would seem to be that their discoidal placentae might be better vascularized and/or more efficient in transplacental exchange. This may permit the mothers to deliver better developed infants than might otherwise be the case.

Accessory placental structures

In all of the emballonurid bats adequately studied thus far, a sac-like haemophagous organ has been found attached to the margin of the placental disc (Gopalakrishna, 1958; Wimsatt and Gopalakrishna, 1958). These begin to develop in the mesometrial region of the uterus shortly after implantation. They consist of irregular chorionic folds and villi that invade and erode the endometrium, releasing maternal blood into the sinuses within the haemophagous organ. Erythrocytes are then phagocytized by trophoblast cells and broken down, presumably releasing iron for transmission to the conceptus. Two functionally specialized regions have been recognized in these organs. The deepest region, closest to the endometrium, consists of chorionic folds and villi covered by an inner layer of cytotrophoblast and an outer layer of syncytiotrophoblast. This appears to be primarily concerned with invasion and localized destruction of the endometrium. In the more superficial (absorptive) region, closer to the conceptus, the folds and villi are covered only by a single layer of highly phagocytic cytotrophoblast cells.

Although comparable haemophagous organs have not been observed in other bats, the villous chorioallantoic folds immediately adjacent to the discoidal placenta of *M. lucifugus* apparently engage in a more limited phagocytosis of extravasated erythrocytes (Wimsatt, 1945b).

These villous chorioallantoic folds along the border of the placenta in *Myotis* sp. (Branca, 1927; Wimsatt, 1945b), similar folds in *M. lyra* (Gopalakrishna and Khaparde, 1978b), the membranous (smooth) chorion of *T. tricolor* and many other bats (Gopalakrishna and Karim, 1979, 1980), and remnants of the involuted diffuse chorioallantoic placentae in molossids (Gopalakrishna *et al.*, 1989b; Rasweiler, 1990) are all foetal membranes in close apposition to the endometrium. These are presumably regions of physiological exchange between the foetus and mother, and should also be considered as likely accessory placental structures.

Morphogenesis of chorioallantoic placentae

Although placental development has now been examined in a considerable variety of bats, it would seem neither feasible nor advisable to attempt to survey the available information for all species. Instead, the essential features of the morphogenesis of the placentae in the molossid *M. ater* will be reviewed and then selectively compared to what takes place in other bats. Placental development has been quite thoroughly examined in *M. ater* using captive-bred animals (Rasweiler, 1990, 1991b, 1992), and some of its details seem to have much broader relevance.

During implantation in *M. ater*, the blastocyst expands in the lumen of the right uterine horn. Its trophoblast invades the endometrium over a broad area and engulfs the most superficial maternal capillaries. This is accompanied by expansion of the yolk sac, creating an avascular yolk sac placenta. With the spread of mesoderm and vitelline blood vessels around the yolk sac, this is converted to an extensive, vascularized choriovitelline placenta. This choriovitelline placenta has only a brief existence, as the yolk sac soon collapses and is replaced by vascularized mesoderm derived from the allantois. This creates an extensive diffuse endotheliodichorial placenta, which functions as the principal site of physiological exchange between the conceptus and mother during mid-gestation. The barrier between the two circulations is classified as being endotheliodichorial because it includes the following cellular layers: moderately hypertrophied maternal endothelial cells, syncytiotrophoblast, cytotrophoblast and foetal endothelial cells. An unusually thick basal lamina (the ‘interstitial membrane’) develops between the maternal endothelium and the syncytiotrophoblast. A similar basal lamina has been observed in the endotheliochorial placentae of many other bats, insectivores, tupaiids, carnivores and elephants (Rasweiler, 1993). In the case of the diffuse placenta of *M. ater*, there is no clear evidence of penetration of the trophoblast further than the endometrial–myometrial border. This is also the point at which the maternal endothelial cells become squamous and their basal laminae revert to a more normal thickness.

As pregnancy progresses, the diffuse endotheliodichorial placenta of *M. ater* involutes and is supplanted by a discoidal haemochorial placenta as the major site of foeto-maternal exchange. Although the maternal vascular tubules disappear from the involuting diffuse placenta, the chorion in that region remains vascularized by some foetal vessels and intimately associated with the endometrium. Thus, continued physiological exchange between foetus and mother via these vessels and/or fluid in the exocoelom (which, in turn, bathes the amnion and the highly vascularized splanchnopleure of the collapsed yolk sac) remains possible until parturition. In other words, even the remnants of the diffuse placenta may retain some placental functions.

Development of the discoidal placenta in *M. ater* is of interest because this always occurs at the cranial end of the right uterine horn. This positioning of the discoidal placenta is related to the presence of an unusual vascular tuft that forms in the superficial endometrium of this region after ovulation. A similar tuft forms in the left horn, and the formation of both tufts does not depend upon whether the female is pregnant or not. These tufts are composed of a closely packed network of capillaries and possibly endothelial tubules connected to specialized arterioles and venules which run parallel to the intramural portion of the oviduct. These vessels are specialized in that they exhibit morphological and histochemical changes which are different from those occurring in other endometrial vessels. As decidual reactions spread through the endometrium late in the nonpregnant cycle or during implantation, the endothelial cells of the tuft vessels hypertrophy and their basal laminae become more prominent in sections stained for glycoproteins (Rasweiler, 1991a, b). In the event of pregnancy, the endothelial cells of the tuft vessels in the gravid horn also become highly proliferative and give rise to what appears to be a stratified endothelium in each of the uteroplacental vessels at the base of the discoidal placenta (Badwaik *et al.*, 1998). This unique epithelium is actually formed, however, by great infolding of the proliferating endothelial cells and their basal laminae. This may serve to strengthen the vessel walls while still permitting the endothelial cells to grow as monolayers.

Although trophoblast invades the decidua all around the embryo, much of that engulfing the maternal vessels within the choriovitelline and diffuse placentae is syncytial. That proliferating around the vessels of the vascular tuft remains cellular. Furthermore, much of the cytotrophoblast in this latter region appears to be growing on the thickened basal laminae of the tuft vessels. These observations suggest that the hypertrophied endothelial cells of the vascular tuft may be secreting factors which control early trophoblastic invasiveness and differentiation, and that these factors may be incorporated at least in part into the endothelial cells' basal laminae. They also raise the possibility that (1) the differences in the secretory activities of the maternal endothelial cells in the nontuft decidua might be at least partially responsible for the disparate pattern of trophoblastic differentiation observed in the diffuse placenta, and (2) trophoblast may not invade the myometrium (at least in early pregnancy) because the maternal endothelial cells in that region differ in their secretory activities. Endothelial cells in the myometrium do not hypertrophy or develop thickened basal laminae like those within the placentae. Regional differences have also been noted in the maternal endothelial cells in the uteri of nonpregnant *M. ater* that have spontaneously decidualized (Rasweiler, 1991a). It is now widely appreciated that basal laminae and other components of the extracellular matrix play important morphogenetic roles in a variety of cells and organs (Rasweiler, 1991b).

At the limb bud stage, the relationship between the cytotrophoblast and maternal blood vessels of the vascular tuft in the developing discoidal placenta of *M. ater* begins to change. Solid trophoblastic tubules then start to sprout from the surface of the trophoblastic cuffs surrounding the tuft vessels and from the mesodermal (embryonic) face of a band of cytotrophoblast extending across the base of the placenta. Many of these new trophoblastic tubules lack any maternal vascular elements. Such sprouting greatly increases the number of trophoblastic tubules and the overall size of the developing placenta, and serves to fill in the center of the placenta (previously occupied by the uterine lumen). Patent tubules then form by a canalization process. This may be assisted by the purely cellular nature of the cytotrophoblast, which facilitates the formation of polarized epithelial cells joined by junctional complexes around central lumina.

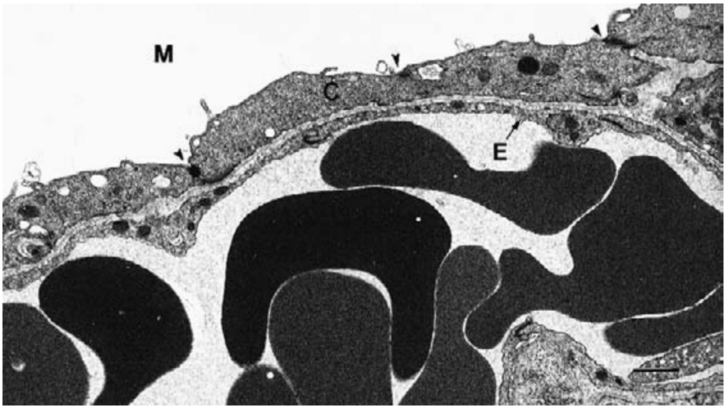
While some maternal blood was evident in many of the smallest tuft vessels up until the completion of implantation, less was seen in these vessels at the somite stage, and little or none was present from the limb bud stage onwards. The first evidence of significant maternal blood flow through the discoidal placenta was not observed until the foetus reached a crown–rump length of about 11.4 mm. This seems significant in at least three respects. First, it suggests that during early placental development the trophoblast is responding to morphogenetic factors of maternal endothelial cell origin, rather than to factors emanating from the maternal blood. Second, it points to the importance of having the diffuse placenta as a site of physiological exchange between embryo and mother during development of the discoidal placenta. Finally, mechanisms must exist for redirecting maternal blood flow when the diffuse placenta involutes and the discoidal placenta becomes functional.

The precise fate of the maternal endothelial cells in the smaller vessels of the vascular tuft and their basal laminae is not known, but neither are evident histologically after the discoidal placenta begins to function or ultrastructurally in near-term placentae (Figures 6.6 and 6.7). Ultrastructural studies pursued with another molossid bat, *T. brasiliensis*, indicate that the endothelial basal laminae in the developing placental labyrinth are penetrated and engulfed by ectoplasmic processes of the cytotrophoblast cells (Stephens, 1969). When maternal blood begins to flow through the placental labyrinth of *M. ater* and *T. brasiliensis*, the interhaemal barrier is haemomonochorial.

Although mitotic activity was commonly seen in the cytotrophoblast cells of the solid tubules in the developing discoidal placenta of *M. ater*, this ceased quite abruptly when the tubules became patent and began to carry maternal blood. From that point on, further growth of the tubules appeared to be due only to hypertrophy of the existing trophoblast cells, and these were converted to cytotrophoblast giant cells as pregnancy progressed (Figure 6.6) (Rasweiler, 1991b).

Development of the discoidal haemochorial placenta has also been examined in *T. brasiliensis* and the Indian molossid *Chaerephon plicata* (Stephens, 1962, 1969; Gopalakrishna *et al.*, 1989b) and, in most respects, the processes appear to be quite similar. Stephens noted, however, that placental development in *T. brasiliensis* initially involves the formation of a thickened pad of cytotrophoblast immediately around the uterine end of the oviduct. Then, at the early limb-bud stage, maternal blood vessels infiltrate the basal portion of this pad, and endothelial cells migrate into the trophoblast. This differs from what has been observed in *M. ater*, where the cytotrophoblast initially uses the vessels of the vascular tuft as a framework on which to grow. In the natalid bat *N. tumidirostris*, the maternal endothelial cells persist and proliferate during development of the paired discoidal placentae, thereby greatly enlarging the maternal labyrinth. These cells are still present in near-term placentae, and the interhaemal barrier at that stage appears, on the basis of both light and electron microscopic observations, to be endotheliomonochorial (L. Rampaul-Persad, J.J. Rasweiler and I. Omah-Maharaj, unpublished observations).

6.6

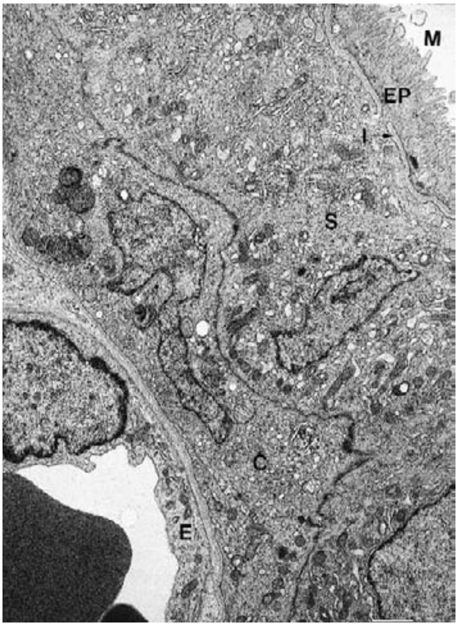


6.7

Figures 6.6 and 6.7 Electron micrographs of the haemomonochorial interhaemal barrier in the chorioallantoic placenta of a *Molossus ater* carrying a near-term (32 mm) foetus. The only cellular elements in the minimal barrier are cytotrophoblast (C) and the endothelial cells (E) of the foetal capillaries. Intercellular junctions (arrowheads) are generally abundant in the trophoblastic layer and indicate that the cytotrophoblast cells must have many interdigitating processes. The barrier is unusual among those of bats because it contains neither an intratrophoblastic lamina nor an interstitial membrane. Infoldings of the trophoblastic basal lamina (arrows in Figure 6.6) are common within the trophoblastic layer. M = maternal blood space. Bar (Figure 6.6) = 1.25 μm. Bar (Figure 6.7) = 0.90 μm.

Early development of the discoidal chorioallantoic placenta in the vespertilionid *M. lucifugus* also seems to exhibit some similarities to that observed in *M. ater*. Implantation in *M. lucifugus* takes place at a hyperaemic site on the antimesometrial wall of the uterus at about the middle of the right horn (Wimsatt, 1944b). Immediately beneath the luminal epithelium at this site is a distinctive tuft of small anastomotic vessels. These are lined by moderately hypertrophied endothelial cells, and there is little connective tissue between the vessels. These endothelial cells are retained during and immediately after implantation, when the tuft vessels are engulfed by trophoblast. During this period the endothelial cells undergo some proliferation, thereby contributing to enlargement of the early placental labyrinth. Further major steps in the development of the definitive haemochorial placenta in *M. lucifugus* involve (1) the penetration of the maternal endothelial basal laminae by ectoplasmic processes of the adjacent syncytiotrophoblast, (2) the elimination of maternal endothelial cells from the labyrinth at a still early stage of pregnancy, and (3) further enlargement and remodeling of the maternal blood spaces, so that the final placental labyrinth differs greatly in form from the original endometrial capillary bed. Components of the maternal endothelial basal lamina appear to persist as part of an interrupted layer, the ‘intrasyncytial lamina’ that is completely engulfed by the syncytiotrophoblast. The intrasyncytial lamina remains as an extracellular layer in the interhaemal barrier until parturition (Wimsatt, 1944b, 1945b; Enders and Wimsatt, 1968; Cukierski, 1987). Development of discoidal haemochorial placentae in a variety of non-molossid bats generally seems to follow a pattern similar to that just described for *M. lucifugus* (Björkman and Wimsatt, 1968; Bodley, 1974; Kimura and Uchida, 1984; Bhiwgade, 1990). In some species (e.g. *N. leporinus* and *M. lyra*), many maternal endothelial cells persist in the labyrinth well into pregnancy or even until parturition; however, the minimal barrier becomes haemodichorial (Figures 6.8 and 6.9).

With rare exceptions (e.g. some of the molossids), ultrastructural studies have confirmed the presence of an intrasyncytial lamina as a regular feature of the interhaemal barrier in the haemochorial placentae of bats. A very similar layer appears to occur in the placental barrier of the hyena (Order Carnivora), several sciurid rodents, and possibly the elephant shrew (Order Macroscelidea) (Enders, 1965, 1982; Oduor-Okelo and Neaves, 1982; Oduor-Okelo, 1985; Wynn *et al.*, 1990). The engulfment of a fragmented basal lamina by adjacent epithelial cells (i.e. trophoblast) is clearly a peculiar morphological relationship. It has been proposed that this may have evolved, in part, as a biological compromise that retains tissue of maternal origin in the interhaemal barrier for morphogenetic reasons (i.e. to influence trophoblastic growth and/or differentiation), but isolates potentially thrombogenic subendothelial components from exposure to the maternal blood (Rasweiler, 1991b). Observations made on placental development in *M. ater* strongly suggest that maternal endothelial cells influence trophoblastic invasiveness, proliferation, and differentiation in that species, and that some of these effects probably depend upon factors incorporated into the endothelial cells’ basal laminae. It is also well established that one of the functions of endothelial cells is to provide a non-thrombogenic lining for the vascular system, while subendothelial components of the vessel walls (including endothelial cell basal



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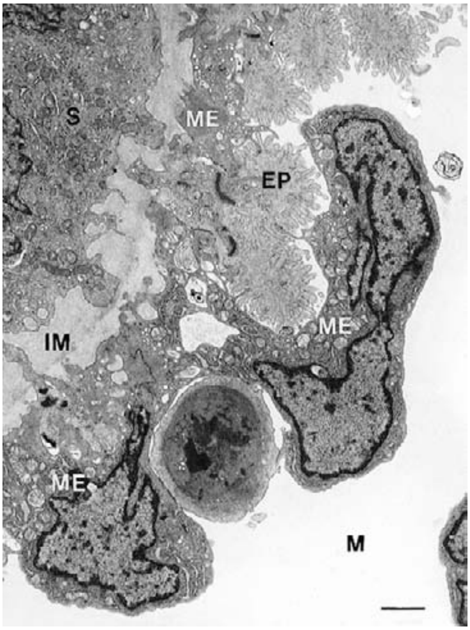


6.9

Figures 6.8 and 6.9 Electron micrographs of the interhaemal barrier in the chorioallantoic placenta of a *Noctilio leporinus* obtained during mid-pregnancy (18 mm foetus). At this stage, the cellular elements present in the minimal barrier (Figure 6.8) include a layer of syncytiotrophoblast (S) containing an intrasyncytial lamina (I), cytotrophoblast (C) and the endothelial lining (E) of foetal capillaries. Scattered maternal endothelial cells (ME) are still present in some portions of the placental labyrinth (Figure 6.9). Where a thickened extracellular layer intervenes between these endothelial cells and the syncytiotrophoblast, this is probably best referred to as an interstitial membrane (IM). EP = ectoplasmic processes of the syncytiotrophoblast. M = maternal blood space. Bar (Figure 6.8) = 1.01 μm. Bar (Figure 6.9) = 1.23 μm.

laminae) can induce platelet adhesion and aggregation (Rasweiler, 1991b). Other possible functions of the intrasyncytial lamina are discussed in the next section.

In some bats, maternal endothelial cells and their basal laminae persist in the interhaemal barrier of the chorioallantoic placenta until parturition (i.e. the endotheliochorial condition). In that situation, both layers could exert morphogenetic influences upon the trophoblast and presumably have other functions as well (e.g. in mediating transplacental transport and contributing to the tensile strength of the barrier).

Nature of the interhaemal barrier

All bats examined thus far have been found to possess chorioallantoic placentae in which the interhaemal barrier is either endotheliochorial or haemochorial (Table 6.4 (page 239)). In the endotheliochorial condition, the maternal blood spaces are lined by a continuous layer of maternal endothelial cells. In the minimal barrier, these are then separated from foetal blood by one or two layers of trophoblast and the endothelial cells of the foetal capillaries. In the haemochorial condition, maternal blood circulating through the placental labyrinth directly bathes the trophoblast. The only cell layers separating the two circulations in the minimal haemochorial barrier are one or two layers of trophoblast and the endothelial cells of the foetal capillaries. When one layer of trophoblast is present, the barrier is monochorial, and when two layers are present it is dichorial. In some bats, scattered maternal endothelial cells persist in the interhaemal barrier, but the *minimal* barrier is haemochorial and should be characterized as such. Because some layers in the interhaemal barrier can become extremely attenuated, ultrastructural studies are always necessary to confirm which components are present.

With relatively few exceptions, the interhaemal barrier of most bats includes a prominent extracellular layer. This is generally rich in glycoproteins and can be visualized readily in histological sections by staining with the periodic acid-Schiff reaction. In endotheliochorial bat placentae, this takes the form of a thickened basal lamina, frequently referred to as an 'interstitial membrane', that intervenes between the maternal endothelium and the trophoblast. In most haemochorial bat placentae, the maternal endothelium is lost (sometimes not completely), but the endothelial basal lamina appears to persist as part of the 'intrasyncytial lamina' that is usually completely engulfed by trophoblastic processes. In early studies, both layers were referred to as interstitial membranes (Wimsatt, 1958). Despite the fact that Enders and Wimsatt (1968) subsequently drew a clear distinction between these two layers based upon their ultrastructural studies of placentation in *M. lucifugus*, some investigators continue to use the terminology inappropriately.

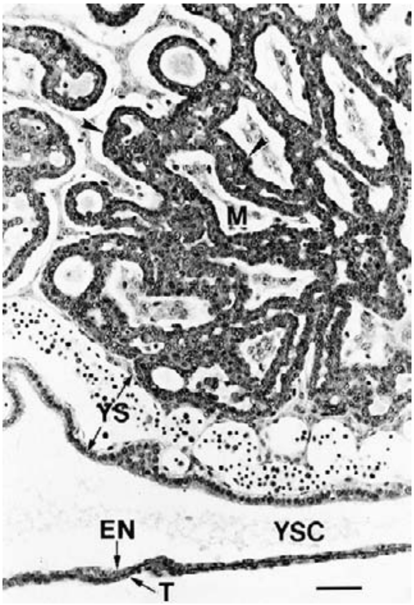
The possible morphogenetic role of the intrasyncytial lamina in placental development was discussed in the previous section. It has also been suggested that the intrasyncytial lamina may have roles in contributing to the immune barrier between the mother and conceptus, increasing the surface area of the apical plasmalemma of the syncytiotrophoblast to facilitate transplacental transport, selective filtration, structural support, and the maintenance of cell polarity (Enders and Wimsatt, 1968; Cukierski, 1987). Recent studies involving *Pteronotus parnellii* (family Mormoopidae) lend further credence to the possibility that the intrasyncytial lamina may contribute significantly to structural support within the chorioallantoic placenta. In many portions of the placental labyrinth of this bat, thickening of the intrasyncytial lamina was noted, and this increased with the size of the vascular channels. As such thickening would presumably decrease the efficiency of physiological exchange by increasing the diffusion distance across the interhaemal barrier, it must have another purpose, such as to strengthen those regions mechanically (Badwaik and Rasweiler, 1998).

Evidence was obtained that the intrasyncytial lamina may make an important contribution to the mechanical strength of the barrier even in the smallest vascular tubules. When these tubules were stained immunocytochemically for cytokeratins, expression by the syncytiotrophoblast was generally very weak in advanced pregnancy. Intermediate filaments composed of cytokeratins usually constitute a major component of the cytoskeleton in cells of epithelial origin. Cytokeratin expression by the cytotrophoblast in the haemodichorial barrier of the smallest tubules in *P. parnellii* was much stronger; however, this layer is often highly attenuated and in that form may make only a limited contribution to the tensile strength of the barrier. It should be noted that significant cytokeratin expression was observed in both the cyto- and syncytiotrophoblast lining the larger maternal vascular channels (Badwaik and Rasweiler, 1998). This may serve to further strengthen the walls of those channels.

A similar dichotomy in cytokeratin expression by the cyto- and syncytiotrophoblast in the smallest maternal vascular tubules of the placenta has been observed during advanced pregnancy in the phyllostomids *D. youngi* and *C. perspicillata* which also have intrasyncytial laminae (Badwaik and Rasweiler, 1998; Badwaik *et al.*, 1998; Rasweiler and Badwaik, 1999b). Interestingly, when exactly the same anticytokeratin antibodies were used on the interhaemal barrier of the human placenta (which does not have intrasyncytial laminae), both the cytotrophoblast and the syncytiotrophoblast reacted strongly (Daya and Sabet, 1991).

It would seem that the interstitial membrane might play a similar mechanical role in bats with endotheliochorial placentae. This suggestion is supported by comparative observations made on seals, which also have endotheliochorial placentae. The interhaemal barrier in the seal placenta is noteworthy for the extreme attenuation of its constituent layers. This may be an adaptation of diving mammals to facilitate the diffusion of gases between the foetal and maternal circulations. Nevertheless, an interstitial membrane is present and relatively thick (when compared to the other layers) even in the thinnest portions of the barrier (Harrison and Young, 1966).

While the nature of the minimal interhaemal barrier for a variety of bats has been summarized in Table 6.4, additional comments deserve to be made for some species. Until now, nothing has ever been published on placentation in either member of the family Furipteridae. We have examined the developing discoidal chorioallantoic placenta from a female *Furipterus horrens* captured in a road culvert in the Western Cordillera of the Colombian Andes. Although she carried only a somite stage embryo, most maternal endothelial cells had already been eliminated from the larger vascular channels (establishing a haemodichorial condition), but were still common in the smaller ones (Figures 6.10 and 6.11). This raises the possibility that by late in pregnancy, the minimal barrier may be haemochorial throughout much of the placenta. In the case of *M. lyra*, *N. leporinus* and *P. giganteus*, some endothelial cells were still evident in the maternal labyrinth of the specimens examined, but the minimal barrier observed in each species was haemodichorial. The *M. lyra* were reportedly in advanced pregnancy, but this was not substantiated with any foetal measurements (Bhiwgade, 1990). The foetus carried by the *N. leporinus* weighed only 1.45 g (in a female weighing 59.7 g with the gravid uterus *in situ*), had a crown–rump length of only 18 mm, and clearly did not appear to be near birth. The largest foetal crown–rump lengths reported for this species have been 41–43 mm (Hood and Jones, 1984). Therefore, it cannot be assumed that the loss of endothelial cells from its placenta was complete. That may also hold for the *P. giganteus*, because the oldest foetus examined seems very small (27 mm) for one supposedly obtained in advanced pregnancy from a very large bat. Marshall (1953) indicated that the foetus and placenta of this species may weigh as much as 118 grams! Furthermore, it was simply difficult to tell from the published



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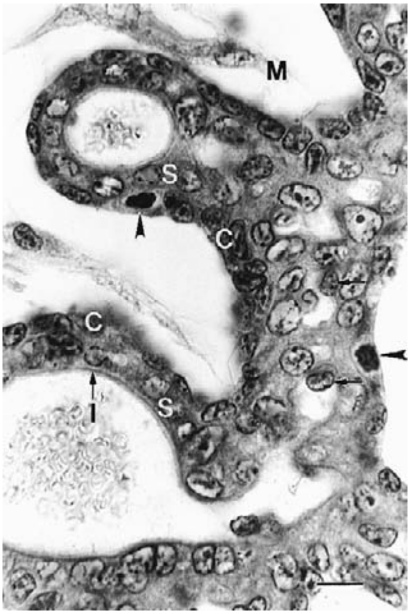

6.11

micrographs how frequent persisting endothelial cells were in the maternal labyrinth (Karim and Bhatnagar, 1996).

In the molossid bats *C. plicata, M. ater* and *T. brasiliensis*, the interhaemal barrier in the discoidal placenta becomes haemomonochorial and is quite unusual because the only trophoblast present is of the cellular variety (Figures 6.6 and 6.7) (Stephens, 1969; Gopalakrishna *et al.*, 1989b; Rasweiler, 1991b). In most mammals with haemochorial placentae, at least one continuous layer of syncytiotrophoblast is present. The only other exceptions presently known are the secondary (accessory) placentae of the vespertilionid bat *M. schreibersii* (Kimura and Uchida, 1984), the jumping mouse (*Zapus hudsonius*), probably the jerboa (*Jaculus jaculus*; King and Mossman, 1974) and the rock hyrax (*Heterohyrax brucei*; Oduor-Okelo *et al.*, 1983). The cytotrophoblast cells of *T. brasiliensis* also contained engulfed masses of basal lamina-like material reminiscent of the intrasyncytial lamina seen in many other bats. This was not seen in the near-term discoidal placentae of *M. ater*.

Placentation in *M. schreibersii* is of interest because, in contrast to other vespertilionid bats, it exhibits the formation of more than one type of discoidal chorioallantoic placenta (Gopalakrishna and Chari, 1983; Chari and Gopalakrishna, 1984). The main or primary placenta is initially endotheliochorial. It then becomes haemodichorial, however, as the maternal endothelial cells are lost, and their basal laminae are engulfed by syncytiotrophoblast to become at least part of an intrasyncytial lamina.

As pregnancy progresses, paired accessory or secondary placentae develop on the margins of the main placenta, and these gradually supplant the latter as a site of foetomaternal exchange. Unfortunately, we still lack a consensus on how these accessory placentae form or the ultimate fine structure of their interhaemal barrier. Studies by Malassiné (1970) indicated that the maternal vascular tubules were lined initially by hypertrophied maternal endothelial cells, an interstitial membrane, and cytotrophoblast. On the other hand, Kimura and Uchida (1984) considered the hypertrophied cells to be cytotrophoblast and reported that these were separated from a second layer of cytotrophoblast by a fragmented ring of homogeneous extracellular material. In the light of what occurs in other bats, it seems likely that Malassiné's interpretation is the correct one and that the homogeneous material represents, at least in part, the remnants of the endothelial

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Figures 6.10 and 6.11 Sections of the developing discoidal chorioallantoic placenta from a *Furipterus horrens* carrying a somite stage embryo. Although the allantois had begun to develop, allantoic vessels had not yet begun to invade the mesoderm (M) surrounding the tubules of the maternal vascular labyrinth (arrowheads). In Figure 6.10, it may be noted that the embryonic side of the disc was covered by vascularized yolk sac (YS), creating a choriovitelline placenta. The remainder of the yolk sac wall was a bilaminar omphalopleure, composed of trophoblast (T) and endoderm (EN) separated by a thick basal lamina (Reichert's membrane). YSC = yolk sac cavity. Figure 6.11 shows a higher power view of the developing chorioallantoic placenta. The larger maternal vascular tubules were lined for the most part by syncytiotrophoblast (S) containing a periodic acid-Schiff positive intrasyncytial lamina (I). Only scattered maternal endothelial cells were present in the large tubules, but these were more common in the smaller ones (e.g. at arrows). Mitotic figures (arrowheads) were frequently seen in the cytotrophoblast layer (C) adjacent to the syncytiotrophoblast. Amylase-PAS-haematoxylin. Bar (Figure 6.10) = 50 μm. Bar (Figure 6.11) = 10 μm.

basal lamina. Near-term, Kimura and Uchida (1984) reported that these accessory placentae become haemomonochorial, and that the only trophoblast present within their interhaemal barrier is of the cellular variety (as in the molossid bats). Other investigators have reported instead that the accessory placentae are endotheliomonochorial and have an interstitial membrane in the interhaemal barrier. In some areas, however, the cytotrophoblast or foetal capillaries are reportedly bathed directly by maternal blood (Malassiné, 1970; Bhiwgade *et al.*, 1992). Some of these discrepancies in the final structure of the interhaemal barrier may have been due to differences in the ages of the placentae examined, but further studies to eliminate the confusion are clearly needed.

Finally, a distinctive region develops deep within each accessory placenta in *M. schreibersii* and has been referred to as the tertiary placenta (Chari and Gopalakrishna, 1984; Bhiwgade *et al.*, 1992). The barrier in these regions is haemodichorial and includes an intrasyncytial lamina.

Although the interhaemal barrier remains haemodichorial in many bats until the time of parturition, the cytotrophoblastic layer frequently becomes highly attenuated (Figure 6.12) (Enders and Wimsatt, 1968; Wimsatt and Enders, 1980; Badwaik and Rasweiler, 1998; Rasweiler and Badwaik, 1999b). This raises the interesting question of why the cytotrophoblast persists at all. In the human, by

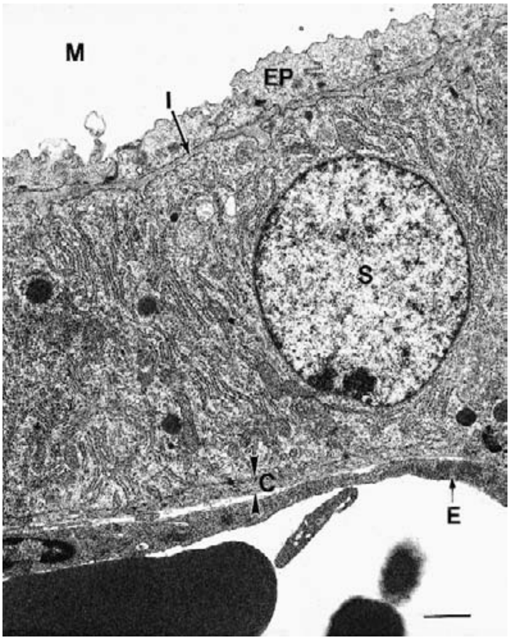


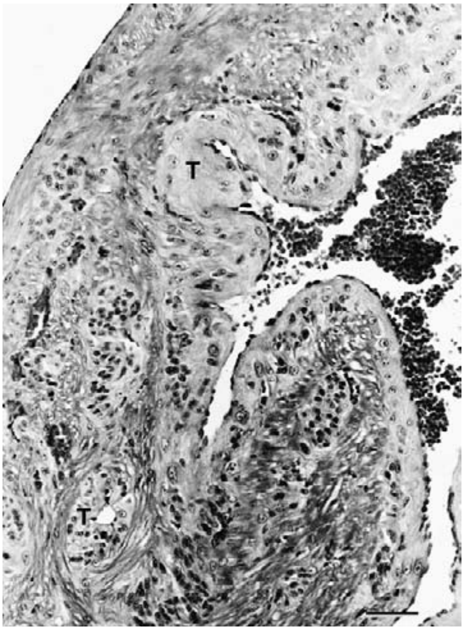
Figure 6.12 Electron micrograph of the interhaemal barrier in the chorioallantoic placenta of a *Carollia perspicillata* carrying a near-term (32.5 mm) foetus. Cellular elements present in the minimal barrier include a layer of syncytiotrophoblast (S) containing an intrasyncytial lamina (I), an attenuated layer of cytotrophoblast (C), and the endothelial lining (E) of foetal capillaries. EP = ectoplasmic process of the syncytiotrophoblast. M = maternal blood space. Bar = 1.03 μm. Reproduced from Rasweiler and Badwaik (1999b).

comparison, much of the cytotrophoblast is eliminated from the placental barrier by late in pregnancy, when the germinal function of these cells is essentially complete. The cytotrophoblast may persist, in part, because in some species (*C. perspicillata, D. youngi* and *P. parnellii*) it is significantly richer in cytokeratins than the syncytiotrophoblast, and thereby contributes to the tensile strength of the interhaemal barrier.

Our studies on *C. perspicillata* indicate that the cytotrophoblast also may be retained for morphogenetic reasons. When the developing placentae of *C. perspicillata* carrying delayed pregnancies are compared to those of normal pregnancies obtained on or about the same postcoital age, many differences in trophoblastic differentiation can be observed. During the period of delay, the developing chorioallantoic placenta typically contains little or less trophospongium (i.e. regions consisting only of syncytiotrophoblast and maternal vascular spaces). Syncytiotrophoblast does line the maternal vascular spaces in the remainder of the placenta, but much of the immediately surrounding cytotrophoblast appears randomly arranged, has few well-developed cell junctions, and lacks a basal lamina. Finally, invasion of the placenta by mesoderm is retarded. Mesodermal invasion of the placenta occurs earlier and is much more vigorous in normal (non-delayed) pregnancies. This does not take place, however, until the cytotrophoblast in the regions to be invaded first becomes organized as sheets of contiguous cells connected by lateral cell junctions exhibiting apical-basal polarity, and having basal laminae. This suggests that one of the functions of cytotrophoblast may be to control or direct invasion of the developing placenta by mesoderm which is intimately associated with vascularization of its embryonic side. Apparently cytotrophoblast continues to perform essential, but still undefined, functions in the interhaemal barrier of *C. perspicillata* and many other bats until the time of parturition.

6.5 HIGHLY INVASIVE TROPHOBLAST

Trophoblastic invasiveness varies greatly between species. At one extreme are the artiodactyls, cetaceans and strepsirhine primates possessing trophoblast that is either noninvasive (pigs) or minimally invasive (sheep) (Hoffman and Wooding, 1993; B. King, 1993; G. King, 1993). At the other extreme is the human, in which large numbers of cytotrophoblast cells penetrate as far as the inner one-third of the uterine myometrium. This takes place via both the lumina of blood vessels, especially the spiral arterioles (i.e. an endovascular route) and the extracellular matrix of the uterine connective tissue (i.e. an interstitial route). This is important physiologically, because the trophoblast is thought to be responsible for dilating the arterioles, thereby permitting them to deliver increasing amounts of maternal blood to the placenta as pregnancy progresses (Pijnenborg, 1996). Cytotrophoblast also invades the uteroplacental arteries of macaques. This is associated with alteration of the vessel walls (e.g. displacement of the smooth muscle fibers and changes in the extracellular matrix) and dilatation of the vessels (Blankenship *et al.*, 1993; Blankenship and Enders, 1997). In baboons, trophoblastic invasion of the uteroplacental arteries is limited to their decidual segments, and interstitial



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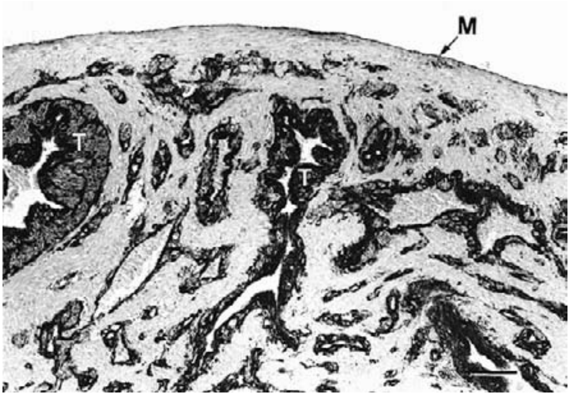

6.14

Figures 6.13 and 6.14 Abundant, highly invasive trophoblast (T) in the walls of myometrial blood vessels in the uterus of a *Diaemus youngi* carrying a primitive streak stage embryo. The section in Figure 6.13 has been stained with haematoxylin and eosin. The section in Figure 6.14 has been stained immunocytochemically with antibody mixture AE1/AE3 which reacts with a variety of cytokeratins and counterstained lightly with haematoxylin. The only cytokeratin positive cells in Figure 6.14 are perivascular trophoblast (e.g., at T) and mesothelial cells (M). No uterine glands are present in this field. Bar (Figure 6.13) = 50 μm. Bar (Figure 6.14) = 100 μm.

trophoblast migration is less frequently observed (Pijnenborg *et al.*, 1996). In cricetid and murid rodents, trophoblast normally does not invade the myometrium except via an endovascular (intraluminal) route along the uterine arteries (Pijnenborg *et al.*, 1981; Carpenter, 1982). In the guinea pig, on the other hand, trophoblast invades the uteroplacental arteries by both intramural and endovascular routes, progresses at least as far as the mesometrial segments, and is suspected of being responsible for pregnancy-induced physiological dilatation of these vessels (Nanaev *et al.*, 1995).

All bats examined thus far examined have trophoblast that normally invades the endometrium and is involved in the formation of either endotheliochorial or haemochorial placentae. Recent work indicates that some species also possess trophoblast that is extraordinarily invasive, penetrating the myometrium, mesenteries of the reproductive tract, the oviducts and even (to a more limited extent) the ovaries. In the best-studied species (*C. perspicillata, D. youngi* and *M. ater*), this was first noted in routine histological sections (Figure 6.13). As it can be challenging to distinguish trophoblast from some maternal cells, these observations were then confirmed by using intermediate filament protein immunocytochemistry. These proteins generally differ according to the differentiation program of cells, with cytokeratins typically being found in epithelial cells (e.g. trophoblast) (Figure 6.14), vimentin in cells of mesenchymal origin and desmin in myogenic cells. Furthermore, their structure has been highly conserved in evolution, so that the available antibodies will frequently cross-react with the intermediate filament proteins in different species.

The highly invasive trophoblast in these bats exhibits both common features and some species' differences. In all three bats, the trophoblast is released by the developing discoidal placenta and then invades the myometrium, as well as adjacent tissues, by means of interstitial migration within the walls of the maternal blood vessels. Invasion via endovascular routes has never been observed in these animals (Badwaik *et al.*, 1998; J.J. Rasweiler, N.K. Badwaik and T.A. Sugarbaker, unpublished observations).

In the case of *M. ater*, the highly invasive trophoblast originates from the cytotrophoblastic shell running along the base of the discoidal placenta and is mononuclear. Although both arterial and venous vessels are invaded, arteries serving the discoidal haemochorial placenta clearly seem to be preferred. These vessels dilate tremendously when the diffuse endotheliochorial placenta that is prominent during midgestation involutes and the discoidal haemochorial placenta becomes functional. It has been suggested that the invasive trophoblast may play a major role in inducing dilatation of these vessels (Badwaik *et al.*, 1998).

In *D. youngi*, the highly invasive trophoblast originates instead from a layer of syncytiotrophoblast on the periphery of the developing chorioallantoic placenta, is multinucleated, and vigorously invades both arterial and venous vessels in the myometrium. It has been suggested that this perivascular trophoblast may be responsible for remodelling and dilating these vessels during pregnancy, thereby increasing blood flow to the placenta as pregnancy progresses. Late in pregnancy, this trophoblast becomes highly branched and extends attenuated processes around many of the myometrial smooth muscle fibers. This raises the possibility that it may also have important local influences upon myometrial contractility.

In *C. perspicillata* (which is a phyllostomid bat like *D. youngi*), the highly invasive trophoblast also originates from a layer of syncytiotrophoblast on the periphery of the developing chorioallantoic placenta; however, it fragments more than in *Diaemus* and, based upon ultrastructural analyses, appears to become mono- or occasionally binucleate in the myometrium and mesenteries. The abundance of this invasive trophoblast varied greatly between animals, and this was related to whether the pregnancies were normal or prolonged by postimplantational delays in embryonic development (J.J. Rasweiler, N.K. Badwaik and T.A. Sugarbaker, unpublished observations).

Delayed development has been observed under two quite different sets of circumstances in *C. perspicillata*. When wild-caught animals were mated in captivity, their gestation periods were highly variable in length. If the females were mated during their first year in captivity, the gestation length was 105–178 days (mean ± SD: 145 ± 19 days). If mated during their second year, the gestation periods tended to be shorter (113–169 days) (mean: 127 ± 12 days). If the females were both born and mated in captivity, the gestation periods were still shorter (113–159 days) (mean: 119 ± 9 days). Most females in the last group had gestation periods of 113–119 days, and this probably represents the normal (nondelayed) gestation period for the species. All of the available evidence suggests that the lengthening of pregnancy in some of these captive-bred animals was probably a response to stress. This was ameliorated somewhat by holding the females in captivity for a longer period prior to breeding them, and it could be largely eliminated by breeding captive-reared animals. Delayed development also occurs seasonally in the wild (Rasweiler and Badwaik, 1997).

Highly-invasive trophoblast was generally abundant in the reproductive tracts of (1) captive-bred females carrying primitive streak stage or more advanced embryos that were significantly retarded in their development, at least up to day 60 *post coitum* (*p.c.*) (the latest stage examined) and (2) pregnant, wild-caught females carrying primitive streak stage embryos during the seasonal period of delayed development. The amount of invasive trophoblast was reduced in females carrying less-delayed pregnancies and absent from the tracts of females carrying normal (nondelayed) embryos (J.J. Rasweiler, N.K. Badwaik and T.A. Sugarbaker, unpublished observations).

The presence of abundant, highly invasive trophoblast in the myometrium and adjacent tissues was also associated with altered development of the placenta. Polarized cytotrophoblast and trophospongium (regions composed only of syncytiotrophoblast and maternal vascular spaces) were much less abundant in the developing placentae associated with delayed pregnancies. These observations suggest that retarded development of the embryo might be the result of altered trophoblastic differentiation and function, which may in turn affect other important morphogenetic events. The invasion of such placentae by mesoderm, which is a prerequisite for vascularization of the placenta by allantoic vessels, was also delayed. Before this can occur, it would appear that a polarized cytotrophoblast with a basal lamina must be present (J.J. Rasweiler and N.K. Badwaik, unpublished observations).

In addition to cytokeratins, the highly invasive trophoblast of *C. perspicillata, D. youngi* and *M. ater* also express vimentin, an intermediate filament protein usually associated with cells of mesenchymal origin (Badwaik *et al.*, 1998; J.J. Rasweiler, N.K. Badwaik and T.A. Sugarbaker, unpublished observations). This is perhaps not surprising because, in order to migrate through the connective tissues of the female reproductive tract, these cells must undergo an epithelial-mesenchymal transformation. This is frequently associated in other cell types with the initiation of vimentin expression (Hay, 1995; Hay and Zuk, 1995), but is an unusual characteristic for trophoblast. Vimentin expression was previously reported only for the invasive interstitial cytotrophoblast of humans, as well as its precursors on the periphery of the placenta (Loke and Butterworth, 1987), and for giant cells in the uteri of pregnant rabbits which are thought to be of trophoblastic origin (Blackburn *et al.*, 1989).

Highly invasive, perivascular trophoblast has also been observed by us in the myometrium and mesenteries of the reproductive tract in *F. horrens* (family Furipteridae) (Figure 6.15), *T. tricolor* (family Thyropteridae) (Figure 6.16), and *P. parnellii* (family Mormoopidae). In all three species, these cells were found to be reactive with antibodies directed against cytokeratins; for evidence of this in *P. parnellii*, see figure 9 in Badwaik and Rasweiler (1998). Because this is an unusual characteristic that has been observed (thus far) in very few mammals, it may prove useful in assessing phylogenetic relationships both within the Chiroptera and between the Chiroptera and other groups. Furthermore, the required immunocytochemical techniques for intermediate filament proteins will work well on field-collected material preserved in Bouin's fluid.

6.6 LITTER SIZE AND GESTATION PERIOD

With the exception of some members of the family Vespertilionidae, bats generally carry only a single conceptus to term during each pregnancy. Litter size in the vespertilionids usually ranges from one to four depending upon species, with the largest being exhibited by some *Lasiurus borealis* (Hayssen *et al.*, 1993). Gestation length varies greatly between species (Gopalakrishna and Badwaik, 1993b; Hayssen *et al.*, 1993) and, in some cases, can also vary considerably within species because of the occurrence of developmental delays (see previous section; also Racey and Swift, 1981; Rasweiler and Badwaik, 1997; Racey and Entwistle, this volume).

In comparison to most nonvolant mammals of similar size, bats generally give birth to large infants. Kurta and Kunz (1987) calculated the neonatal body mass as a percentage of maternal body mass for representatives of some of the best-studied families. This averaged about 28.8% for the rhinolophids, 26.6% for the phyllostomids, 21.1% for the vespertilionids, and 17.5% for the pteropodids. Whenever possible, the body mass of postpartum females was used to make these calculations. While this is a reasonable approach, it may underestimate the maternal investment in pregnancy for some species. When we examined this in the phyllostomid, *C. perspicillata*, the neonatal mass was found to be 24.0–31.9% (mean: 28.1%) of the mother's mass on the day of birth. However, the neonatal mass was 26.9–36.9% (mean: 32.9%) of the mean mass of adult females weighed on days 1–3 *p.c.* There was no question concerning the maturity of these females, because all carried either large preovulatory follicles or newly released eggs. The observed difference in relative neonatal mass was attributable to the fact that mother bats weighed on the day of birth had a mean mass of 20.41 ± 2.70 grams, while the recently mated adult females had a mean mass of only 17.32 ± 1.06 grams. This difference was significant ($P < 0.001$) and probably due in large part to fat which had been deposited in many of the postpartum females during their

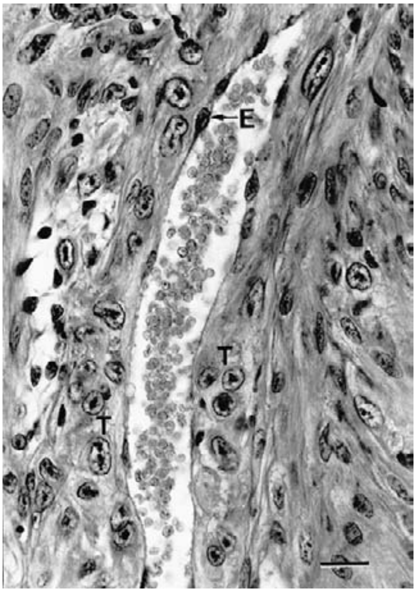

Figure 6.15 Section of myometrium from a *Furipterus horrens* carrying a somite stage embryo. Many highly invasive cytotrophoblast cells (T) containing nuclei with prominent nucleoli are present in the wall of the blood vessel passing across the figure. The vessel is still lined by maternal endothelial cells (e.g. at E), and the trophoblast is subendothelial. Stained with haematoxylin and eosin. Bar = 20 μm.

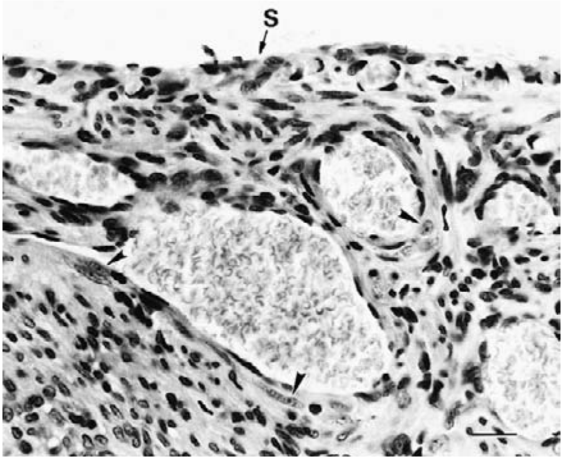

Figure 6.16 Section of myometrium from a *Thyroptera tricolor* carrying a 12 mm embryo. Highly invasive trophoblast (e.g. at arrowheads) may be noted in a subendothelial position in the walls of some of the blood vessels. Although impossible to illustrate here, the trophoblast cells were more basophilic than collagenous and smooth muscle fibers present in the field, and almost completely enveloped each of the vessels. S = serosal surface of uterus. Haematoxylin and eosin. Bar = 20 μm.

pregnancies. As pregnancy progresses, abdominal fat becomes noticeably more abundant in many *C. perspicillata* (Rasweiler and Badwaik, 1999a). It must be cautioned that these observations were made on captive maintained animals; maternal weight gain not including the products of conception could conceivably be less in free-ranging bats.

Kurta and Kunz (1987) have suggested two major ways in which a relatively large neonatal body mass might be advantageous to bats. First, bats do not provide insulating nests for their offspring. The relatively large size and resulting thermal inertia of neonates might ameliorate drops in body temperature during the mother's absence. Second, large body size may be associated with greater neuromuscular maturity which, in turn, probably enables newborn bats to cling tightly to their mothers or to their roosts. The ability of infant bats to remain close to where they have been left by their mothers is presumably of particular importance because of the absence of protective nests (Rasweiler and Badwaik, 1999a). For example, one cannot imagine the altricial young of murid rodents hanging by themselves from the walls or ceilings of a cave within a day or two of birth, as is done by many bats.

Kurta and Kunz (1987) noted that differences in neonatal body size among chiropteran families appear to be mainly phylogenetic, but could not be correlated with placental fine structure, brain size or wing loading (total weight of the bat/wing area). We have observed, however, that the placentae of *C. perspicillata* and *N. leporinus* become positioned in the uterus so that each half becomes vascularized by different sets of major maternal vessels. This dual vascularization probably plays an important role in permitting these bats to produce larger and more precocious young (Rasweiler and Badwaik, 1999a). How this compares to the situation in other bats that produce relatively smaller young requires further research.

6.7 THE ENDOCRINOLOGY OF PREGNANCY

In most eutherian mammals, progesterone must be secreted throughout gestation to maintain a uterine environment conducive to pregnancy and, at least early in pregnancy, this is synthesized by the corpus luteum. Depending upon the species, progesterone of luteal origin may be required throughout pregnancy (rabbit, pig), or the synthesis and secretion of this hormone may be shifted at some point to the placenta or foetoplacental unit (rat, guinea pig, sheep, horse, macaque, human) (Niswender and Nett, 1988). Although their corpora lutea of pregnancy persist into lactation, the luteoplacental shift is completed in macaques by 21 days after ovulation and in humans by 49 days (Mossman and Duke, 1973; Hodgen and Itskovitz, 1988). The ovaries of the horse do not contain corpora lutea during the last 200 days of its 350 day gestation period (Amoroso and Perry, 1977).

In the case of bats, morphological observations on the corpus luteum of pregnancy are available for many species (Table 6.6). These indicate that the corpus luteum disappears completely during gestation in some and involutes at least partially well before the end of pregnancy in others. This suggests that if a requirement for progesterone continues throughout pregnancy, responsibility for producing it must be shifted partially or completely to other steroidogenic tissues,

Table 6.6 Bats exhibiting complete or partial regression of the corpus luteum during pregnancy based upon morphological evidence

Species exhibiting complete regression	Species exhibiting partial regression
Family Megadermatidae	Family Pteropodidae
Megaderma lyra (Gopalakrishna and Badwaik, 1988)	*Cynopterus sphinx* (Krishna and Dominic, 1983)
	Haplonycteris fischeri (Heideman, 1989)[a]
Family Molossidae	*Pteropus poliocephalus* (Towers and Martin, 1995)
Otomops martiensseni (Kayanja and Mutere, 1975)	*Rousettus leschenaulti* (Gopalakrishna and Badwaik,1988)
Family Nycteridae	
Nycteris luteola (Matthews, 1941)	Family Molossidae
Nycteris thebaica (Bernard, 1982)	*Molossus ater* (Rasweiler, 1988)[b]
	Molossus fortis (Krutzsch and Crichton, 1985)
Family Rhinolophidae	*Mormopterus planiceps* (Crichton and Krutzsch, 1987)[b]
Hipposideros caffer, Hipposideros fulvus, Hipposideros lankadiva, Hipposideros speoris (Bernard and Meester, 1982; Gopalakrishna and Badwaik, 1988, 1989)	Family Vespertilionidae
Rhinolophous rouxi (Gopalakrishna and Badwaik, 1988)	*Chalinolobus morio* (Kitchener and Coster, 1981)
Triaenops afer (Matthews, 1941)	*Eptesicus furinus, Eptesicus regulus* (Myers, 1977; Kitchener and Halse, 1978)
Family Rhinopomatidae	*Lasiurus ega* (Myers, 1977)
Rhinopoma microphyllum (Gopalakrishna and Badwaik, 1988)	*Miniopterus schreibersii* (Crichton *et al.*, 1989; Bernard *et al.*, 1991)
Family Vespertilionidae	*Myotis albescens, Myotis nigricans, Myotis tricolor* (Myers, 1977; Bernard, 1982)
Pipistrellus mimus (Krishna, 1985)	*Pipistrellus pipistrellus* (Racey and Swift, 1981)
	Plecotus townsendii (Pearson *et al.*, 1952)

[a] During late pregnancy, most lutein cells were much smaller and the CL was about 1% of the volume of a new CL.
[b] Lutein cells were smaller than earlier in pregnancy.

e.g. the placenta or foetoplacental unit, other components of the ovaries, or the adrenal glands. It should be cautioned that even when the corpus luteum persists until parturition, for most species the available evidence indicates little about its functional capabilities.

Plasma and/or tissue sex steroid levels have been examined in a few bats during pregnancy. Where such studies have focused solely upon delayed implantation and/or delayed development, they will not be reviewed here to avoid overlap with the contribution by Martin and Bernard and Racey and Entwistle in this volume.

In a nicely conducted study of the pteropodid *Pteropus poliocephalus* by Towers and Martin (1995), peripheral plasma progesterone concentrations showed relatively little change around the time when the females were breeding or during early pregnancy, but increased significantly from mid- to late pregnancy. During the first several months of pregnancy (April–July), the corpora lutea also appeared healthy and functional upon histological examination. The lutein cells possessed large nuclei, prominent nucleoli and extensive cytoplasm. Furthermore, the corpora lutea were well vascularized and contained few or no leucocytes. When progesterone levels were compared between the ovaries from a pregnant female in July, much higher levels were detected in the ovary ipsilateral to the gravid uterine horn. The corpora lutea then began to regress both in overall size and in histological appearance. By late pregnancy, they contained few or no recognizably functional lutein cells, but included much more connective tissue and leucocytes. On the other hand, as gestation progressed, placental weight and plasma progesterone levels were significantly correlated, and homogenates of placentae contained large amounts of progesterone. There was no evidence that plasma progesterone concentrations fall before parturition, but they declined rapidly after parturition. Taken together, all of this evidence suggests that in *P. poliocephalus* the placenta is the major source of progesterone in late pregnancy.

In the molossid bats *Mormopterus planiceps* and *T. brasiliensis*, the corpora lutea of pregnancy persist until term, although the lutein cells decrease in size in *M. planiceps* late in pregnancy. Plasma progesterone levels peak late in pregnancy in both species; however, nothing is known about the relative contributions of the corpus luteum and placenta to this (Jerrett, 1979; Crichton and Krutzsch, 1987).

In the phyllostomid *M. californicus*, plasma/serum progesterone levels rise to a peak late in pregnancy but then drop prior to parturition (Burns and Easley, 1977, Burns, 1981; Crichton *et al.*, 1990). Again, the relative roles of the corpus luteum and placenta in progesterone secretion late in pregnancy remain uncertain. Burns (1981) found significant 3β-hydroxysteroid dehydrogenase activity in the placenta at that time. However, he concluded that the ovary is essential for maintaining gestation at all stages, because removal of the right ovary (which always contains the corpus luteum) at various times during gestation resulted in abortion in nearly all cases. This requires confirmation because, in related phyllostomid and noctilionid bats, the implantation and placentation site receives a major part of its maternal blood supply from the uterine branch of the uteroovarian artery (Rasweiler and Badwaik, 1999a). If the same arrangement exists in *M. californicus*, the performance of an ovariectomy might damage important vessels supplying the implantation and placentation site. This may not have been properly addressed by the sham operations that were performed. In most cases, these did not result in abortions (Burns, 1981).

When Crichton *et al.* (1990) dissociated corpora lutea obtained from *M. californicus* at several different stages of pregnancy, they observed a reduction in the number of lutein cells that stained for 3β-hydroxysteroid dehydrogenase, and a reduction in the diameter of the lutein cells, just before parturition. When lutein cells from nondissociated corpora lutea were examined ultrastructurally at that time, the steroidogenic organelles were found to be reduced. Some of these cells contained moderately abundant lipid droplets (which could be indicative of decreased steroidogenesis and the accumulation of steroidogenic precursors), while others exhibited a few lysosomes and autophagic vacuoles (which were interpreted as possible early signs of luteolysis). The full significance of these apparent changes is unfortunately difficult to assess in the absence of a morphometric analysis and studies on the progesterone content or synthetic capability of these cells.

Plasma progesterone levels have been examined during late pregnancy in several vespertilionid bats. In *Antrozous pallidus*, these rise to a peak immediately prior to parturition (Oxberry, 1979), while in *Pipistrellus pipistrellus* they reached a peak about six days before the first parturition and then fell significantly (Racey and

Swift, 1981). The corpus luteum of pregnancy in *A. pallidus* persists to the end of pregnancy (although in an undefined condition), while that of *P. pipistrellus* exhibited a peak in overall volume which coincided with peak plasma progesterone levels and then began to decline. No information is available for either species on the possible role of the placenta in secreting this hormone. In *M. lucifugus*, plasma progesterone levels were found to increase as pregnancy progressed and plateau prior to parturition (Currie *et al.*, 1988; also see Buchanan and Younglai, 1986). As progesterone was present in the placenta in amounts far in excess (about 15- to 20-fold) of what could be explained by the blood content of this organ, Currie *et al.* (1988) concluded that placental steroidogenesis was likely in *M. lucifugus*.

In *M. schreibersii*, plasma progesterone levels were found to peak and decline prior to parturition. During the final month of gestation, lutein cell size also decreased and exhibited some ultrastructural evidence suggestive of reduced steroidogenenic capability (e.g. a reduced content of smooth endoplasmic reticulum, lipid accumulation, and the increased presence of secondary lysosomes) (Crichton *et al.*, 1989; Bernard *et al.*, 1991). However, evidence amassed by van Aarde *et al.* (1994) indicates that much of the increase in plasma progesterone observed in *M. schreibersii* is actually of placental origin. No notable increase in ovarian progesterone occurred in the second half of pregnancy, when plasma levels increased tenfold, while adrenal progesterone varied little during gestation. On the other hand, placental progesterone content increased significantly with foetal weight. When placental progesterone reached its peak level (1163 ± 272 ng/placenta) in late pregnancy, it was present in these other organs at the following mean levels: 1.198 ng/ovary; 8.88 ng/adrenal. Earlier histochemical studies (Peyre and Malassiné, 1969) had established that steroidogenic enzymes are present in the paired secondary (accessory) chorioallantoic placentae at this time.

6.8 POSTPARTUM OESTRUS

With the exception of some of the vespertilionids, bats are monotocous (i.e. they bear only a single infant during each pregnancy). In tropical areas of the world, where environmental conditions are often less constraining, many bats increase their annual reproductive potential by exhibiting a postpartum oestrus (Table 6.7). For most of these species, the interval between parturition and the next oestrus has not been well defined. The phyllostomid *C. perspicillata* is an exception. When captive-maintained, pregnant females were checked once per day between 5 : 30 and 9 : 00 hours, the interval between the discovery of a new baby and the subsequent detection of a sperm-positive vaginal aspirate was 3–6 days for 20 bats, and 8 or 10 days for two others. One female exhibited a split oestrus, with sperm-positive smears on days 3–5 and 7–9. The only females included in these data are those that successfully reared the first infant because, in some species, loss of the young can bring on a new oestrus. Interestingly, *C. perspicillata* will also exhibit a postpartum oestrus with similar timing following the abortion of obviously premature infants (Rasweiler and Badwaik, 1996a).

Table 6.7 Bats exhibiting a postpartum oestrus

Family	Species	References
Pteropodidae	*Cynopterus sphinx*	Ramakrishna, 1947; Sreenivasan *et al.*, 1974; Sandhu and Gopalakrishna, 1984; Krishna and Dominic, 1983;
	Eonycteris spelaea	Beck and Lim, 1973; Bhat *et al.*, 1980
	Epomops buettikoferi, Epomops franqueti	Okia, 1974a; Thomas and Marshall, 1984
	Epomophorus gambianus, Epomophorus labiatus	Okia, 1974b; Thomas and Marshall, 1984
	Haplonycteris fischeri	Heideman, 1988
	Micropteropus pusillus	Thomas and Marshall, 1984
	Ptenochirus jagori	Heideman and Powell, 1998
	Rousettus leschenaulti	Gopalakrishna, 1964; Gopalakrishna and Choudhari, 1977
Emballonuridae	*Coleura afra*	McWilliam, 1987b
	Peropteryx kappleri	Rasweiler, 1982
	Taphozous longimanus	Gopalakrishna, 1955; Krishna and Dominic, 1982
Molossidae	*Chaerephon pumila*	van der Merwe *et al.*, 1987; McWilliam, 1987a
	Molossus ater	Marques, 1986; Rasweiler, 1988
	Molossus fortis	Krutzsch and Crichton, 1985
Nycteridae	*Nycteris luteola*	Matthews, 1941
Phyllostomidae	*Anoura caudifera*	Taddei, 1976
	Artibeus jamaicensis	Fleming *et al.*, 1972; Willig, 1985
	Artibeus lituratus	Tamsitt and Valdivieso, 1963; Taddei, 1976
	Carollia perspicillata	Willig, 1985; Rasweiler and Badwaik, 1996a
	Chiroderma doriae	Taddei, 1976
	Desmodus rotundus	Wimsatt and Trapido, 1952; Willig, 1985
	Diaemus youngi	Badwaik *et al.*, 1998
	Glossophaga soricina	Taddei, 1976
	Stenoderma rufum	Gannon and Willig, 1992
	Sturnira lilium	Taddei, 1976
	Uroderma bilobatum	Fleming *et al.*, 1972
	Vampyrops lineatus	Taddei, 1976; Willig, 1985
Vespertilionidae	*Myotis nigricans*	Wilson and Findley, 1970; Wilson, 1971
	Pipistrellus dormeri	Madhavan, 1978
	Pipistrellus mimus	Krishna, 1985

Unfortunately, the interval between parturition and a postpartum oestrus in *C. perspicillata* tells us nothing about what the interval might be in other bats. This could conceivably vary considerably depending upon uterine anatomy and the frequency of alternation of successive ovulations between the two ovaries, the rate at which debris from the first pregnancy (which may interfere with sperm and/or embryo transport) can be cleared from the female tract, the speed with which the uterus can be repaired to receive a new embryo, the rate and the site of early development of the second conceptus, the duration of lactation, whether the second pregnancy can be delayed during lactation, the mother's nutritional reserves, and environmental constraints (e.g. food availability and ambient temperature).

In the case of *C. perspicillata*, young conceived at a postpartum oestrus can be delivered after what are probably normal/nondelayed gestation periods of 113–119 days, despite the fact that the mother is nursing an infant that is already large at birth (about 24–31.9% of the mother's mass on the day of birth). *Carollia perspicillata* has a number of reproductive adaptations which may help the mother to accomplish this. In captivity at least, many of the females accumulate extra body fat during gestation that can presumably be mobilized and utilized in milk production following the birth of the first infant. Fluid secreted into the uterus around the time of ovulation (which occurs in at least some female *C. perspicillata*) may facilitate the transport of spermatozoa to the site of fertilization and the clearance of debris. *Carollia perspicillata* has a nonpregnant cycle in which most endometrial growth and postmenstrual repair occurs after ovulation, rather than before (as would be the case in anthropoid primates) (Rasweiler and Badwaik, this volume). The fertilized egg is held in the oviduct for an unusually long time (until days 12–13 *p.c.* – among the longest tubal journeys of the embryo exhibited by any mammal), and this provides considerable time for repair of the endometrial lining of a postpartum uterus. Finally, early development of the embryo is relatively slow. The most rapidly developing embryos appear to still be at the primitive streak stage and responsible for only slight swelling of the uteri at day 30 *p.c.*, and to have a maximum crown–rump length of about 13.5 mm (with a mass of < 0.35 g) on day 60 *p.c.* By comparison, newborn young have masses of 4.66–6.39 g on the day of birth (Rasweiler and Bonilla, 1992; Badwaik *et al.*, 1997; Rasweiler and Badwaik, 1997, 1999a). Since *C. perspicillata* appears to nurse its young for less than two months (Kleiman and Davis, 1979), the first infant is probably weaned before the second (conceived at the postpartum oestrus) begins to make significant physiological demands upon the mother.

These observations have been presented in some detail because they suggest that the advantage of having a postpartum oestrus (where environmental conditions permit) may have been one of the major forces driving the evolution of female reproductive mechanisms in bats. Unfortunately, at present we have only a very incomplete picture of how the postpartum period is dealt with by other species of bats. It would seem that having bicornuate uteri, functional reproductive asymmetries (e.g. the dominance of one side of the tract or the ability to alternate pregnancies from one side of the uterus to the other), and short tubal journeys of the embryo might create opportunities and/or problems for many other bats that are quite different from those faced by *C. perspicillata*.

ACKNOWLEDGMENTS

Original research reported for the first time in this chapter was supported by the Department of Obstetrics and Gynecology, Cornell University, and by National Institutes of Health Grants AI-10050, HD-17739 and HD-28592. Manuscript preparation was also supported by Cornell and NIH Grant HD-28592.

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7

Sperm Storage and Fertilization

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7.1 INTRODUCTION: THE WHAT, WHEN, WHERE AND WHY OF SPERM STORAGE

Sperm storage involves the retention of viable spermatozoa within the reproductive tract for prolonged periods. Implied in this definition is the caveat that no new sperm are being added to these stores, i.e. that spermatogenesis has ceased. Sperm-storing species are therefore seasonal breeders that have become adapted to retain the male gamete for extended time intervals. Usually, prevailing environmental conditions are not conducive to the establishment of pregnancy at the time sperm are produced. Other reasons for storing sperm may be social conditions that do not allow the sexes to meet in order to copulate at a time most opportune for pregnancy to ensue immediately. Sperm storage typically occurs in females whose gametogenic cycle is not in synchrony with that of the male; however, the term can equally be applied to the male that retains gametes beyond the cessation of spermatogenesis. Furthermore, for sperm storage to be established in the female, it must be demonstrated that conception does not depend upon re-insemination at a time closer to ovulation, a scenario often made possible by concurrent sperm retention in the male of the species.

Reproductive Biology of Bats
0-12-195670-9

Sperm storage is a phenomenon that is usually associated with poikilothermy or heterothermy. It is perhaps best developed among insects, for some of which sperm storage has been reported to extend into many years (Thornhill and Alcock, 1983; Tschinkel, 1987). Among vertebrates, sperm storage is well documented for various species of reptiles (Haines, 1940; Carson, 1945; Hoffman and Wimsatt, 1972; Fox, 1963; Saint-Girons, 1975; Halpert *et al.*, 1982), amphibians (Dent, 1970; Boisseau and Jolly, 1975; Sever and Hamlett, 1998), fish (Jalabert and Billard, 1969) and birds (Van Krey *et al.*, 1967; Friess *et al.*, 1978; Hatch, 1983; Bakst and Bird, 1987; Bakst, 1987; Brillard and Bakst, 1990; Bakst *et al.*, 1994) as well as in some mammals other than bats, e.g. hares (Martinet and Raynaud, 1975) and hybrid mice (Ullmann, 1976).

Sites which are utilized by animals for the purpose of storing spermatozoa vary. Within the male vertebrate, the excurrent ducts of the reproductive tract, or (glandular) specializations of these, are utilized. Typically, it is the cauda epididymidis that is the principal storage organ; this organ has evolved to be such because spermatogenesis, once established, is a continuous process whereas ejaculation is not. Sperm storage has also been reported in the testis of some bat species (Krutzsch, 1975; Krutzsch and Crichton, 1986). Within the female, sperm are stored within the reproductive tract (uterus, vagina or oviduct) or, in invertebrates, in specialized receptacles (spermathecae). Within their storage organs, sperm are generally held in a mass that seemingly has no relationship with the epithelium. However, this does not hold true for some regions of the female tract for some bat species wherein a relationship with the epithelium is suggested by the orientation of the stored sperm (Racey, 1975). This point will be elaborated upon later.

For conventional mammals, it is generally accepted that the life span of spermatozoa in the male is in the order of a few weeks (Thibault, 1973). Storage of sperm for longer periods is unusual. In the female, the ‘normal’ life-span of the spermatozoa of a species approximates the duration of oestrus, i.e. usually only a few days. In many species of bats, oestrus is attenuated (16–200 days: Racey, 1979) and, as a result, fertile sperm are retained within their tracts for periods that greatly exceed the normal for mammals. Some species of bats, primarily those that reside in temperate regions of the world, are reported to retain viable sperm in the female and/or male tracts beyond the cessation of the annual spermatogenic period for upwards of six months (Racey, 1972b, 1973). In these instances, storage largely coincides with winter and hibernation when metabolism is greatly reduced. However, insemination takes place several weeks before the bats enter hibernation when they are still actively foraging and laying down fat stores. Moreover, many species of bats that store sperm arouse periodically during hibernation and feed (e.g. Avery, 1985). Thus, sperm storage, *per se*, does not depend entirely on the maintenance of low body temperatures. Indeed, sperm storage for periods of several weeks is also displayed by some tropical species of bats which are nevertheless regarded as being hetero- rather than homeothermic despite the more constant environmental conditions in which they reside (Medway, 1972; Myers, 1977; Gopalakrishna and Madhavan, 1978).

The retention of sperm in the reproductive tract of bats residing in temperate regions is associated with delayed ovulation and fertilization and is one of several strategies that allow bats to interrupt their reproductive cycle by hibernating (Wimsatt, 1960, 1969). The testicular and ovarian gametogenic cycles are initiated in the early and late summer, respectively and are followed by the growth of the pre-ovulatory follicle(s) in the female and the accessory sex glands in the male, the latter being driven by increasing Leydig cell activity in the early fall. Spermatogenesis is ceasing at this time. An apparent disparity exists between European and American species in the status of Leydig cells during the ensuing winter months. Nevetheless, although they are seemingly involuted in many American species (Gustafson, 1987), their androgenic secretions are apparently sufficient to maintain libido and accessory gland secretory activity during hibernation. Low levels of circulating testosterone also maintain accessory gland status in the European noctule, as demonstrated by castration experiments during hibernation (Racey, 1974). These experiments also demonstrated that epididymal sperm viability could be maintained for at least one month in the absence of testicular androgens (Racey, 1972a). A cauda epididymidal store of sperm throughout winter ensures a supply of gametes for further insemination during winter and/or upon spring arousal from hibernation. Sperm are stored in the female tract until ovulation upon arousal in the spring. Thus, sperm storage in temperate zone Chiroptera represents an asynchrony between the spermatogenic and accessory sex cycles of the male and of the production of gametes by both sexes.

7.2 THE HISTORY OF SPERM STORAGE IN CHIROPTERA

Sperm storage in Chiroptera was first reported last century (Pagenstecher, 1859) in the species *Vesperugo (= Pipistrellus) pipistrellus*. Other nineteenth-century observations of sperm within the uteri of European bats were made by Eimer (1879), Benecke (1879), Fries (1879) and Rollinat and Trouessart (1895a, b, 1896, 1897). The number of sperm-storing species identified by these various workers was about 12. Since this time, the list of sperm-storing species has grown to include over 40. Significant contributions to our understanding of sperm storage in bats were made by Courrier (1921, 1925, 1927) who was the first to show sperm lined up to the epithelial wall in the female. Hartman (1933) reviewed the status of information as it was known at the time but cautioned that ‘proof has not yet been forthcoming finally to establish the view that the sperms ejaculated in the fall are the ones that will fertilize the ovum in the spring’. This concern was addressed by Wimsatt (1942, 1944) and Folk (1940) who performed the first isolation experiments which convincingly demonstrated that stored sperm indeed were capable of fertilization many months later (as opposed to fresh sperm reintroduced into females by subsequent copulations). Furthermore, although true sperm storage has been implicated in females of a large number of bats, isolation experiments that have separated the sexes at the time of initial insemination followed by proof of fertilization at a later time have only been undertaken for a handful of species, a fact that should be kept in mind when attributing sperm storage status categorically to the vast majority of species in which it has been inferred. To the list of eight confirmed sperm-storing species (all vespertilionids) named by Racey (1979), we can add *Mormopterus planiceps* (at least 56 days: Crichton and Krutzsch, 1987) and *Chalinolobus gouldii* for which, using progesterone assays, Hosken *et al.* (1996) concluded that sperm storage persists for at least 33 days in females.

The status of our knowledge of the subject of sperm storage in bats was last summarized by Racey in 1979. The present review focuses on knowledge that has accumulated since then. It introduces all of the species that have been placed in the category of possible sperm storers since 1979 and addresses information that has been generated on the mechanism of the sperm storage phenomenon. It will be seen that while our knowledge of this subject has increased, we are still far from understanding its scope, underlying mechanisms and implications. This is somewhat surprising given the far-reaching applications that such knowledge could have.

7.3 THE OCCURRENCE OF SPERM STORAGE IN CHIROPTERA

Interestingly, the vast majority of species that store sperm belong to either the rhinolophid or vespertilionid families. Most of these reside in temperate parts of the world where a period of hibernation interrupts the reproductive cycle. Since the review of Racey (1979), an ever-increasing number of vespertilionid (and to a lesser extent, rhinolophid) species have been reported to exhibit this pattern of reproduction in both northern and southern hemispheres. These include *Murina leucogaster* (Mori *et al.*, 1989), *Chalinolobus morio* (Kitchener and Coster, 1981), *C. gouldii* (Kitchener, 1975; Schlawe, 1983), *Pipistrellus subflavus* (Krutzsch and Crichton, 1986), *P. mimus* (Krishna, 1985), *P. endoi, P. coreensis, P. savii* (Uchida and Mori, 1987), *P. rusticus* (van der Merwe and Rautenbach, 1990), *Nycticeius schlieffenii* (van der Merwe and Rautenbach, 1987), *Nyctophilus gouldi* (Phillips and Inwards, 1985), *Rhinolophus clivosus* (Bernard, 1983) and possibly *Myotis tricolor* (Bernard, 1982), *M. formosus, M. ikonnikovi, M. leucogaster* (Uchida and Mori, 1987), *Vespadelus* (= *Eptesicus*) *regulus* (Kitchener and Halse, 1978), and *V.* (= *Eptesicus*) *vulturnus* and *V.* (= *Eptesicus*) *darlingtoni* (Tidemann, 1993). Interesting variations upon the theme have been reported in the genus *Miniopterus*. In Japan, *M. schreibersii fuliginosus* appears to store sperm in the uterotubal junction for a short time prior to ovulation (Mori and Uchida, 1980). In temperate Australia *Miniopterus australis* and *M. schreibersii* store epididymal spermatozoa for a month or so prior to ovulation and during the ensuing several months of hibernation, even though conception has already occurred (Richardson, 1977; Krutzsch and Crichton, 1990). In view of the reduced secretory status of the Leydig cells and the atrophied, non-secretory state of the sex accessory glands, such sperm stores are presumably not available for further inseminations even if needed. No doubt their persistence is related to the influence of hibernation on the processes that would normally eliminate residual sperm. Another Australian bat, *Rhinolophus megaphyllus* (Rhinolophidae) likewise retains an epididymal store of sperm over winter (at 27°–32°S, Krutzsch *et al.*, 1992) as also does *R. capensis* in South Africa (33°S, Bernard, 1985, 1986, 1988) and *R. clivosus* at 25°S in South Africa (Wessels and van der Merwe, 1997), preparatory for ovulation in late winter or early spring. Sperm storage does not occur in the females of these species at the latitudes at which they were studied although uterine sperm storage has been recorded in some northern hemisphere rhinolophids (Mori *et al.*, 1982; Matthews, 1937; Gaisler, 1965, 1966).

Not all bats that store spermatozoa reside in temperate environments and not all are members of either the family Rhinolophidae or Vespertilionidae. Sperm have been seen in the reproductive tracts of females of several tropical dwelling species prior to ovulation. Sperm storage *per se* has not been conclusively established for any (by isolation experiments) – if indeed it occurs, its duration is shorter than for temperate species. *Tylonycteris pachypus* and *T. robustula* (Medway, 1972; Racey *et al.*, 1975), *Scotophilus heathi* (Gopalakrishna and Madhavan, 1978; Krishna and Dominic, 1978), *Pipistrellus ceylonicus chrysothrix* (Gopalakrishna and Madhavan, 1971), *P. mimus* (Krishna, 1985), *P. nanus* (Bernard *et al.*, 1997), *Lasiurus ega, Eptesicus furinalis,* and *Myotis albescens* (Myers, 1977) are all such vespertilionid species. Males of tropical species in which sperm retention has been documented include *Lasiurus ega* (Myers, 1977), *Rhinopoma hardwickei* (Karim and Banerjee, 1985), *Scotophilus heathi* (Singh and Krishna, 1995) and *Hipposideros speoris* (Gopalakrishna and Bhatia, 1980).

Families other than the Vespertilionidae and Rhinolophidae in which storage of sperm has been described have been expanded to include the Molossidae (genus *Mormopterus*: both sexes) and possibly the Pteropodidae (genus *Macroglossus:* females), Emballonuridae (genus *Taphozous*: both sexes) and Rhinopomatidae (genus *Rhinopoma:* both sexes) and, in males only, the Phyllostomidae (genus *Hipposideros*). *Mormopterus planiceps* is the only molossid in which sperm storage has been described to date; in South Australia, storage of sperm occurs for a period of at least two (females) to six months (males) over winter when the animals are torpid (Crichton and Krutzsch, 1987; Krutzsch and Crichton, 1987). In another molossid, *Molossus ater*, Rasweiler (1987) has described persistence of sperm in the female reproductive tract for some time after conception. However, it seems more likely that this is related to prolonged oestrus and continued insemination, rather than a case of sperm storage. Hood and Smith (1989) noted sperm in the uterus of *Macroglossus minimus* (Pteropodidae) in Papua New Guinea at times that seemed unrelated to imminent ovulation, but more definitive information relative to the reproductive cycles of both sexes is required before this can be regarded as a conclusive record of sperm storage in this family. A similar situation applies to *Rhinopoma hardwickei* (Rhinopomatidae) which may store sperm for a couple of weeks prior to ovulation (Karim and Banerjee, 1989). Gopalakrishna and Bhatia (1980, 1983) and Gopalakrishna *et al.* (1992) have noted prolonged storage in the epididymides of *Hipposideros speoris* at 19°57′ N in eastern India. Epididymal stores of sperm throughout the year have also been described in *Rhinopoma microphyllum* (= *kinneari*) (Anand Kumar, 1965) and in the emballonurid *Taphozous georgianus* in tropical Australia (with fertilization probably being effected by sperm stored for at least three months: Jolly and Blackshaw, 1987). Male *T. hilli* appear to be fertile (?storing sperm) throughout the year (Kitchener, 1983) while female *T. nudiventris* in southern Iraq store sperm (Al-Robaae, 1968).

7.4 THE HOW OF SPERM STORAGE

7.4.1 Anatomy

In a few species that store sperm, storage is accompanied by a vaginal plug (e.g. *Mormopterus planiceps*: Crichton and Krutzsch, 1987; *Nyctophilus gouldi*: Phillips and Inwards, 1985; *Rhinolophus ferrumequinum*: Oh *et al.*, 1983). A vaginal plug has also been described in *Miniopterus schreibersii fuliginosus* (Mori and Uchida, 1981), a species that stores sperm for a limited time prior to ovulation and fertilization and in *Molossus ater*, a species in which sperm are noted in female tracts long after conception (Rasweiler, 1987). The vaginal plug of bats appears to be of either female (vaginal cornification as, for example, in pipistrelles) or male (coagulated urethral, e.g. in some rhinolophids; or seminal vesicle, e.g. *Pteropus poliocephalus*: Martin *et al.*, 1995) gland origin and may contain decapitate sperm and leucocytes. The plug seals the vaginal orifice and presumably prevents sperm loss. Several researchers have argued that the plug also plays a critical role in sperm competition by preventing the introduction of sperm from competing males. Interestingly, female greater horseshoe bats 'cheat' by voiding the plug (Racey, 1975). Sperm-storing bats provide an interesting model for research into sperm competition (e.g. Fenton, 1984). Hosken (1997) has found that testis mass is increased in bat species that store sperm versus those that do not; he suggests that sperm competition is greater in sperm-storing species and that an increase in investment in spermatogenesis has resulted.

Of considerable interest have been many observations at the light microscope level of a perpendicular orientation of stored spermatozoa with their heads towards the reproductive tract epithelium (Racey, 1975; Uchida and Mori, 1987). This does not appear to be restricted to the Chiroptera, having been described also in other vertebrates that retain sperm (e.g. garter snake: Hoffman and Wimsatt, 1972; guppy: Jalabert and Billard, 1969; birds: Van Krey *et al.*, 1967; dogs: Doak *et al.*, 1967; shrew: Pearson, 1944). In bats, orientation of sperm towards the epithelium is apparently established very soon after copulation (Racey *et al.*, 1987). It is particularly evident for the relatively small populations of sperm that are housed in the caudal oviduct (utero-tubal junction) but pertains, to a lesser extent, to peripheral sperm of the populations that are seen stored in the uterus and vas deferens of some species (e.g. Gopalakrishna and Madhavan, 1971; Racey *et al.*, 1975; Racey, 1979; Krutzsch *et al.*, 1982; Mori *et al.*, 1982; van der Merwe and Rautenbach, 1987). Close contact with the plasma membrane of adjacent epithelial (particularly non-ciliated) oviductal and uterine cells and associated microvilli has been recorded (Racey and Potts, 1970; Uchida and Mori, 1987; van der Merwe and Rautenbach, 1990) with some spermatozoa actually indenting (see Figure 7.1) these cells to the point that their heads are enveloped in epithelial cell cytoplasm (e.g. *Pipistrellus kuhli*: Andreuccetti *et al.*, 1984; *Myotis lucifugus* and *M. velifer*: Krutzsch *et al.*, 1982; Racey *et al.*, 1975). Such a relationship was shown, in *Pipistrellus kuhli*, to resist trypsin digestion but succumb to treatment with EDTA and hyaluronidase, suggesting that attachment is mediated by proteoglycans or Ca++ rather than a protein (Andreuccetti *et al.*, 1984). Granular exocytosis of substances into the intercellular spaces between epithelial cells and closely adjacent sperm gives rise to speculation that transfer of material occurs that might be of nutrient value to the sperm. Studies that have sought a trophic role for the oviductal epithelium of sperm-storing bats include those of Racey (1975) and Racey and Potts (1970). However, the significance, if any, of the close relationship between sperm and the epithelium in certain sites, is unknown. Counter-productively, it would seem, spermiophagy by oviductal epithelial cells and connective tissue cells of the underlying lamina propria has also been observed (e.g. *Myotis lucifugus* and *M. velifer*: Krutzsch *et al.*, 1982; Racey *et al.*, 1987; *Pipistrellus abramus*: Mori and Uchida, 1974; *Rhinolophus ferrumequinum nippon*: Mori *et al.*, 1982; Uchida *et al.*, 1984; Uchida and Mori, 1987). Racey *et al.* (1987) have suggested that this may be a mechanism for removing defective or dead sperm thus permitting optimal use of limited storage sites by good sperm, though no definitive proof of this has been forthcoming.

The role of the oviduct, particularly the isthmic portion, and of the uterotubal junction as a specialized reservoir that houses the select population of sperm destined to proceed to the site of fertilization, has been documented for other mammalian species; it is speculated that the life-span of sperm residing here is prolonged by an interaction with oviduct-specific apical membrane fragments (Smith, 1998). Research has strongly supported a role for this epithelium in the elaboration of proteins (King *et al.*, 1994) and lipids (Henault and Killian, 1993) that may be correlated with capacitation, a biochemical process that leads to the acrosome reaction which in turn permits sperm to gain entry to the egg in the process of fertilization. In the bat system, a delay in capacitation is obviously of paramount importance and a necessary correlate to the sperm storage process.

Leucocytes are responsible for the removal of dead spermatozoa prior to and following ovulation. As reported for other mammalian species (Flechon and Hunter, 1981; Hunter *et al.*, 1987) the incidence of polymorphonuclear leucocytes is low in the lumen of the bat oviduct when sperm are present (Krutzsch *et al.*, 1982; Mori *et al.*, 1982, Racey *et al.*, 1987) but high in the uterus (Uchida *et al.*, 1984); it may increase in some species as hibernation progresses (Oh *et al.*, 1983). This observation lead Uchida and Mori (1987) to suggest an obvious difference between the uterus and the oviduct in their capacity to store sperm. The uterotubal junction may serve to separate sperm and leucocytes, thus aiding sperm survival (Uchida and Mori, 1974). Interestingly, Mori and Uchida (1982) reported leucocytic phagocytosis of sperm in the right (non-ovulatory side) oviduct but not on the left (ovulatory) side of *Miniopterus schreibersii fuliginosus*, a species wherein the early cleaving zygote migrates from the left oviduct to the right uterine horn where implantation occurs and in which limited sperm storage occurs in the female prior to fall copulation and conception.

Little attention has been directed towards the epididymal epithelium and its possible role in sperm storage. There is no evidence of a trophic relationship between this epithelium and stored sperm (e.g. Bernard, 1988) though orientation of stored sperm towards the epithelium of the vas deferens and the epididymis has been reported (Racey, 1975). Transmission electron microscope views of epididymal tissue display nothing extraordinary that might relate to its specialized ability, in bats, to support extended sperm longevity. These cells appear to contain the usual complement of smooth and rough endoplasmic reticulum, free

Figure 7.1 Electron micrograph of utero-tubal epithelium in *Myotis lucifugus* showing sperm head (SP) indenting cytoplasm of cell. Note abundant glycogen granules (arrowed) and presence of lipid (LD). CL=cilia. (× 18240). Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., from Krutzsch *et al.* (1982).

ribosomes, Golgi apparatus and vacuoles of various sizes, consistent with the role of this epithelium in synthetic, secretory and absorptive activities. Electron-dense tight junctions are evident at the apical borders. In contrast to oviductal epithelial cells that lie adjacent to stored sperm, glycogen is not present. Leucocytic invasion of the lumen is rare. Freeze fracture studies, which split cells at the level of the phospholipid bilayer and expose the structural details of tight junctions (zonulae occludens), revealed the presence, in the cauda epididymidis of *Antrozous pallidus* (Crichton *et al.*, 1993b), of a complex morphology such as that previously only seen in mammals between Sertoli cells (blood-testis barrier), where such junctions serve to provide the utmost in protection to the developing spermatozoa,

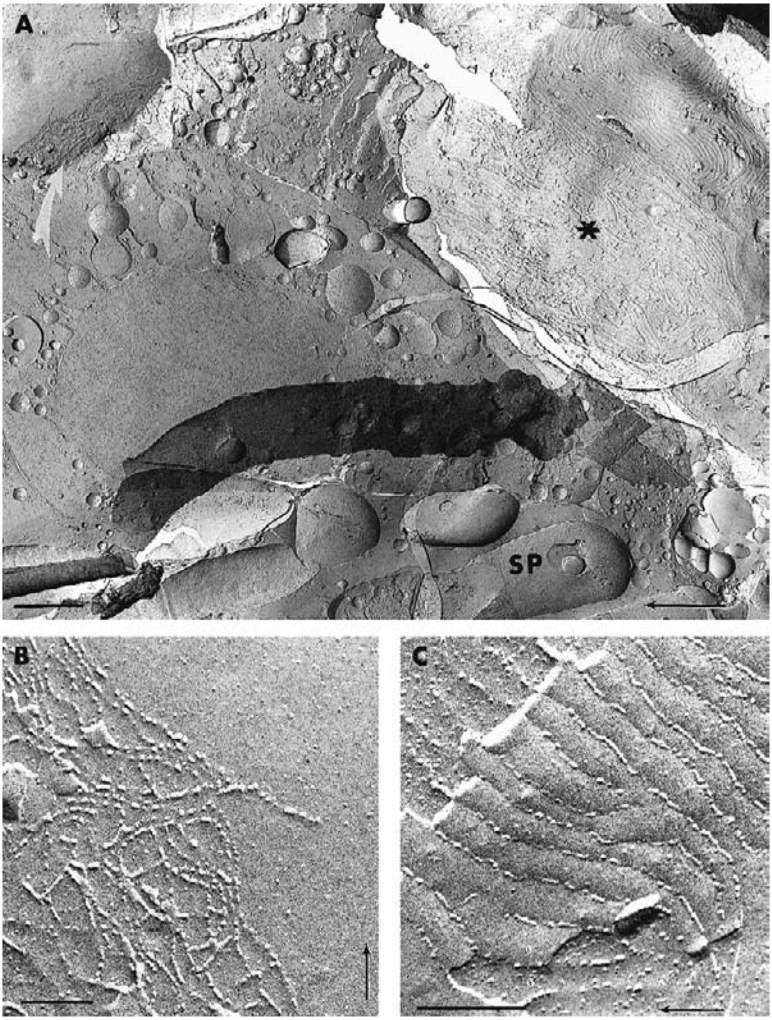

Figure 7.2 Freeze-fracture micrographs of cauda epididymidal epithelium of *Antrozous pallidus* showing ordinary (arrowed) and specialized Sertoli cell-like (*) tight junctions between adjacent cells at low (A: bar = 2 μm) and high (B: ordinary tight junctions, bar = 200 nm; C: specialized tight junctions, bar = 500 nm) magnification. Note spermatozoa (SP) in lumen. Arrows at lower right show direction of shadowing. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc., from Crichton *et al.* (1993b).

shielding them from any adverse influences that might reach them from plasma. This blood-epididymal barrier, like its homologue the blood-testis barrier, would allow for the maintenance of a micro-environment that is highly specialized and vital for the survival of its contained gametes. Structurally, this barrier took the form of multiple (often more than 70) long parallel rows of strands that contained many particles (proteins) on their external (E) faces (Figure 7.2). Such a complex morphology contrasted with that normally found between epididymal cells and which takes the form of lesser (about 12) numbers of anastomosing strands. Furthermore, observations suggested that this specialized epithelium is established seasonally in that its appearance coincided with the sperm storage season; outside the storage season, tight junction morphology reverted to fewer rows of anastomosing strands that are more typical of mammalian epididymal tight junction morphology (Friend and Gilula, 1972; Suzuki and Nagano, 1978a, b). This morphology is unique to bats as far as knowledge of mammalian epididymal morphology has accumulated to date. Its significance is apparent in relation to the luminal chemistry (see section 7.4.2). However, a similar morphology could not be demonstrated by the same procedures in another sperm-storing species, *Myotis lucifugus*. Further research along these lines would be of great value to the elucidation of the sperm storage mechanism.

Several papers have addressed the morphology of sperm that are stored and some have compared these sperm to those of non sperm-storing bat and other mammalian species (e.g. Fawcett and Ito, 1965). The large numbers and differential staining reactions of the mid-piece mitochondria in *M. lucifugus* (Wimsatt *et al.*, 1966) are of interest. Although their significance remains unknown, it may be related to increased amounts of phospholipids available for metabolism. Except for the absence of a cytoplasmic droplet and a reduction in the size of the well-developed membranous scroll, no differences have been noted at the transmission or scanning electron microscope level between sperm that are housed in the female and those residing in the epididymis. Nor do stored sperm show evidence of any structural modification that would seem to underlie their longevity. No evidence of the acrosome reaction has been seen in stored uterine or oviductal sperm (Krutzsch *et al.*, 1982).

Using freeze fracture techniques, Hoffman *et al.* (1987) described novel aggregates of particles at the acrosomal-postacrosomal junction of *Myotis lucifugus* and just anterior to the posterior ring. Though the presence of these particles is unknown for other species of mammals, their significance remains elusive. Also of interest is the observation of a difference between uterine and epididymal sperm in the presence, in the former, of a linear particle aggregation in the membrane of the principle piece (Hoffman *et al.*, 1987).

7.4.2 Physiology

Although the number of papers that address this subject are surprisingly few, and the level of scrutiny not detailed, the phenomenon of sperm storage in Chiroptera has invited some research into the underlying physiology – a fascinating question, the answer to which, in all probability, holds the key to this process.

Although sperm storage is associated with winter temperatures and hibernation it is becoming apparent, that, contrary to the suggestions of Wimsatt (1960), the relationship may not be obligatory. Sperm storage does not completely coincide with this reduced metabolic state. Furthermore, not all species of sperm-storing bats are completely torpid throughout storage nor all sperm-storing bats hibernators (e.g. Medway, 1972). Indeed, in many temperate-dwelling species, sperm reach the epididymides several weeks before entry into hibernation, and in tropical species there may be no periods of torpor. Thus, the relationship between the duration of sperm storage and body temperature is in question although heterothermy *per se* is an element of the sperm storage process. Research by Lambert (1981) suggested a relationship between temperature and the process of capacitation in *Myotis lucifugus*. However, in the present author's experience, the process of inducing the acrosome reaction in bat sperm is difficult, requiring more than just temperature to set the chain of events in motion (Krutzsch and Crichton, 1991).

Racey (1975) provided a comprehensive light microscopical report on the histochemical analysis of the presence of lipids (especially phospholipids), proteins and carbohydrates in sperm-storing tissues in male and female *Pipistrellus pipistrellus*. Subsequent studies at the electron microscope level have confirmed the presence of glycogen in adjacent oviductal and uterine epithelial cells (Krutzsch *et al.*, 1982; Andreuccetti *et al.*, 1984) giving rise to speculation that this may be an energy source for stored sperm, albeit it would only be available as a break-down product. However, it should be noted that glycogen-rich oviductal cells are not restricted to sperm-storing species among bats (e.g. Rasweiler, 1977, 1978). Racey (1975) considered the role of glucose and provided some evidence of the possible transfer of this metabolite from uterine cells to stored spermatozoa. Crichton *et al.* (1981) considered another sugar, fructose (the principal reducing sugar of semen) in *Myotis lucifugus* and *M. velifer*; they demonstrated seasonal variation in its production in the accessory male sex organs in a cycle that correlated with maximum gland activity, sperm storage and hibernation. However, lesser quantities (probably largely of seminal origin) were found in the uteri and there was none in the epididymides. Fructose is a substrate that can contribute acetyl moieties to carnitine which then becomes acetylated yielding acetyl groups that can be oxidized and available to spermatozoa for their metabolism. A survey of levels of carnitine in *Myotis lucifugus* and *M. velifer* yielded encouraging results for a possible role for this substance in the epididymis, but a role in the female, if any, was not convincingly indicated by the data (Krutzsch *et al.*, 1984). Suppressors of sperm motility and metabolism have also been studied in the bat reproductive system. The metallic ion zinc is one such substance to inhibit the activity of sperm and also of polymorphonuclear leucocytes which could phagocytose stored sperm. Its presence in high quantities in the storage organs and also the male accessory organs in *Myotis lucifugus* and *M. velifer* is highly suggestive of a role, though no experimental manipulations were performed to test the response of sperm to the presence and absence of zinc (Crichton *et al.*, 1982). In the same vein, Hunter *et al.* (1971a, b) observed a protein in seminal plasma of *M. lucifugus* that inhibited uterine motility and the phagocytic system, and was thus conducive to sperm retention. No further light has been shed on this observation. Racey (personal communication) was unable to confirm further claims by these authors that this protein was toxic when injected into rabbits and mice.

Given that sperm that have undergone the acrosome reaction are doomed to destruction, Krutzsch *et al.* (1982) commented that prolonged sperm storage in bats must involve a delay in capacitation; thus the events that delay capacitation in bats likely serve as the ultimate clue to the longevity of bat sperm. This is an exciting area of research, given the possible implications for the storage of mammalian sperm for IVF and artificial insemination procedures. Research by Uchida and Mori (1987) and Uchida *et al.* (1988) suggested that sperm require at least 50 days of residence in the female tract of *Pipistrellus abramus* before capacitation can take place; however, in the hands of Racey (1973), conceptions occurred immediately following the introduction of sperm into female *Nyctalus noctula*. These differing observations may be due, in part, to the timing of the experiment with respect to ovulation. Nevertheless, bat sperm seem to be remarkably tolerant cells, resisting all attempts *in vitro* to induce them to undergo the acrosome reaction using methods successful in other species (*Myotis* and *Antrozous*: Krutzsch and Crichton, 1991). Studies initiated to compare the resilience of the bat sperm plasma membrane compared with other mammalian species tentatively concluded that a unique composition is not a component of the inherent longevity of these cells since they displayed no greater tolerance to various perturbing agents than did the sperm of other species (Crichton *et al.*, 1993a). However, this is obviously an area for more research.

An insight into a possible mechanism underlying the longevity of bat sperm was obtained by Crichton *et al.* (1994) who looked to the composition of the luminal microenvironment of the cauda epididymidis for clues. Epididymal fluids collected from *Myotis lucifugus, M. septentrionalis* and *Eptesicus fuscus* by micropuncture and electroejaculation were analyzed in a nanoliter osmometer. Freezing-point depression revealed extraordinarily high osmolalities, in the region of 1.5 to 4.5 above blood plasma values and considerably higher than ‘normal’ for mammals wherein the cauda epididymidal environment is usually only slightly hyperosmolar to plasma (~300 mOsm/kg water: Cooper, 1986). Furthermore, a correlation was evident between osmolality and time in the sperm storage period, suggesting that this specialized hyperosmolar microenvironment is established then gradually diluted as the storage season progresses. Figure 7.3 depicts some of the events that occur in the mammalian epididymis and presents a possible scenario by which hyperosmolality might be generated in the bat epididymis by movement of large amounts of solutes from plasma into the caudal lumen where they might be converted into a (?larger) form in which they are impermeable to the epithelium. In this way, quantities sufficient to excessively dehydrate the contained spermatozoa might remain entrapped. Specialized tight junctions may form between epithelial cells to withhold these excessively high concentrations (Figure 7.3e). Towards the end of the sperm storage season, these solutes may be converted back into a form in which their elimination from the lumen might be possible leading to a reduction in osmolality and activation of sperm.

The probability that sperm are held in a state of suspended animation by dehydration was confirmed by *in vitro* experiments wherein epididymal sperm were expressed into fluids of high osmolality and then diluted (Crichton *et al.*, 1994). Sperm that were initially immotile and shrunken (monitored by resistance pulse spectroscopy) were rendered motile and swollen under these conditions. Furthermore, experiments that measured the respiration (by conversion of [^{14}C] glucose to lactate) of sperm held in fluids of high (500 and 1000 mOsm/kg water) versus normal (300 mOsm/kg water) osmolality demonstrated that metabolism is reduced by hyperosmolality. Extrapolating from these data it would seem likely

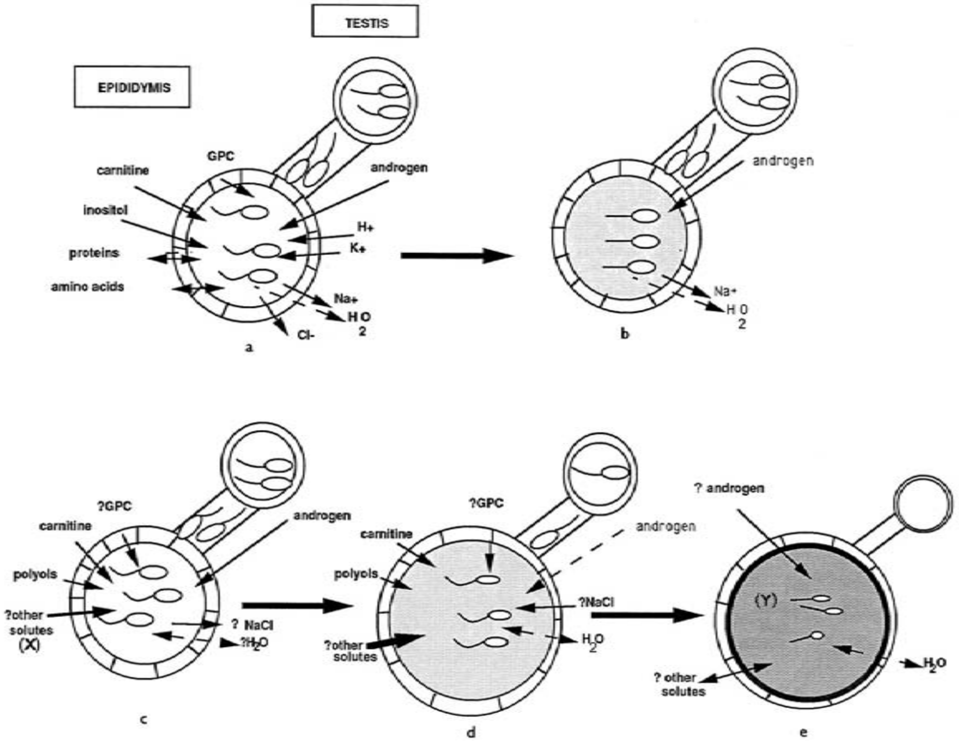

Figure 7.3 Diagram of events that may occur in mammalian (a, b) and bat (c, d, e) epididymides in preparation for the receipt and maintenance of sperm. (GPC: glyceryl phosphorylcholine). In the bat it is hypothesized that solutes enter the cauda epididymidal lumen across the epithelium and become converted to another form (X to Y). Their egress back into blood plasma is prevented by the formation of tight junctions and their voidance to the exterior is presumably hindered by the cessation of muscular contractions. Hyperosmolality (indicated by stippling) rises and is tolerated by specialized tight junctions. Sperm are shrunken and rendered immotile by dessication. Adapted, with permission of the American Physiological Society, from Crichton *et al.* (1994).

that sperm longevity in male bats is controlled by the establishment and ultimate removal of conditions that dessicate and thus preserve these cells. Hyperosmotic conditions apparently preserve viability and prevent the initiation of forward motility in bat sperm as well as induce their quiescence by reducing respiration. Unfortunately, there is no information on the luminal microenvironment of the female so at the present time, one can only speculate as to whether similar conditions might prevail. Indeed, observations by Racey (personal communication) in pipistrelles suggest that stored uterine sperm are highly motile, giving credence to his 1975 statement that it is likely that 'the mechanisms of sperm storage may differ among male and female bats' and throwing doubt on extrapolations from data generated using males.

Temporal dehydration which is reversible when conditions become conducive to the return of biological function is not an unusual phenomenon in biological systems. It has been adopted by many organisms (invertebrates, plants; Leopold, 1986) as a function of severe environmental stress (e.g. salinity or temperature) and a role for hyperosmolality in the imposition of quiescence has also been described for sperm in some fish and amphibian species (Hardy and Dent, 1986; Morisawa *et al.*, 1983). Although raised levels of potassium in the seminal versus the blood plasma have been implicated in freshwater cyprinid fishes (Morisawa *et al.*, 1983), the levels of this ion in epididymal fluids were lower than in the blood plasma in *M. septentrionalis* and *M. velifer* and further preliminary analyses to identify potential osmolytes (sugars, amino acids, ions) in bat epididymal fluids using gas chromatography and mass spectrometry did not yield conclusive data (Crichton, unpublished data). The elucidation of the controlling molecules and mechanisms remains an exciting area for future research.

In all probability, the ultimate control of the mechanism underlying sperm storage may be hormonal. On this note, Racey (1972a) ovariectomized pipistrelles during hibernation with highly detrimental effects on the survival of stored sperm, when females were examined up to two weeks later and compared with controls which unfortunately were not sham operated. There is little information pertaining to the hormonal events surrounding the commencement, duration and completion of storage and this surely is also a potentially fascinating area for future research on the mechanism of sperm storage. Interpretation of the available data is complicated by the dearth of knowledge regarding seasonal fluctuations in the levels of specific steroid binding proteins and of their role in steroid action in bats. In female *Scotophilus heathi*, a sperm-storing species, Gopalakrishna and Madhavan (1978) recorded some intriguing observations that could relate sperm destruction to progesterone, a hormone that is not produced in any quantity until after ovulation. Interestingly, research in recent years in the human (e.g. Blackmore *et al.*, 1990) has forecast a role for progesterone in capacitation. Thus, if it were produced in any quantity prior to conception, progesterone would likely destroy stored sperm. Levels of progesterone have been recorded in some species of sperm-storing bats. Though they were 'elevated' in *Myotis lucifugus* during November and February, plasma levels of this hormone are generally low during hibernation: (4.0 to 7.5 ng/ml, Buchanan and Younglai, 1986) compared with 20–30 ng/ml prior to implantation and 100–200 ng/ml during pregnancy (Buchanan and Younglai, 1986) and 8.4 ng/ml after parturition (Currie *et al.*, 1988). They are also 'low' in *Antrozous pallidus* (about 5 ng/ml during hibernation: Oxberry, 1979), and in *Chalinolobus gouldii* (Hosken *et al.*, 1996). These levels are comparable to those recorded in *Miniopterus schreibersii*, a species in which the female does not store sperm and which is pregnant (though not implanted) during hibernation (3.5 ng/ml during hibernation, Crichton *et al.*, 1989).

Circulating levels of androgens that would logically be required for the maintenance of sperm integrity and viability in the male are known for only a few bat species. With the exception of *P. pipistrellus* and *Nyctalus noctula* in England (Racey, 1974; Racey and Tam, 1974), testosterone levels measured in North American and Australian sperm-storing species are low during sperm storage (<10.0 ng/ml) at least relative to pre-hibernal levels (e.g. in *Myotis lucifugus*: 59.1

ng/ml (Gustafson and Shemesh, 1976); 85.5 ng/ml (Crichton, unpublished observations)) when testicular activity reaches its zenith. These values are consistent with the involuted status of the testicular tubules during hibernation (e.g. *Nyctalus noctula*: Racey, 1974; *Rhinolophus capensis*: Bernard, 1986, 1987; *Myotis lucifugus*: Gustafson and Shemesh, 1976; Gustafson and Damassa, 1984). A similar trend in peripheral androgen levels was demonstrated in *Vespadelus* (= *Eptesicus*) *vulturnus* (Tidemann, 1993). Racey (1974) demonstrated that small testicles (lower levels of circulating testosterone) in noctules were sufficient to maintain accessory gland hypertrophy during hibernation and suggested that stimulation by high prehibernal levels of androgens may be sufficient to maintain accessory gland activity throughout hibernation. It is highly likely, though as yet unproven, that this conjecture applies also to the maintenance of stored sperm. Also unknown for bats are the local levels of androgens within the epididymides (these are usually higher than peripheral plasma: Pujol *et al.*, 1976) or the specific metabolites required for sperm maintenance.

Unpublished data generated by this author suggest that androstenedione (A_4: a precursor hormone of lesser androgenicity than testosterone) may be present in elevated levels in animals storing sperm (see Figure 7.4). The results from a series of radioimmunoassays run in triplicate for each sample indicated that A_4 levels were elevated, averaging around one μg/ml during spermatogenesis (n=3 *M. septentrionalis*, range 450–1796 ng/ml, n=3 *M. lucifugus* (range 415–1577 ng/ml in August; n=8 *M. lucifugus*, range 400–1350 ng/ml in September). During hibernation and sperm storage (November to April), A_4 levels recorded in *M. septentrionalis* averaged between 250 and 450 ng/ml (n=26, range

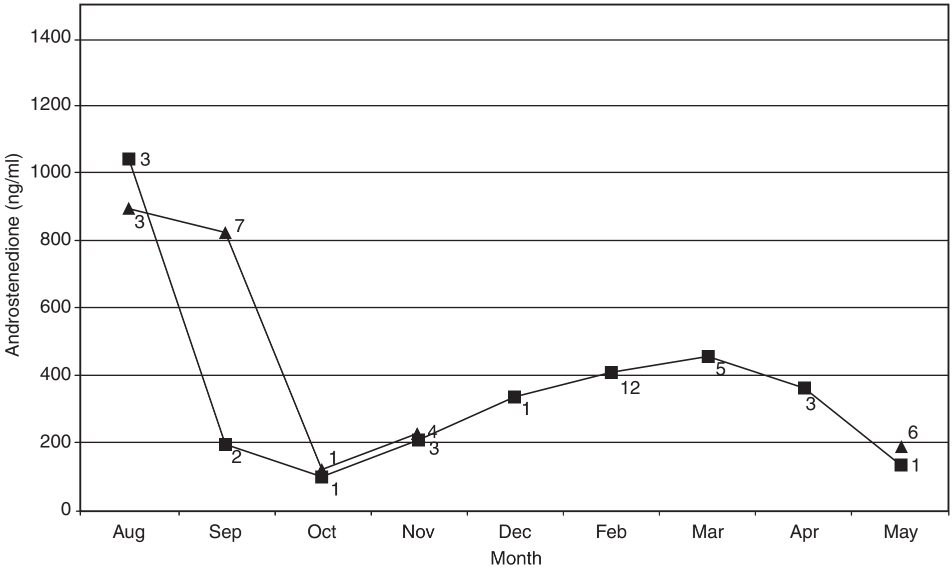

Figure 7.4 Plasma androstenedione (mean ± SEM) in male *Myotis lucifugus* (▲) and *M. septentrionalis* (■). Figures at each point indicate numbers of animals in the sample.

100–896 ng/ml); values recorded in *M. lucifugus* were also reduced (mean=277 ng/ml, range=139–539, n=6) in May at the time of arousal and termination of sperm storage. Of great interest also were the results of assays performed on several females; two *M. lucifugus* sampled just prior to hibernation (September) had extraordinarily high levels of A_4 (24.5 and 38.2 μg/ml) which dropped to around 150 ng/ml in November (n=3 *M. septentrionalis*, n=1 *M. lucifugus*). These assays were performed using an Androstenedione-^{125}I kit (ICN Biomedicals Inc, Costa Mesa, CA) with a highly specific antiserum (stated cross-reactivity with other steroids being <1.79% except with DHEA-SS (4.4%) and DHEA (3.5%)), on plasma separated by centrifugation from blood collected by cardiac puncture from bats anesthetized with chloroform (*Myotis lucifugus* and *M. septentrionalis*) and frozen (–70 °C) prior to analysis. During the hibernation phase of the reproductive cycle, bats were sampled from a population that had been placed in an artificial hibernaculum (7 °C) in October. Preliminary tests to validate the RIA predicted high levels of A_4 by revealing that bat plasma had to be diluted by a factor of 1 : 100 with assay buffer in order to be on the standard curve and for parallelism to be achieved with standards. All subsequent assays were run in the 1 : 100 to 1 : 500 dilution range, depending on the amount of hormone present. No extraction was necessary. Controls for these assays included the running of a pooled bat plasma sample and other replicates in each assay. The assay was validated by spiking pooled bat plasma with a known amount of labelled hormone followed by extraction (ETOH and hexane), and by dextran/charcoal stripping of bat plasma. The interassay and intra-assay coefficients of variation were 18 and 8% respectively. The limit of sensitivity of the RIA was 0.1 ng/ml. Levels of A_4 were undetectable in juvenile males; levels of 200 to 350 ng/ml were recorded in juvenile females collected in October and November.

Androstenedione levels have been recorded only in a few species of male bats; in none are they comparable to those measured in *Myotis*. Krutzsch and Crichton (1987, 1990) reported plasma values of up to 4.0 ng/ml and 0.5 ng/ml during the male reproductive cycles of *Mormopterus planiceps* (Molossidae) and *Miniopterus schreibersii* (Vespertilionidae) respectively. Neither of these sperm-storing species resides in climates as extreme as that experienced by *Myotis* in the eastern United States. Jolly and Blackshaw (1989) recorded a mean plasma A_4 concentration of 1.30 ng/ml with no seasonal variation, in *Taphozous georgianus* in Australia. ‘Unusually high’ concentrations of circulating A_4 were found in female (Abhilasha and Krishna, 1996) and male (Krishna and Singh, 1997) *Scotophilus heathi* during early delayed ovulation (100 to 250 ng/ml) and at the time of peak spermatogenesis (165 ng/ml) which is also the time that fat is deposited in preparation for dormancy, suggesting a possible cause-and-effect relationship between these two processes. The origin of this androgen in bats is unknown – in the female it may be the ovarian thecal cells (Abhilasha and Krishna, 1996), as it is in other mammals, and in the male it may be the adrenal glands (2.220 μg/mg, n=1 male *M. lucifugus*: Crichton, unpublished findings). It is known to accumulate in the interscapular brown fat (9.75 ng/mg, n=1 female *M. lucifugus*; 13.5 ng/mg, n=1 male *M. lucifugus*: E.G. Crichton, unpublished findings). Its role in sperm maintenance is unknown.

Dihydrotestosterone (DHT) has also been assayed by radioimmunoassay (Crichton, unpublished). For these assays, *Myotis lucifugus* and *M. septentrionalis* plasma was extracted with ether-acetone/dry ice and samples were run in triplicate using an ICN kit (Costa Mesa, CA). The antiserum was specific for 5α-DHT; however, it did show cross-reactivity with testosterone (22.7%), androstenedione (2.4%) and 5α-androstane-3,17-dione (17.14%). Preliminary data indicated that values were highest at the time of spermatogenesis (approximately 15–17 ng/ml) but they dropped to around 6–8 ng/ml during sperm storage (October–May; n=4 *M. lucifugus*, n=11 *M. septentrionalis*).

7.5 PREPARATION OF SPERM FOR FERTILIZATION

Information on the preparation of sperm for and the process of fertilization in bats is limited and few data have accumulated since the review of Krutzsch and Crichton (1991) which addressed literature recording fertilization both *in vivo* and *in vitro*. As already mentioned, in the experience of the present author, the spermatozoa of sperm-storing bat species are remarkably resilient cells, resisting attempts to induce the acrosome reaction with agents that work for other mammalian species. The acrosome reaction-inducing agent calcium ionophore has been used with success on the sperm of *Myotis lucifugus* (Krutzsch and Crichton, 1991) and the non-sperm-storing *Pteropus poliocephalus* (Cummins *et al.*, 1986), and the ultrastructure of the process in the latter species has been described by Cummins *et al.* (1988). However, attempts by Krutzsch and Crichton to test the viability and fertilizability of Ca-ionophore-treated *Myotis* sperm in the hamster zona pellucida-free oocyte system failed (when mouse controls succeeded) as did efforts to have these sperm penetrate their homologous oocytes. These results may have been due, in part, to an incompatibility of hamster oocytes with bat sperm and to an inadequate maturation of bat oocytes *in vitro*, respectively. Future research should be directed at developing techniques for the *in vivo* or *in vitro* maturation of bat oocytes and for its verification. Given the largely monoestrous and monotocous nature of the majority of sperm-storing species, this is not an easy area to address. Nevertheless, it is regrettable that there are not more researchers working in this very exciting arena.

7.6 CONCLUSIONS AND FUTURE DIRECTIONS

Considering the fascination provoked by the uniqueness of bat sperm to survive for such extended periods and the enormous potential for the application of the findings underlying the mechanism of this phenomenon, it is indeed surprising that so little research has been directed towards this goal and that so little information is available. Unfortunately, the accumulation of data is as much hampered by the nature of the model as to any other cause. The small size of most bats that store sperm poses many difficulties in the form of obtaining sufficient fluids (blood, uterine and epididymal) to perform analyses; however, this handicap does not apply to all sperm-storing bat species. The European species *Nyctalus* *noctula* and *Pipistrellus pipistrellus*, in which females store large volumes of seminal plasma, offer particularly exciting potential models for future research on the analysis of fluids in which sperm are housed *in vivo*. An additional problem relates to the fact that, in many countries, bats are protected species which imposes barriers on their collection for research purposes.

The data that have accumulated to date suggest that the spermatozoa of sperm-storing bats survive in the epididymis for extended intervals as a function of the provision of specialized (unknown) molecules that raise the osmolality of their surrounding milieu to a level whereby sperm remain viable by desiccation. The provision of such molecules may be regulated by hormonal events; high levels of androstenedione may play a role in this process, either directly (by sustaining stored sperm) or indirectly, by regulating the production of substances vital to sperm survival. At this time, no similar data are available for the female; indeed, given that the female tract, unlike the epididymis, has not evolved as a sperm-storing organ, it may well be that a completely different mechanism supports the longevity of sperm in the uterus and/or oviduct. In the female, hormonal changes accompanying ovulation may release the sperm from inhibition and trigger the events that capacitate and acrosome react them in preparation for fertilization.

An analysis of the microenvironment in the female reproductive tract during sperm storage along with the elucidation of the molecules that raise the osmolality in the epididymis is surely where future research might most profitably be directed. Such knowledge could have far-reaching applications to the short-term preservation of sperm of human and agricultural animal species as an alternative to cryopreservation (which is potentially accompanied by structural damage as a function of ice crystallization) or even to long-term cryobanking of such sperm by the provision, in the freezing medium, of molecules that may be less toxic to sperm than those currently used as cryoprotectants.

ACKNOWLEDGMENTS

Dr. Dan Deaver (Department of Animal Sciences) and Dr. R.H. Hammerstedt (Department of Cell and Molecular Biology), Pennsylvania State University are gratefully acknowledged for their guidance and for the use of their facilities as a Visiting Scientist. Helpful advice for radioimmunoassay work was also provided by Jana Peters (Pennsylvania State University), Dr. Mildred Rodriguez (Pennsylvania State University) and Dr. D. Damassa (Tufts University). Drs Ryuzo Yanagimachi (University of Hawaii) and Philip H. Krutzsch (University of Arizona) provided a constant source of inspiration as well as training and guidance.

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8

Bat Mating Systems

Gary F. McCracken and Gerald S. Wilkinson

8.1 INTRODUCTION

Although bats are the most gregarious of mammals, most research documenting the complexity of their social interactions dates from only the last 30 years. Twenty years have passed since the last comprehensive review of this literature (Bradbury, 1977a). Bradbury documented the rich diversity of bat social systems by (1) describing the structural diversity that exists in the seasonal and long-term associations of bats, and (2) reviewing the information then available on social interactions and communication in several species. Bradbury's review and earlier papers by Dwyer (1970, 1971), refuted the then common presumptions that bats are forced to aggregate because of thermoregulatory demands or limited roost sites, and that these constraints result in interactions among individuals that are largely passive and random.

Bradbury (1977a) categorized 120 bat species (about 12% of the order) by social structure, but mating systems were described for only a fraction of these species.

Reproductive Biology of Bats
0-12-195670-9

Table 8.1 Families of bats, species diversity, and information on bat mating systems

Family	Total species	Information on mating systems	
		# species	% total
Vespertilionidae	330	17	5.2
Pteropodidae	175	16	9.2
Phyllostomidae	147	11	7.5
Emballonuridae	51	7	13.7
Rhinolophidae	129	5	3.9
Molossidae	89	4	4.5
Nycteridae	12	3	25
Megadermatidae	5	2	40
Noctilionidae	2	1	50
All others	23	0	0
Total	963	66	6.9

Species diversity from Findley, 1993

'Year-round harems' were described for six species based either on association patterns among adults, or association patterns coupled with long-term observations of marked individuals. Eleven other species were listed as monogamous, but this categorization was a best guess. Discussing this category, Bradbury (1977a) stated that '. . . the existence of monogamous families in any species of bat remains to be proven by year-round studies'. With some exceptions, little or nothing was known about the mating systems of the species that Bradbury placed in his other social structure categories (e.g. 'sexes separate except for mating', 'sexual segregation at parturition; sexes together at other times', 'year-round multi-male, multi-female groups'). In large part, Bradbury's review was a call to the need for more research.

This chapter shows that the call has been answered by a large number of studies. Here, we focus specifically on the mating systems of bats, emphasizing research from the last 20 years. For completeness, we list all species where mating systems were defined by Bradbury, but we discuss only those where there is new information or where additional interpretations are possible. In total, we include information on the mating systems of 66 species of bats in 10 families. Bats from the largest families (Vespertilionidae, Pteropodidae, Phyllostomidae) are best represented in this literature (Table 8.1). In proportion to their species diversity, bats in the Rhinolophidae and Molossidae have received the least attention (Table 8.1). Bats from the zoogeographic regions with the greatest numbers of species (Ethiopian, Neotropical, Oriental) also have received the most study. In proportion to regional species richness, mating systems have been least studied in bats from the Australian and Oriental regions (Table 8.2).

8.2 MATING SYSTEMS IN BATS AND OTHER MAMMALS

Over 90% of the mammal species that have been studied have some form of polygynous mating system in which one male mates with several females (Kleiman, 1977; Clutton-Brock, 1989). In contrast to birds, where about 90% of all

Table 8.2 Classical zoogeographic regions, species diversity, and information on bat mating systems

Region	Total species	Information on mating systems	
		# species	% total
Neotropical	221	17	7.7
Nearctic	40	4	10.0
Palearctic	85+	6	7.1
Ethiopian	186	21	11.3
Oriental	268	14	5.2
Australian	166	7	4.2

Zoogeographic data from Findley, 1993

species were thought to be monogamous, a view now refuted by numerous molecular studies of bird mating systems (Birkhead and Moller, 1992; Westneat and Webster, 1994), monogamy has been attributed to only about 3% of mammal species (Kleiman, 1977; Whittenberger and Tilson, 1980). Bats conform to the general mammalian pattern. Most bat species are apparently polygynous, while fewer, yet a surprising number, appear to be monogamous.

Polyandrous mating systems have been attributed to very few mammals (e.g. Malcolm and Marten, 1982; Terborgh and Goldizen, 1985). However, there are many mammals where a female mates with several males. Molecular studies have documented numerous examples of multiple paternity in birds (e.g. Gowaty and Karlin, 1984; Burke *et al.*, 1989; Westneat, 1990), but also in some mammals (Birdsall and Nash, 1973; Hanken and Sherman, 1981), including bats (Mayer, 1995; Wilkinson, unpublished data). In some bats, females mate in sequence with several males, and paternity outside of apparent mating groups has been documented in a few species. Available evidence suggests that multiple mating by females is common in bats, and it deserves further study.

Many mammals are promiscuous in that the individuals that mate have no continuing relationship before or after mating (Clutton-Brock, 1989). Many bats are promiscuous, and earlier studies (e.g. Ognev, 1928; Wimsatt, 1945; Pearson *et al.*, 1952) described mating in bats that appeared to be totally anarchic. More recent studies on bats have described mating as both ‘promiscuous’ and ‘random’ (Thomas *et al.*, 1979; Fenton, 1984; Wai-Ping and Fenton, 1988), but these terms must not be confused. Promiscuous mating can be highly structured and nonrandom. For example, lek mating in the Hammer-headed bat *Hypsignathus monstrosus* (Bradbury, 1977b) is promiscuous, but highly nonrandom because only a few males mate. There is no convincing evidence that mating in any bat is random.

8.2.1 Categorizing Mating Systems

There are many ways to categorize animal mating systems (e.g. Bradbury and Vehrencamp, 1976, 1977; Emlen and Oring, 1977; Clutton-Brock, 1989), but any

Table 8.3 Bat mating systems

Family	Species	Region	Diet	Day roost	References
1. Single-male/multi-female groups					
1.1. Year-round harems with stable female composition					
Phyllostomidae					
	Phyllostomus hastatus	Neotropical	Omnivore	Caves	Bradbury, 1977a McCracken and Bradbury, 1977; 1981 McCracken, 1987
Noctilionidae					
	Noctilio leporinus	Neotropical	Insects, fish	Caves, tree hollows	Brooke, 1997
Vespertilionidae					
	Myotis bocagei	Paleotropical (Africa)	Insects	Rolled leaves	Brosset, 1976
Molossidae					
	Tadarida pumila	Paleotropical (Africa)	Insects	Buildings	McWilliam, 1988
Emballonuridae					
	Coleura afra	Paleotropical (Africa)	Insects	Caves	McWilliam, 1987a
1.2. Year-round harems with less stable female composition					
Phyllostomidae					
	Artibeus jamaicensis	Neotropical	Fruit Leaves	Caves, tree hollows, tents	Morrison, 1979 Morrison and Handley, 1991 Kunz *et al.*, 1983 Kunz and McCracken, 1996 J. Ortega, personal communication
	Carollia perspicillata	Neotropical	Fruit	Caves, tree hollows	Williams, 1986 Porter, 1979 Fleming, 1988
	Phyllostomus discolor	Neotropical	Fruit	Tree hollows	Bradbury, 1977a Wilkinson, 1987
Vespertilionidae					
	Tylonycteris pachypus	Paleotropical (Malaysia)	Insects	Bamboo hollows	Medway, 1969 Medway and Marshall, 1972
	Tylonycteris robustula	Paleotropical (Malaysia)	Insects	Bamboo hollows	Medway, 1969 Medway and Marshall, 1972
	Miniopterus australis	Paleotropical (Malaysia)	Insects	Caves	Medway, 1971

Emballonuridae					
	Saccopteryx bilineata	Neotropical	Insects	Tree holes	Bradbury and Emmons, 1974; Tannenbaum, 1975 Bradbury and Vehrencamp, 1976, 1977; McCracken, 1984
Pteropodidae					
	Cynopterus sphinx	Paleotropical (India)	Fruit	Tents	Bhat and Kunz, 1995 Balasingh *et al.*, 1995
	Pteropus tonganus	Paleotropical (Samoa)	Fruit	Trees	G.S. Grant and S.A. Banack, unpublished
	Pteropus mariannus	Paleotropical (Guam, Mariana Islands)	Fruit	Trees	Wiles, 1987
1.3. Seasonal single-male/multi-female groups					
Phyllostomidae					
	Ectophylla alba	Neotropical	Fruit	Tents	Brooke, 1990
Vespertilionidae					
	Myotis adversus	Subtropical (Australia)	Insects	Caves	Dwyer, 1970
	Nycticeius humeralis	Temperate (N. America)	Insects	Buildings	Watkins and Shump, 1981 Bain and Humphrey, 1986
	Nyctalus noctula	Temperate (Europe)	Insects	Tree holes	Sluiter and Van Heerdt, 1966 Mayer, 1995
	Pipistrellus pipistrellus	Temperate (Europe)	Insects	Tree holes, bat boxes	Gerell and Lundberg, 1985 Lundberg and Gerell, 1986 Gerrell-Lundberg and Gerell, 1994
	Pipistrellus nanus	Paleotropical (Africa)	Insects	Rolled leaves, buildings	O'Shea, 1980 LaVal and LaVal, 1977 Happold and Happold, 1996
	Pipistrellus nathusii	Temperate (Europe)	Insects	Tree holes, bat boxes	Heise, 1982 Gerell-Lundberg and Gerell, 1994
1.4. Other single-male/multi-female groups					
Phyllostomidae					
	Vampyressa nymphaea	Neotropical	Fruit	Tents	Brooke, 1987
	Uroderma bilobatum	Neotropical	Fruit	Tents	Timm and Clauson, 1990 Timm and Lewis, 1991 Lewis, 1992b Kunz and McCracken, 1996
	Artibeus cinereus	Neotropical	Fruit	Tents	Kunz and McCracken, in prep.

Table 8.3 (continued)

Family	Species	Region	Diet	Day roost	References
Molossidae					
	Tadarida midas	Paleotropical (Africa)	Insects	Rock crevices	Verschuren, 1957; Bradbury, 1977a
	Otomops martiensseni	Paleotropical, temperate (Africa)	Insects	Buildings	Richardson and Taylor, 1997
Pteropodidae					
	Cynopterus brachyotis	Paleotropical (Malaysia)	Fruit	Tents	Tan *et al.*, 1997
	Cynopterus horsfeldi	Paleotropical (Malaysia)	Fruit	Foliage	Tan *et al.*, 1997
	Pteropus seychellensis	Paleotropical (Comoro Islands)	Fruit	Trees	Cheke and Dahl, 1981
	Pteropus rodricensis	Paleotropical (Captivity)	Fruit	Trees	Carroll and Mace, 1988
	Pteropus hypomelanus	Paleotropical (Captivity)	Fruit	Trees	J. Seyjagat, personal communication
	Pteropus vampyrus	Paleotropical (Captivity)	Fruit	Trees	J. Seyjagat, personal communication
	Pteropus pumilus	Paleotropical (Captivity)	Fruit	Trees	J. Seyjagat, personal communication
2. Multi-male/multi-female groups					
2.1. Year-round multi-male/multi-female groups that mate at the roost					
Phyllostomidae					
	Desmodus rotundus	Neotropical	Blood	Caves, hollow trees	Wilkinson, 1985a, b; 1987
Emballonuridae					
	Rynchonycteris naso	Neotropical	Insects	Tree holes, cliffs	Bradbury and Vehrencamp, 1976; 1977
	Balantiopteryx plicata	Neotropical	Insects	Caves	Bradbury and Vehrencamp, 1976; 1977
	Peropteryx kappleri	Neotropical	Insects	Hollow trees	Giral *et al.*, 1991

2.2 Year-round multi-male/multi-female groups that mate away from the roost					
Pteropodidae					
	Hypsignatus monstrosus	Paleotropical (Africa)	Fruit	Foliage	Bradbury, 1977b
	Epomophorus crypturus	Paleotropical (Africa)	Fruit, flowers	Foliage	G.S. Wilkinson, unpublished observations
	Epomophorus wahlbergi	Paleotropical (Africa)	Fruit, flowers	Foliage	Wickler and Seibt, 1976 Bradbury, 1977a; 1982
	Epomops franqueti	Paleotropical (Africa)	Fruit	Foliage	Bradbury, 1977a; 1982
2.3 Seasonal multi-male/multi-female groups					
Phyllostomidae					
	Macrotus californicus	Temperate, subtropical (N. America)	Insects, fruit	Caves	Berry and Brown, 1995
Vespertilionidae					
	Myotis lucifugus	Temperate (N. America)	Insects	Caves, buildings	Thomas *et al.*, 1979 Wai-Ping and Fenton, 1988 Watt and Fenton, 1995
	Myotis myotis	Temperate (Europe)	Insects	Buildings	Zahn and Dippel, personal communication
	Miniopterus minor	Paleotropical (Africa)	Insects	Caves	McWilliam, 1990
	Plecotus auritus	Temperate (Europe)	Insects	Buildings	Speakman and Racey, 1987 Speakman *et al.*, 1991 Burland, 1998
Molossidae					
	Tadarida brasiliensis	Temperate (N. America) Neotropical (Captivity)	Insects	Caves, buildings	Lollar, 1995 A. Lollar, personal communication B. French, personal communication
Rhinolophidae					
	Rhinolophus ferrumequinum	Temperate	Insects	Caves, mines	G. Jones, personal communication
Pteropodidae					
	Pteropus poliocephalus	Paleotropical, subtropical (Australia)	Fruit	Trees	Nelson, 1965 Martin *et al.*, 1995

Table 8.3 (continued)

Family	Species	Region	Diet	Day roost	References
3. Single-male/single-female monogamous groups					
Phyllostomidae					
	Vampyrum spectrum	Neotropical	Carnivore	Tree hollows	Vehrencamp *et al.*, 1977
Vespertilionidae					
	Kerivoula picta	Paleotropical (S. Asia, India)	Insects	Foliage	Brosset, 1962 Bradbury, 1977a
	Kerivoula harrisoni	Paleotropical (Africa)	Insects	Foliage	Brosset, 1966 Bradbury, 1977a
	Kerivoula papillosa	Paleotropical (Malaysia)	Insects	Bird and wasp nests	Medway, 1969 Bradbury, 1977a
Emballonuridae					
	Saccopteryx leptura	Neotropical	Insects	Tree boles	Bradbury and Vehrencamp, 1976; 1977
	Taphozous peli	Paleotropical (Africa)	Insects	Tree hollows	Kingdon, 1974 Bradbury, 1977a
Megadermatidae					
	Cardioderma cor	Paleotropical (Africa)	Insects	Caves	Vaughan, 1976 McWilliam, 1987b
	Lavia frons	Paleotropical (Africa)	Insects	Foliage	Wickler and Uhrig, 1969 Vaughan and Vaughan, 1986; 1987
Nycteridae					
	Nycteris arge	Paleotropical (Africa)	Insects	Tree hollows buildings	Bradbury, 1977a
	Nycteris nana	Paleotropical (Africa)	Insects	Tree hollows	Bradbury, 1977a
	Nycteris hispida	Paleotropical (Africa)	Insects	Buildings, trees, tree hollows	Bradbury, 1977a
Rhinolophidae					
	Rhinolophus sedulus	Paleotropical (Malaysia)	Insects	Culverts	Heller and Volleth, 1989 Heller *et al.*, 1993
	Rhinolophus luctus	Paleotropical (India)	Insects	Caves, buildings	Bradbury, 1977a
	Hipposideros beatus	Paleotropical (Africa)	Insects	Foliage	Brosset, 1982
	Hipposideros brachyotis	Paleotropical (Sri Lanka)	Insects	Caves	Bradbury, 1977a
Pteropodidae					
	Pteropus samoensis	Paleotropical (Samoa)	Fruit	Trees	Cox, 1983 Pierson and Rainey, 1992

attempt to categorize bat mating systems is a compartmentalization of a continuum. Consequently, all categorizations, simple or complex (e.g. Clutton-Brock, 1989), suffer because many species do not fit neatly into any one category. Having wrestled with diverse categorizations, we opt for simplicity, which we believe enhances, rather than detracts from, the ability to emphasize common themes.

Following Bradbury's approach (1977a), we categorize species according to the structural roosting and mating associations of males and females, and the stability and seasonality of these associations (Table 8.3). Our basic structural association categories are (1) single male/multi-female mating groups, (2) multi-male/multi-female mating groups, and (3) single male/single female mating groups. We subdivide these categories into associations that persist throughout the year versus those that occur only during the mating season, and with regard to the compositional stability of groups (Table 8.3).

Mating groups that consist of a single male and multiple females are typically called harems. This use of the term 'harem' has been criticized for ambiguity and for carrying anthropocentric connotations of male dominance and female subservience (Lewis, 1992a). We intend no such connotations, and find 'harem' useful for ease of discussion in describing the mating systems of many bat species. As we use the term, a harem is a single male/multi-female roosting group of adults in which the resident male has, or appears to have, preferred opportunities for mating and reproducing with those females. In using the term 'harem', we recognize that the preferred reproductive ability of a resident male has been documented for very few species of bats, and that one or both sexes also may mate outside of their harems.

Ambiguity is a separate issue. Mating groups are not called harems if they lack, or appear to lack, the basic criterion of one male reproducing preferentially with a roosting group of multiple females. All single male/multi-female mating groups are not harems. This is illustrated by the African banana leaf-roosting bats *Pipistrellus nanus*, in which single males establish themselves at ephemeral roosting sites where they are visited briefly for mating by roving females. Males are often visited simultaneously by several females, and some males are visited more frequently by more females than others. However, both males and females appear to mate promiscuously (Happold and Happold, 1996).

Other bat species (e.g. *Macrotus californicus*, *Miniopterus minor* and *Tadarida brasiliensis*) also have mating systems in which single males establish themselves at sites where they are visited by roving females. Although it has been suggested that these species may mate in harems or perhaps leks, we categorize these bats as mating in 'multi-male/multi-female polygynous groups' because mating occurs in mixed-sex colonies and it is not clear whether their mating systems satisfy the criteria for harems or leks. The category 'multi-male/multi-female polygynous groups' is an umbrella for a diversity of mating systems, but we clarify the form of each mating system by subdividing this category and by providing the information that is available for each species.

There are other difficulties in classifying bat mating systems. For example, male *Pteropus poliocephalus* defend seasonal territories in mixed-sex colonies where some males are polygynous and other males are monogamous. For convenience, we categorize *P. poliocephalus* as mating in multi-male/multi-female polygynous groups. Also, we refer to species that typically roost and mate in single male/single female groups as 'monogamous', and place *Saccopteryx leptura* in this category. However, there is evidence that some male *S. leptura* are polygynous. The fact that this diversity of mating systems defies categorization may vex an orderly human mind, but it points to the extensive variability of mating behaviors in bats.

8.3 SINGLE MALE/MULTI-FEMALE POLYGYNOUS GROUPS

Depending on taxonomy, 34 or 35 bat species are reported to mate in groups that consist of a single adult male and multiple adult females (Table 8.3). Almost all of these (i.e. 33 or 34 species) have been cited as mating or possibly mating in harems, but, as discussed above, all of these species do not mate in harems. None the less, current literature suggests that harems are common, perhaps the most common mating system in the Order.

8.3.1 Year-round Harems with Stable Female Composition

Highly stable aggregations of females that remain together for several years have been described for five species of tropical bats (Table 8.3). *Phyllostomus hastatus* forms clusters of 7 to 25 females (Figure 8.1) with an annual group compositional stability of 87% (McCracken and Bradbury, 1977, 1981). The same marked females have remained together for over 10 years (G.F. McCracken and G.S. Wilkinson, unpublished observations) and some females appear to have lifetime associations. Stable associations lasting two or more years also have been documented for groups of three to 10 female *Noctilio leporinus* (Brooke, 1997), and two to seven female *Myotis bocagei* (Brosset, 1976). Groups of three to 21 adult female *Tadarida pumila* showed an annual group compositional stability of 70% (McWilliam, 1988), and females in clusters of two to 20 *Coleura afra* have remained together throughout a 14-month study (McWilliam 1987a).

The stable female groups in all of these species each are attended by a single adult male (Figure 8.1) that aggressively repels intrusions by other males. Paternity studies using allozymes showed that resident males sire between 60 to 90% of the pups born to the females in the harems of *P. hastatus* (McCracken and Bradbury, 1977, 1981). Paternity estimates are not available for the other species.

In all of these species harem male replacements occur without disrupting female roosting associations, suggesting that males attach themselves to existing female groups and that grouping is the result of female gregariousness. In *P. hastatus* and *N. leporinus* females evicted from a roost site remain together at new sites. All-male 'bachelor' clusters can occur in the same roosts as harems in *P. hastatus*, *N. leporinus* and *C. afra*, but McWilliam (1987a) reports that bachelor males in *C. afra* are more likely to roost singly. Singly roosting bachelors also have been reported for *M. bocagei*. Single 'satellite' males may attend larger female clusters in *C. afra*.

Relative age indices based on toothwear show that in *P. hastatus* and *N. leporinus* harem males are on average older than bachelor males, but in both species harem males were not larger than bachelors (McCracken and Bradbury, 1981; Brooke, 1997). Harem males in *N. leporinus* had larger testes and larger areas

Figure 8.1 A harem group of *Phyllostomus hastatus*. The harem male is at the periphery of the group of females (photograph by G.F. McCracken).

covered with subaxial secretions that are used in social communication (Brooke, 1997). There was a correlation between male body size and harem size in *T. pumila*, with larger males attending larger harems (McWilliam, 1988).

In *P. hastatus* and *N. leporinus* female groups consist largely of similarly aged bats, suggesting that female groups form as age cohorts of females born in the same year. This has been documented for *P. hastatus* (McCracken and Bradbury, 1981), but young females also are occasionally recruited into natal groups of both species (Brooke, 1997; Wilkinson, personal observation). The recruitment of females into natal groups led McWilliam (1987a, 1988) to suggest that harems in *T. pumila* and *C. afra* may be kin groups. Genetic information documents low average relatedness (r = 0.037) among harem females in *P. hastatus* (McCracken, 1987). This level of relatedness would result if approximately 15% of the females in a *P. hastatus* harem were half-sisters. The age cohort structure of harems of *N. leporinus* also suggests low average relatedness within female groups (Brooke, 1997), but relatedness was not estimated. Brosset (1976) did not observe recruitment of female *M. bocagei* into natal groups, but he did observe one harem male that assumed residence in its natal harem after that male's mother had disappeared from the harem.

8.3.2 Year-round Harems with Less Stable Female Composition

Year-round harems have been reported for several other tropical bats, but female group composition is more fluid with females often moving between groups. *Artibeus jamaicensis* (Morrison, 1979; Morrison and Handley, 1991; Kunz *et al.*, 1983; J. Ortega-R., personal communication) and *Phyllostomus discolor* (Bradbury, 1977a, Wilkinson, 1987) both form groups of one male with two to 15 females in hollow trees or caves. Cave-roosting groups of female *A. jamaicensis* with more than 14 females are sometimes attended by two resident males (J. Ortega-R., personal communication). Smaller groups of two to six *A. jamaicensis* also roost in modified leaves ('tents') in associations that suggest harems (Kunz and McCracken, 1996). *Carollia perspicillata* roosts in groups of one male and two to 17 females (Williams, 1986; Fleming, 1988), and *Tylonycteris pachypus* and *Tylonycteris robustula* both roost in apparently year-round groups that typically consist of one male and up to 12 or seven females, respectively (Medway, 1969; Medway and Marshall, 1972; Bradbury, 1977a). Groups with multiple males and females have been reported for both *T. pachypus* and *T. robustula*, but only during the months after weaning, suggesting that these additional males are juveniles (Medway and Marshall, 1972). *Saccopteryx bilineata* roosts on the sides of large trees and in buttress cavities in colonies of one to a few dozen individuals. Colonies consist of a single group or several adjacent groups, each group with one male and up to eight females (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1976, 1977). Groups of bachelor males or bachelors roosting singly are found at the same roosts as female groups in *A. jamaicensis*, *C. perspicillata* and *S. bilineata*, and at separate roost sites in all of the above six species.

These roosting associations suggest harems, which have been attributed to all of these species. However, the reproductive success of the males attending female groups has not been documented for any of these bats, and the fluidity of female group compositions suggests that females may mate with multiple males. The presumption of harem mating is supported where it is known that, despite their movements, many of the same females are typically found with the same male.

Female groups of *A. jamaicensis*, in caves in Mexico, had a compositional stability of 65% between October to February, but a compositional stability of 95% from March to September, with the latter period including the mating season (Ortega-R., personal communication). In Panama, many of the same females remained together in the same tree hole for over three months (Morrison, 1979), further indicating that female *A. jamaicensis* show affinity for a site or a roosting group (Wilkinson, 1987). In the wild, female *C. perspicillata* moved between an average of 3.8 harems every six months, but individuals showed greater affinity for particular groups and sites, being at their favored site in 62% of all observations and at their two most favored sites in 82% of all observations (Williams, 1986).

Even so, only 3.3% of the same female pairs were together on 50% or more of all observations. In captivity, female *C. perspicillata* showed a similar pattern of frequent movement between groups but preference for roosting at particular sites (Porter, 1979). Harems of *S. bilineata* censused outside of the November/December mating season every 10 days from April to August consisted of one to four resident females (i.e. the same individuals present in the same group on at least 2/3 of all censuses). However, the annual compositional turnover of females in *S. bilineata* colonies was high, ranging from 80% to 100% (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1977). In both *Tylonycteris* species, females changed roost sites frequently and Medway and Marshall (1972) suggested that they may associate randomly. Random associations among females suggest that mating in these *Tylonycteris* species may be promiscuous. Except that compositional turnover among females is high, little has been published about female group stability in *P. discolor* (Bradbury, 1977a). In *S. bilineata* (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1976) and *C. perspicillata* (Porter, 1979; Williams, 1986) the male's fidelity to a group's roost site exceeds that of females, suggesting that site or group associations among females are an indicator of resident males' opportunities for reproduction.

Unlike *P. hastatus* and *N. leporinus*, there is no evidence that any of these species form female groups that are age cohorts. Most young female *S. bilineata* disperse widely and there is no evidence that females join natal groups as adults (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1976; Tannenbaum, 1975). However, Tannenbaum (1975) reported that 76% of young male *S. bilineata* remained in their natal colonies, where they established territories as adults. Only one of 31 young female *A. jamaicensis* was captured as an adult in the hollow tree where she was born (Morrison and Handley, 1991), but Williams (1986) reported that 58% of yearling female *C. perspicillata* returned to their natal caves.

Using the information available on group stability and dispersal patterns, Wilkinson (1987) modeled the expected kin relationships among females in groups of *P. discolor*, *C. perspicillata* and *A. jamaicensis*. Where the best information was available he concluded that average relatedness among females was low for groups of *C. perspicillata* and *A. jamaicensis* ($r = 0.010–0.058$ and $r = 0.000–0.001$, respectively). Wilkinson (1987) thought that relatedness may be higher among groups of *P. discolor* ($r = 0.019–0.083$). Allozyme genetic assays of *S. bilineata* roosting groups showed no evidence for non-random genetic relationships among adult females (McCracken, 1984). However, these assays revealed non-random allelic distributions among males in different colonies, suggesting that males within *S. bilineata* colonies may be related as a consequence of male philopatry to their natal colonies (McCracken, 1984).

At least three species of Old World fruit bats and one other microchiropteran have been reported to live for all or most of the year in harem groups with labile female composition. Tent roosting *Cynopterus sphinx* in India have two annual breeding periods which coincide with biannual periods of tent construction by the resident males (Balasingh *et al.*, 1995). Single adult males expend considerable effort constructing tents, which are then used as day roosts by the male and variable numbers of adult females. Single tents can be used for a year or more, and adult males have been observed defending tents from intrusions by other bats

(Balasingh *et al.*, 1995; Bhat and Kunz, 1995). Female groups of two to 19 adults per tent varied substantially in size and in their fidelity to particular tents, indicating that harem composition in *C. sphinx* is labile (Balasingh *et al.*, 1995).

Pteropus tonganus, in American Samoa, roosts in largely defoliated trees in groups of two to 16 females per male (Grant and Banack, 1995; Figure 8.2). Females typically roost about 25 cm apart in group-specific roosting areas defended by individual males using scent marking and aggressive interactions at boundaries. Male bachelor groups and single males roost at the periphery of these colonies. This social structure was maintained throughout the year, except for a two-month period (November–December) when all bats left the study area. Copulations were frequent and observed throughout the study, and pregnant females and lactating females with young were found at all times. Biweekly surveys showed frequent fluctuation in the size of female groups suggesting that group composition is highly labile (Grant and Banack, 1995). *Pteropus mariannus* in Guam and the Northern Mariana Islands, has a mating system that is similar to that of *P. tonganus*. *Pteropus mariannus* lives year-round in groups of two to 15 females per male, has aseasonal reproduction, and groups fluctuate in size over periods of days (Wiles, 1987).

In Malaysia, *Miniopterus australis* roosts in erosion concavities in caves in highly labile clusters of up to six females with one male (Medway, 1971). These apparent harems exist in October, November, and February to May, spanning the

Figure 8.2 A presumed harem group of *Pteropus tonganus* (photograph by A.P. Brooke).

mating season, as well as much of pregnancy and lactation. Harems also exist outside of the reproductive season in July, suggesting that they are maintained throughout the year (Medway, 1971). Interestingly, in Australia (Dwyer, 1968) and the New Hebrides (Baker and Bird, 1936) both sexes are together during most of the year, but females are segregated from males in maternity colonies during parturition (Bradbury, 1977a). The contrasting reports about *M. australis* may reflect problems with taxonomy, or, alternatively, support the notion that within single species mating systems can adapt to local ecological contexts (Clutton-Brock, 1989).

8.3.3 Seasonal Single Male/Multi-Female Groups

A large number of temperate as well as many tropical bat species show a three-phase annual social structure that Bradbury (1977a) termed the 'temperate cycle'. Two phases of this cycle consist of mixed sex associations in winter, and sexually segregated groups at parturition. Bradbury (1977a) pointed out that the third phase of the cycle, mating, was the least known but was largely assumed to be random and promiscuous. Several temperate cycle species are now known to mate in single male/multi-female associations.

The annual cycles and mating systems of three European species are similar. *Nyctalus noctula* (Sluiter and van Heerdt, 1966), *Pipistrellus nathusii* (Heise, 1982; Gerell-Lundberg and Gerell, 1994), and *Pipistrellus pipistrellus* (Gerell and Lundberg, 1985; Lundberg and Gerell, 1986; Gerell-Lundberg and Gerell, 1994) hibernate during the winter in mixed sex groups. After emergence in the spring they roost briefly in mixed sex associations, but during parturition and nursing females form maternity colonies apart from males. All three species form summer roosts in trees and buildings, and studies of these bats were facilitated by their acceptance of boxes as alternative roosts (Heise, 1982; Gerell and Lundberg, 1985; Lundberg and Gerell, 1986; Gerell-Lundberg and Gerell, 1994).

In all three species, single males establish seasonal territories for mating in roost boxes where they roost and mate with multiple females. However, the timing and duration of territory establishment and mating varies among species. Male *P. pipistrellus* establish territories in June while females are in maternity roosts, and the solitary distribution of males is stable throughout the summer. Mating occurs in August and September with 93% of the male *P. pipistrellus* remaining at the same box roost from June to September (Gerell and Lundberg, 1985; Gerell-Lundberg and Gerell, 1994). In contrast, male *P. nathusi* do not establish territories until July, when females are leaving their maternity roosts, and mating occurs shortly afterward in mid-July and August (Heise, 1982; Gerell-Lundberg and Gerell, 1994). Comparing the mating behaviors of these bats in southern Sweden, Gerell-Lundberg and Gerell (1994) suggest that these differences occur because *P. nathusi* migrates out of the study area in September, and because the locations of maternity roosts and mating sites are stable and predictable from year to year in *P. pipistrellus*, but not so in *P. nathusi*. Male *N. noctula* also do not establish individual territories until later in the summer as the females are leaving their maternity colonies (Sluiter and van Heerdt, 1966; Gerell and Lundberg, 1985). Sluiter and van Heerdt (1966) reported that male *N. noctula* are solitary and dispersed earlier in the summer, whereas Gerell and Lundberg (1985) state that male *N. noctula* establish bachelor groups during the nursing period.

In all three species, multiple females aggregate at some male territories. Female group sizes ranged from one to 10, with an average of 2.9 females/territorial male in *P. pipistrellus*, and from one to nine with an average group size of 3.5 females/territorial male in *P. nathusi* (Gerell and Lundberg, 1985; Gerell-Lundberg and Gerell, 1994). The group composition of females at male roost sites changes throughout the mating season (Sluiter and van Heerdt, 1966; Gerell and Lundberg, 1985; Gerell-Lundberg and Gerell, 1994), suggesting that females also may mate with several males. DNA tests show that the twins produced by *N. noctula* are sometimes sired by different males (Mayer, 1995), and that the sperm of one to five different males is present in the uteri of females (Mayer, personal communication). Mayer's studies using DNA suggest that levels of polygamy in female *N. noctula* may be as great as or exceed those of males.

Even though female composition in roost boxes is labile, both male and female *P. pipistrellus* show some fidelity to the same roost sites. Eleven of 13 marked males that attracted females in two successive years did so at the same box, and 25 of 46 marked females returned during these years to the same site. This resulted in some females roosting with the same male in successive years (Gerell and Lundberg, 1985). Female group size did not correlate with male size or age, but there was a significant correlation over years with the number of females that visited particular sites. Transient adult males that had not established territories were often captured in roost boxes, providing additional evidence that males compete for territorial roost sites, and that the number of available sites limits the population of breeding males. It was suspected that these surplus males attempted to 'steal' matings from territorial males (Lundberg, 1990).

In some of the first studies to reveal structured mating systems in bats, Dwyer (1970, 1971) reported seasonal harems in *Myotis adversus*. Mating occurs twice each year, in the fall and again in the spring. The colony of *M. adversus* in an abandoned railroad tunnel varied greatly in size, with few bats present there in winter (Dwyer, 1971). However, when present, males were usually solitary and largely faithful to particular holes in the wall of the tunnel, while females were typically in unisexual groups, or in groups with other females and pups. During the mating seasons, groups of up to 12 females ($\bar{x} = 7.6$), and females with their pups during the spring mating period (Bradbury, 1977a), roosted with the single males at their apparently territorial roost sites. Monthly censuses showed that these associations of females are labile and apparently short-term, occurring only during the mating season (Bradbury, 1977a).

Available evidence suggests that *Nycticeius humeralis* mate in seasonal single male/multi-female groups. In the northern part of its range in Missouri, *N. humeralis* form female-only maternity colonies during the summer (Watkins and Shump, 1981). In the fall, females and young of both sexes gain fat and migrate to unknown southern locations. In Florida, two males alternately roosted and mated with a group of 30 females between October and February (Bain and Humphrey, 1986). Because individual males were resighted, but did not roost together, it appears that males defend females from other males. Throughout the species' range, female *N. humeralis* show natal philopatry (Watkins and Shump, 1981;

Bain and Humphrey, 1986), but the same females use multiple roost sites which may provide mating opportunities for multiple males (Bain and Humphrey, 1986).

Neotropical *Ectophylla alba* appear to mate in seasonal harems (Brooke, 1990), roosting in tents that are occupied during most of the year by mixed sex groups of one to 17 bats (Figure 8.3). After young are born in April, tent groups segregate into small groups consisting of mothers and pups with a single adult male, or into bachelor groups of males only. This change of roosting group composition at birth led Brooke (1990) to suspect a post-partum oestrus and a harem mating system. The same bats roosted together for up to 45 days, and remained together after moving to different tents, suggesting substantial stability in roosting group composition.

Pipistrellus nanus in Africa roost in rolled-up banana leaves as well as in palms, thatch roofs, crevices and culverts (LaVal and LaVal, 1977; O'Shea, 1980; Happold and Happold, 1996). Throughout Africa *P. nanus* are currently recognized as a single species, but O'Shea (cited in Happold and Happold, 1996) has suggested that the thatch-roosting bats, which have a pair of glands on their tails, and the leaf-roosting forms, which lack these glands, might represent different species. The existence of cryptic species cannot be discounted even for well-known bats, as is

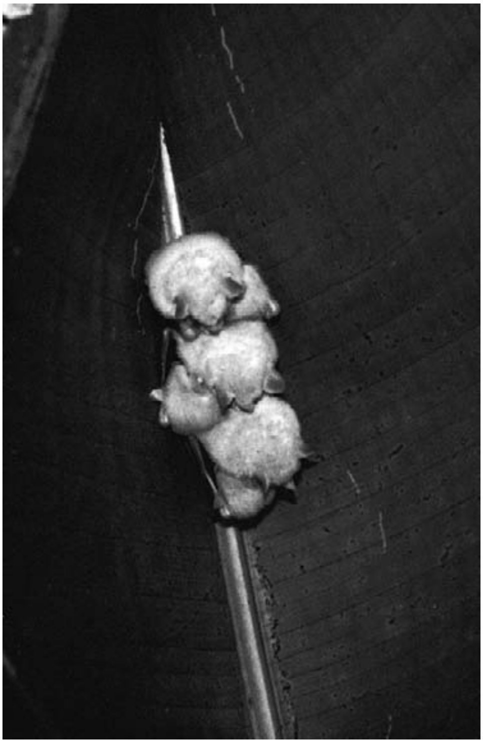

Figure 8.3 *Ectophylla alba* roosting in a *Heliconia* leaf, a bat that apparently mates in seasonal harems (photograph by A.P. Brooke).

illustrated by the recent discovery of two sibling species in what was believed to be *Pipistrellus pipistrellus* (Barlow and Jones, 1997; Barratt *et al.*, 1997).

Two studies have examined mating behaviors in *P. nanus*. One of these studies focused on a thatch-roosting population in Kenya (O'Shea, 1980), the other study on a leaf-roosting population in Malawi (Happold and Happold, 1996). In both studies, the bats roosted alone or in small groups during most or all of the year. Males were almost always solitary or with small groups of females. Females were solitary or in small single- or mixed-sex groups. One difference between the leaf-roosting and thatch-roosting forms is that thatch-roosting *P. nanus* appear to segregate into maternity colonies of 100 or more females and pups between November and January (LaVal and LaVal, 1977; O'Shea, 1980), whereas leaf-roosting forms segregate during the same period into smaller groups consisting of single females roosting alone with their pups or of two to three females with young.

In both studies, mixed-sex clusters were formed after young were weaned, with small groups of females aggregating temporarily around single males at the male roosting sites. Clustering behavior peaked at the beginning of the mating season (May or June to August) and persisted until November. The average size of these mixed sex clusters was small ($\bar{x} = 2.6$ bats (O'Shea, 1980); $\bar{x} = 2.4$ (Happold and Happold, 1996)), but groups of up to 10 or 12 females were occasionally found with single males. Census data from both populations showed that the composition of these female groups was highly labile, with no associations among particular females. In both studies, success in attracting females varied among males.

In the thatch, the average duration of a female's residence with a male was 1.8 days, and females roosted with several different males during the mating season (O'Shea, 1980). However, females showed preferences for particular males, and were found with their favored male 2.6 times more frequently than their next favored male. Of 44 color-banded resident males, the two most successful males accounted for 56% of all visits by females.

In the leaves, 19 males that were monitored repeatedly each roosted with between four to 21 ($\bar{x} = 9.1$) different females (Happold and Happold, 1996). Between May and June, 14 males captured in at least three censuses were found with between one to seven ($\bar{x} = 2.9$) different females, usually with one or two at a time, but sometimes with as many as five. Similarly, seven females that were captured at least three times during this period were found roosting with two to seven ($\bar{x} = 4.3$) different males.

The roost sites used by these two populations differ in important respects, as do the mating behaviors that were observed in the two studies. Significantly, thatch roost sites are stable and persist for years, whereas rolled banana leaf roost sites persist for only one to three days, until the leaf roll opens. In the thatch roosts, males maintained territories throughout the year, and 77% of all marked males were within 1 m of their previous roost site during all censuses. As a banana leaf provides a usable roost for only one to three days, leaf-roosting bats must move frequently to different leaves. However, multiple rolled leaves were always available either on the same banana plant or on nearby clumps of plants, and almost half (44%) of all males were found only in leaves located on the same plant or in leaves on a small number (< 5) of nearby clumps of plants. Such roost site fidelity was not seen in the leaf-roosting females.

Thatch-roosting males also showed obvious territorial behaviors. Calling by males, both in the roost and while flying nearby, was common and most intense during the mating season. Fights among males were common, as were injuries to males that were associated with this fighting (O'Shea, 1980). O'Shea (1980) found a positive correlation between the average number of females with a male and his calling activity.

Evidence for territorial behavior was equivocal in leaf-roosting *P. nanus* (Happold and Happold, 1996). Although males rarely roosted with other males, obvious territorial behaviors, including calling by males and injuries due to fights, were not noted. It appeared that competition among males for leaves or preferred clumps was not necessary because the numbers of rolled leaves and the numbers of clumps of plants with suitable leaf roosts always exceeded the number of males in the population. Roost site fidelity by males increased with an index of roost site 'quality' (assessed as the percentage of surveys in which a clump provided one or more suitable rolled leaves). However, the numbers of females that a male attracted did not increase with this index.

O'Shea (1980) concluded that *P. nanus* has a harem mating system based on territorial defense of roost sites and display behaviors by males. Happold and Happold (1996) argued that unstable groups of roving females that visit multiple males in quick succession are not harems. As leaf roosts provided a resource to the transient groups of females using them, they also argued that this mating system is not a lek. Happold and Happold (1996) suggested that the super-abundance of ephemeral roost sites results in minimal competition among males for resources (i.e. roost sites) needed by females. Thus, possession of a roost is not a reliable indicator of a male's competitive ability or fitness. Happold and Happold (1996) also note that *P. nanus* has a long (two- to three-month) mating season during which females are receptive. They argue that the lack of male–male competition and protracted female receptivity result in a promiscuous mating system, and they propose that levels of polygyny are determined largely by sperm competition.

8.3.4 Other Single-Male/Multi-Female Groups

Harem mating systems have been reported or suspected in at least 13 other tropical bat species (Table 8.3). *Pteropus seychellensis*, from the Comoro Islands, are reported as having harem groups (Cheke and Dahl, 1981), and both in the wild and captivity *Pteropus rodricensis* forms apparent harem groups of one male with up to eight adult females (Carroll and Mace, 1988). Captive groups of several other Old World fruit bats, including *P. hypomelanus*, *P. vampyrus* and *P. pumilus*, appear to mate in small harems in captivity (J. Seyjagat, personal communication). Paternity information obtained using variable microsatellite DNA markers has demonstrated a high level of polygyny in the captive breeding colony of *P. vampyrus* at the Lubee Foundation, Inc. (Comeaux and McCracken, unpublished). Although this colony contained as many as 10 adult males, the DNA studies proved that three of these males sired 25 of the 26 pups that were born in the colony (the three males sired 13, 10 or two pups and the paternity of one pup was not identified). Interestingly, independent assignations of paternity based on observations of the roosting associations of males and females and male dominance were often incorrect, demonstrating that female *P. vampyrus* often mate outside of their apparent harems (L.B. Comeaux and G.F. McCracken, unpublished).

Kunz and McCracken (1996) hypothesize that many of the bats that live in tents (reviewed in Kunz *et al.*, 1994) have harem mating systems, but little is known about group composition, seasonality, or group stability for most of these bats. In Trinidad, Kunz and McCracken (1996) found groups of two to six adult female *Uroderma bilobatum* roosting with single males, some of the females with pups. If these are harems, information from Costa Rica suggests that female group composition is highly labile (Lewis, 1992b). Seasonal changes in population size at study sites (Timm and Lewis, 1991; Lewis, 1992b) also suggest that female group formation in *U. bilibatum* may be seasonal, and that many roosting clusters may be maternity groups rather than harems (Lewis, 1992b). Ten of 11 roosting groups of *Vampyressa nymphaea* captured by Brooke (1987) contained one adult male with enlarged testes and up to three pregnant or lactating females and their young. The same individuals used the same tent for at least eight days and one tent was used for more than nine months, but there is no information on the compositional stability of groups. Similarly, 12 complete tent roosting groups of *Artibeus cinereus* captured by T.H. Kunz and G.F. McCracken (unpublished) on Trinidad consisted of

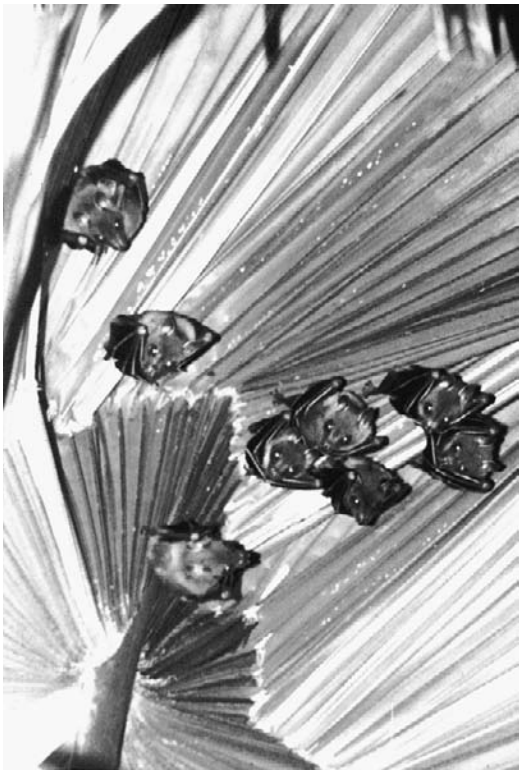

Figure 8.4 Tent-making bats *Cynopterus brachyotis* roosting in a *Livistonia chinensis* palm (photograph by T.H. Kunz).

up to five females (x̄ = 1.6 females/group) with a single adult male. In Pennisular Malaysia, *Cynopterus brachyotis* roost alone or in groups in tents formed when bats modify the leaves of palms, orchids, philodendrons and other plants (Tan *et al.*, 1997; Figure 8.4). Individuals roosting alone were always males, while groups consisted of a single adult male and up to four adult females. Harem composition appears stable as the same bats roosted in the same tent for several months. The capture of three subadult females roosting with a subadult male suggests that harems in *C. brachyotis* may form as age cohorts (Tan *et al.*, 1997). A harem mating system also has been suggested for *C. horsefeldi*. Four groups of *C. horsefeldi* that were captured in naturally occurring concavities of epiphytic ferns consisted of one adult male and two or three adult females, some with young (Tan *et al.*, 1998).

In Durban, South Africa, single adult male *Otomops martiensseni* roost with small groups of adult females and their young in buildings (Richardson and Taylor, 1997). Roosting females are spaced 100 to 300 mm apart with the male about 1 m from the female group. Adult males are about 5% larger than adult females and resident males are more aggressive than females; all suggest that *O. martiensseni* may mate in harems (Richardson and Taylor, 1997). In contrast, populations of the same species in Kenya roost tightly packed in caves, and appear to lack sexual dimorphism (Mutere, 1973).

8.4 MULTI-MALE/MULTI-FEMALE GROUPS

Many bats mate in multi-male/multi-female colonies. In many of these species males establish themselves at preferred roosting sites where males may be dominant or territorial. The males at preferred sites may have greater access to females and greater success at mating than males at other sites. Territorial males often call, have display behaviors, and may mark females, their territories, or themselves with glandular secretions. Several other bat species roost in mixed sex colonies, but males establish display sites or territories for mating away from their regular roost sites.

8.4.1 Seasonal Multi-Male/Multi-Female Polygynous Groups

Tadarida brasiliensis, *Myotis myotis*, *Rhinolophus ferrumequinum*, *Macrotus californicus* and *Miniopterus minor* mate in seasonal male territories within mixed-sex colonies. *Tadarida brasilensis* overwinters in mixed sex colonies that either hibernate (*T. b. cynocephala*, Sherman, 1937; *T. b. mexicana*, Cockrum, 1969) or migrate (*T. b. mexicana*, Villa-R., 1956; Villa-R. and Cockrum, 1962; Cockrum, 1969). In summer, females and pups live in largely sexually segregated maternity colonies that include some of the largest aggregations recorded for any mammal (Davis *et al.*, 1962). Until recently, the mating system of *T. brasilensis* has been an enigma. Sherman (1937) reported that in spring all individuals of a nonmigratory population of *T. b. cynocephala* in Florida left their usual roost for a one-week period during which they mated at an unknown site. Others (Villa-R. and Cockrum, 1962; Davis *et al.*, 1962; Constantine, 1967; McCracken *et al.*, 1994) have suggested that mating in migratory populations occurs in winter roosts shortly before migration, or at sites along their spring migratory routes (Cockrum, 1969; McCracken *et al.*, 1994).

Recent evidence of their mating system comes from observations in captive and wild colonies. Lollar (1995), A. Lollar (personal communication) and B. French (personal communication) report that between November and February males in captive colonies establish small territories in the roost that are visited briefly by multiple females for mating. Male territorial behavior involves aggressive interactions among males, vocalizations and scent marking of territories and females. A. Lollar and B. French both noted differential success among males in attracting females and mating with them.

In the wild, mating was observed during a three-week period between mid-March and early April in caves and under bridges in Central Texas (A. Nicklaus and B. Keeley, personal communication). Nicklaus and Keeley also observed male vocalizations and scent marking, but mating interactions seemed far more chaotic than were observed in captivity. Two distinct copulatory strategies seemed evident: (1) the male aggressively pulled a female from a group, restricted her movements, and mated with her while vocalizing; or (2) the male moved slowly over a female in a group and they quietly mated with no apparent reaction from the female and no male vocalizations. Marked individuals of both sexes mated multiple times, and some of the same individuals engaged in both types of matings. Vaginal plugs that were either ejected or removed from females littered the floors below roosts. Available evidence shows that *T. brasiliensis* is promiscuous, but these observations in captivity and the wild suggest that they may employ as many as three different strategies for mating. This species clearly demands additional research.

Male *Myotis myotis* establish and defend roosting sites of ca. 250 cm^2 in area in attics that may also be occupied by females and juveniles (Zahn and Dipple, personal communication). Males are not faithful to one site, and may roost at different sites on different days. Different males may also use the same sites sequentially. However, the fidelity of males to preferred sites increases from May to August, and peaks during the August–September mating season. Some of the same males used the same sites for up to six years (Zahn and Dipple, personal communication).

Female *M. myotis* visit and mate with males at these sites. Females typically remain with a male for a few days, they visit several different males during the mating season, and may return to visit the same male several times. Males apparently show no obvious behaviors to attract females, but females visit and mate with some males more frequently than with others. During one mating season, five males were each visited by a minimum of four to nine females, and the most successful male accounted for 14 of 31 observed copulations (Zahn and Dipple, personal communication).

While mating in *M. myotis* usually occurs in male roosts with females visiting from different roosts, some males also roost with females at maternity sites. Banding studies and genetic analysis based on a microsatellite locus and mtDNA sequence information indicate that females are philopatric to their natal maternity sites, that males disperse, and that males at maternity sites are not related to the females (Petrie *et al.*, 1997). Microsatellite paternal allelic contributions for 46 mother/pup pairs in a large (ca. 700 adult females) maternity colony in Bavaria showed that no males monopolize paternity. The maximum likelihood of paternity for 19 males that roosted at the maternity site was 0.178 (SD = 0.1), consistent with the conclusion that most females mate with males at other sites (Petrie *et al.*, 1997). Interestingly, the genetic tests suggested that a group of males roosting about 16 km from the maternity site were both more closely related to the females and had higher success at mating with them. The suggestion that female *M. myotis* may selectively mate with related males demands more study (Petrie *et al.*, 1997).

Rhinolophus ferrumequinum appears to have a polygynous mating system that involves male territoriality (G. Jones, personal communication). Beginning in September, male *R. ferrumequinum* in England establish themselves at apparently territorial sites in mines and caves. These sites are marked with brown stains that are presumably from scent marks of males. Multiple females gather at these sites and mating can occur through the hibernation period. Males maintain large testes throughout the winter and secretions from males form a vaginal plug that is shed by females in April, which is presumably the time of fertilization (G. Jones, personal communication).

In September, males in a California population of *Macrotus californicus* establish and defend preferred roosting locations in an abandoned mine that was not their normal roost site (Berry and Brown, 1995). Displays involving wing flapping and vocalizations attract females for copulation and some males were more successful than others in mating. Berry and Brown (1995) also report that in summer small clusters of females and pups are attended by a single displaying male.

In coastal east Africa, McWilliam (1990) reports that male *Miniopterus minor* aggressively compete for access to a dome in the ceiling of a cave every year when their testes are enlarged. Fifteen of 25 males were faithful to the dome from May to July and several returned for three successive years. Males roosting in the dome cover their venters with urine and emit a conspicuous odor during the mating period. At the beginning of the mating period, males occupying the dome were heavier and older than males roosting elsewhere in the cave. Although McWilliam (1990) did not observe copulations, he interprets the competition among males as evidence that mating is concentrated in the dome. During the mating season females move between roosting sites within and between caves and do not roost with males for extended periods. All females sampled were pregnant by the end of July when males abandoned the mating site. Pregnant females leave coastal mating caves and move inland where they give birth in sexually segregated maternity colonies (McWilliam, 1990). Because males appear to aggregate in a traditional location for mating, McWilliam (1990) argues that *M. minor* exhibits a lek mating system. Additional studies including direct observations of copulations or paternity analysis are needed to confirm this conclusion.

During the summer *Plecotus auritus* forms stable mixed-sex colonies of five to 55 ($\bar{x} = 16.8$) individuals in attics (Speakman *et al.*, 1991). Mating has been observed rarely, but is reported to occur in summer colonies and in transient roosts or hibernation sites in fall and winter. Females store sperm and fertilization occurs in early spring (Speakman and Racey, 1987). Recent paternity testing using six microsatellite loci (Burland, 1998) established that a third of assigned paternities involved males from the same summer roost sites as their mates, although males from up to 10 km away sired young. The long mating season, absence of a vaginal plug, and sperm storage appear to provide considerable opportunity for sperm competition. Nevertheless, paternal allelic contributions in 86 offspring indicated no skew in male reproductive success.

While both sexes of *P. auritus* are philopatric, Burland's (1998) genetic data indicate that females are more likely than males to remain in their natal roosts. The average relatedness among females within 23 colonies was 0.009 to 0.042, whereas average relatedness among males was 0.007 to 0.022. Males and females within colonies had average relatedness of 0.002 to 0.039. The presence of related males and females within a colony creates the possibility for inbreeding. Burland (1998) did, in fact, identify four parental pairs with pair-wise estimates of relatedness greater that 0.2; consistent with consanguineous mating. However, all data across all loci showed an inbreeding coefficient of –0.001. Thus, despite natal philopatry by both sexes, inbreeding appears to be avoided in *P. auritus*.

In several species of Australian *Pteropus*, including *P. alecto*, *P. gouldi* (= *alecto*), *P. poliocephalus* and *P. scapulatus* (Nelson, 1965; Martin *et al.*, 1995), both sexes aggregate in spring at traditional camps located in trees (Figure 8.5). Here, females give birth and raise their young, and males set up territories around females. After copulation, both sexes disperse from the traditional mating sites and may live either in sexually segregated winter camps (Nelson, 1965) or as individual nomads (Martin *et al.*, 1995).

There is little information on mating for most of these species but, in the best-known species, individuals appear to be both monogamous and polygynous. In

Figure 8.5 A camp of *Pteropus poliocephalus* in Brisbane, Australia (photograph by G.S. Wilkinson).

Pteropus poliocephalus, a male's mating success appears to be related to the location of his territory within a camp. Nelson (1965) reports that males with territories near the center of the camp are polygynous, while males with territories away from the camp's center are monogamous. Males roosting on the periphery of the camp rarely roost with females. Nelson (1965) also noted higher monogamy among males that set up territories around females that were caring for a pup of the previous year (Nelson, 1965).

The case of Myotis lucifugus

Myotis lucifugus has been 'the bat' for many studies on reproduction (Fenton, 1984), and it is the best known temperate cycle species in which mating has been cited as random and promiscuous (Thomas *et al.*, 1979; Wai-Ping and Fenton, 1988). *Myotis lucifugus* hibernates in mixed sex groups in caves and mines, and is largely sexually segregated in maternity colonies in spring and summer (Fenton, 1969; Humphrey and Cope, 1976). In Ontario, 'swarming behavior' begins in July when groups, mostly of males and nulliparous females, briefly visit the hibernation sites. Hibernating populations begin to build up in August when adults of both sexes and subadults arrive in groups during a second period of swarming (Fenton, 1969). Mating activity peaks in August, but continues even among hibernating bats through the fall, winter and spring (Figure 8.6).

Figure 8.6 *Myotis lucifugus* copulating in August in a mine at Renfrew, Ontario (photograph by M.B. Fenton).

During the peak of mating activity, males visit small crevices or holes in the walls of the roost where they land briefly, and from which they make short, 2 to 10 m, looping flights. Males frequently move to different crevices and holes where they repeat these behaviors. Small groups of bats join males at these sites, and 77% of 90 copulations observed in an abandoned mine occurred at holes (Thomas *et al.*, 1979). However males showed no fidelity to holes, no territorial behavior, and no evidence for displays or social interactions (Barclay and Thomas, 1979; Barclay *et al.*, 1979; Thomas *et al.*, 1979). Copulation occurred immediately upon contact between a male and female. The short looping flights of males could serve to attract females, but there was no apparent relationship between flight activity and the numbers of females attracted to holes. Thomas *et al.* (1979) concluded that mating was indiscriminant. Subsequently, Wai-Ping and Fenton (1988) found no relationship between the size and mass of males that were captured in copula versus those not copulating, providing further support for indiscriminant mating.

Indiscriminant mating in *M. lucifugus* is supported by frequent observations of males copulating with torpid females. Copulating pairs have been found in hibernacula throughout the hibernation period (Wimsatt, 1945; Fenton, 1969), and Thomas *et al.* (1979) observed matings when males that had been disturbed into flight landed on single or grouped torpid bats and attempt to ‘force’ copulations. At this time, males not only copulate with females, but also with other males (Thomas *et al.*, 1979).

Despite this evidence, the recent examination of DNA fingerprints shows that fertilization in *M. lucifugus* is non-random (Watt and Fenton, 1995). Multilocus DNA fingerprint profiles of mothers and their single nursing pups revealed that the percentage band sharing between pups was significantly greater than the band sharing observed between the mothers or between a cohort of other adult females captured in the vicinity. The closer DNA fingerprint similarity among pups indicates that successful fertilizations were non-random and skewed toward particular males or male lineages. While it seems likely that non-random fertilization is achieved as a consequence of non-random mating, sperm competition in multiply mated females cannot be eliminated (Watt and Fenton, 1995). *Myotis lucifugus* provides an excellent example of the fact that mating systems in bats, and other social interactions as well, can be exceedingly difficult to determine even with careful observational studies.

8.4.2 Year-round Multi-Male/Multi-Female Groups

Groups of eight to 12 adult female vampire bats (*Desmodus rotundus*) roost throughout the year in hollow trees or caves with groups of two to 10 adult males (Wilkinson, 1985a, b; Figure 8.7). Males fight with one another for locations at the top of roosts that contain females, and top males have the highest mating success. In tree hollows, 20 of 42 adult males that were observed in top positions accounted for 16 of 21 observed copulations, with the second highest males achieving two matings, and lower males the remaining three (Wilkinson, 1985b). Females were observed mating with top and lower males on successive days, and females were also known to mate with and have offspring from males of other roosts (Wilkinson, 1985b). Genetic data show that top males sire about 46% of the pups born in their

Figure 8.7 A cave-roosting group of common vampire bats *Desmodus rotundus* (photograph by G.S. Wilkinson).

colony, and that top males sire about two times as many offspring as an average male (Wilkinson, 1985b).

Groups of female vampire bats are stable with a new female entering a group about every two years. Average adult male tenure is 17 months, and adult males move among groups more frequently than do females. Female offspring are recruited into their mother's group, while young males disperse (Wilkinson, 1985a, b). Because of these dispersal patterns, a roosting group of vampire bats consists of adult males that are unrelated, and sets of females that are related through different matrilines. Male movements and high juvenile mortality suggest that it is unlikely that females in groups are related through common paternity. Genetic data show that average relatedness among female group members is 0.02 to 0.03 (Wilkinson, 1985b).

A similar vertical structure in male roost-site location and mating success is seen in *Pteropus giganteus*. In India, *P. giganteus* aggregate in trees in permanent, year-round colonies of several 100 or more bats (Neuweiler, 1969). Adult males are faithful to particular roost site locations, with a vertical rank-order in which dominant males roost higher in the tree than subordinate males. Females and their pups also aggregate in the upper branches of these trees. The higher roost sites of dominant males appear to provide reproductive advantage. During the period from birth (March) through mating (July) many young males roost in separate trees apart from the major colony.

While they did not detect any relation between roosting position and male dominance, Bradbury and Vehrencamp (1976) suggested that *Rhynchonycteris naso* has a polygynous mating system involving a dominance hierarchy among males. In Trinidad and Costa Rica, colonies typically consist of five to 11 individuals with both sexes in approximately equal numbers. Colonies roost on the exposed boles of trees or on cliffs, with individuals spaced 2 to 4 cm apart, often in a vertical line (Figure 8.8). Male membership in colonies fluctuated over a period of months, but a particular male was always present at each colony. These 'most-constant' males frequently returned to the day roost at night, whereas other males rarely did so. Most-constant males also were often observed foraging at the periphery of female colony-specific foraging areas, and harassing intruders from other colonies. Some males appeared to have preferred access to matings and copulated without interruption from nearby males, while other males were unsuccessful at copulating.

Two other emballonurids, *Peropteryx kappleri* and *Balantiopteryx plicata* roost year-round in mixed sex colonies. Colonies of *P. kappleri* in Costa Rica were found

Figure 8.8 *Rhynchonycteris naso* in their typical 'in a line' roosting configuration (photograph by A.P. Brooke).

in hollow logs and tree boles and averaged 4.3 bats per colony (Bradbury and Vehrencamp, 1976), whereas in Columbia colonies in abandoned mines were substantially larger with five to 147 bats per colony (Giral *et al.*, 1991). A colony of *B. plicata* in a cave in Costa Rica had a population size of 1500 to 2000 bats. Colonies of both species were subdivided into smaller roosting clusters, but the two sexes were interspersed with no evidence for male territoriality or female grouping.

Within clusters, *P. kappleri* maintain the individual spacing of a few cm that is typical of many emballonurids, and the same individuals remained in the same roosting locations throughout a 14-month study (Giral *et al.*, 1991). However, Bradbury and Vehrencamp (1976) reported that *P. kappleri* roost in dorsal-ventral contact except when disturbed, at which time they then assume individual spacing. Bradbury and Vehrencamp (1976, 1977) suspected that *P. kappleri* may be monogamous because clusters sometimes consisted of one or two adult male/female pairs. In the larger colonies in Columbia, adult females always outnumbered adult males with an average colony sex ratio of 1.4 females/male. For this reason, Giral *et al.* (1991) suspected a harem mating system. At present, we can conclude only that *P. kappleri* mate in mixed sex colonies, and that some level of polygyny seems likely.

The colony of *B. plicata* in Costa Rica was divided into clusters of 50 to 200 bats that roosted in cracks, tunnels, and concavities of the cave surface (Bradbury and Vehrencamp, 1976). A similar colony structure was documented in Guerrero, Mexico (Lopez-Forment, unpublished; cited in Bradbury and Vehrencamp, 1976). In contrast to Giral *et al.*'s (1991) observations on *P. kappleri*, colonies of *B. plicata* in both Costa Rica and Mexico showed a consistent male sex ratio bias. Over ten months of censuses, only 23% of the adults captured in the Costa Rica roost were females. In both Costa Rica and Mexico this superbundance of males peaked in the dry season when mating occurs, and thereafter the fluctuating excess of males decreased. This seasonal pattern led both Bradbury and Vehrencamp (1976) and Lopez-Forment (unpublished) to conclude that, while males are always in abundance, male excesses decrease for much of the year because males move away from these sites after the mating season. Bradbury and Vehrencamp (1977) described mating in *B. plicata* as a possible 'mating swarm'.

8.4.3 Multi-Male/Multi-Female Groups That Mate Away From the Roost

Males of several paleotropical fruit bats establish nocturnal display sites where females visit males for mating. Details of this behavior are available for *Hypsignathus monstrosus*, which mates in leks (Bradbury, 1977b), and for *Epomophorus wahlbergi* (Wickler and Seibt, 1976), and *Epomops franqueti* (Brosset, 1966; Bradbury, 1977b). Unpublished observations by G.S. Wilkinson indicate similar behavior in *Epomophorus crypturus*.

Leks are defined by four criteria: (1) absence of male parental care, (2) the mating arena is significantly smaller than the normal home ranges of both males and females, (3) male territories contain no resources, and (4) females have the opportunity to select mates (Bradbury, 1977b; 1982). While the mating systems of many bats satisfy the first two criteria, little is known for many species with regard to the resources provided by mating sites, such as protection or food, or with regard to mate selection. Thus, in bats, leks have been convincingly documented only for *H. monstrosus* (Bradbury, 1977b).

Hypsignathus monstrosus roosts in trees during the day, mostly as singles of either sex, but also in mixed sex groups of up to 17 individuals. Males typically roost at the periphery of larger groups (Bradbury, 1977b). During biannual breeding seasons that occur in Gabon, males fly at dusk to traditional display areas. There, males establish hanging sites in trees from which they call with loud, repetitive vocalizations. Male sites are spaced approximately 10 m apart, and individual males use the same site each night. Display areas that were studied in detail during the peak of the July mating season ranged between 1.6 to 6.4 ha in size, and included the hanging sites of 25 and 132 males, respectively. Different display areas were located 5 to 18 km apart, all in riparian forest along major streams or rivers. All of the display areas were traditional sites that had been used for many years.

Females enter the display area and hover near the calling male. Females visit some males much more frequently than others, and males respond to female visits by greatly accelerating the rate of their calling, giving a ‘staccato buzz’. After several visits, the female lands and copulates with a chosen male. By monitoring the frequencies of staccato buzzes, Bradbury (1977b) determined that males at sites located near the centers of the display areas receive 10 to 20 times more visits from females than males at more peripheral sites. Direct observation of 50 copulations indicated high polygyny, with 6% of the males accounting for 79% of the observed matings.

Studies on the distribution of food resources and the foraging movements of males and females demonstrate that, except for a mate, the male display areas do not provide resources to females. *Hypsignathus monstrosus* also show obvious evidence of the strong sexual selection that is typical of other species with lek mating systems (Bradbury, 1982). *Hypsignathus monstrosus* has the most extreme sexual dimorphism of any bat. Males are almost twice the weight of females, and males have a large hypertrophied larynx that fills more than half of their body cavities.

Epomophorus wahlbergi roosts in palm leaves or thatch roofs in mixed sex colonies of three to over 100 individuals (Wickler and Seibt, 1976). Except for female-pup pairs, individuals are spaced a minimum of 2.5 cm apart. They maintain this roosting configuration year-round, and consistently maintain preferred roosting locations, but there are no apparent associations between males and females. Observations during two successive January mating seasons showed that individuals leave the roost independently at dusk, and males fly various distances from the roost, calling audibly as they fly. Males continue to call from apparently traditional hanging sites that are in or close to their feeding areas, but rarely closer than 30 m from the calling site of another male. Other bats, presumably females, are attracted to calling males and hover in front of them as males increase the repetition rate of their calls and evert their whitish yellow epaulets. Males and visitors were observed touching noses and licking, but copulations were not observed.

Like *Epomophorus wahlbergi*, single male *E. crypturus* and *Epomops franqueti* call from roost sites that are more dispersed than those of *H. monstrosus*. Bradbury (1977b) also observed female or juvenile *E. franqueti* feeding near the territory of a calling male, suggesting that male territories may provide females or juveniles with other resources. Male *Epomophorus crypturus* call from roosts in trees that are spaced about 50 m apart along rivers. Calling males are visited by other bats that are presumably females, but mating was not observed (G.S. Wilkinson, unpublished observations). With the information available, the mating systems of *E. wahlbergi*, *E. crypturus* and *E. franqueti* do not fulfill the definitional requirements for a lek. There is no information on levels of polygyny in *E. wahlbergi*, *E. crypturus* or *E. franqueti*.

8.5 SINGLE MALE/SINGLE FEMALE GROUPS

Since Bradbury's (1977a) review, monogamy has been reported for five other bat species: *Pteropus samoensis* (Cox, 1983; Pierson and Rainey, 1992), *Rhinolophus sedulus* (Heller and Volleth, 1989; Heller *et al.*, 1993), *Cardioderma cor* (Vaughan, 1976; McWilliam, 1987b), *Saccopteryx leptura* (Bradbury and Vehrencamp, 1976; 1977), and questionably, *Peropteryx kappleri* (Bradbury and Vehrencamp, 1977). Additional information is available for two of the 11 species listed by Bradbury as monogamous: *Vampyrum spectrum* (Vehrencamp *et al.*, 1977; A.P. Brooke, G.F. McCracken, and G.S. Wilkinson, unpublished observations) and *Hipposideros beatus* (Brosset, 1982), and for *Lavia frons* (Wickler and Uhrig, 1969; Vaughan and Vaughan, 1986, 1987), a species that Bradbury discussed as monogamous, but did not include in his table. Thus, monogamy has been reported for a total of 17 bat species (Table 8.3).

Lavia frons and *C. cor* are sit and wait predators that hang from low branches on trees or bushes, listening for prey that are usually on the ground. Both species approach and attack prey in short flights from habitual roosting sites that are within feeding territories. In *L. frons*, male/female pairs roost in the same territory during the day, and pair-specific roost sites and foraging areas remain stable for at least several months (Wickler and Uhrig, 1969; Vaughan and Vaughan, 1986, 1987).

In constrast, *C. cor* roost by day in colonies of 80 or more individuals in hollow trees (Vaughan, 1976) or caves (McWilliam, 1987b). In the evening these bats leave their day roosts for habitual hanging sites where the bats sing and maintain foraging territories. Working in inland southern Kenya, Vaughan and Vaughan (1986) reported that singing was seasonal, occurring only during the March–April wet season. Vaughan and Vaughan (1986) reported individual, but not pair-specific territories. At a different site in coastal Kenya, McWilliam (1987b) found that singing was exclusively a male behavior that occurred primarily in the long dry season. At the coastal site, pairs were observed together from June to November, often sharing the same hanging site in close contact, and showing joint circling flights. Pregnant or lactating females and juveniles were present throughout the year, but peak pregnancy occurred in December.

Vampyrum spectrum is the only bat known to form long-term pair-bonds and to live in extended family groups. Adult pairs have roosted together in the same hollow tree for over a year, and the apparent offspring of up to three successive reproductions have remained with their parents (Vehrencamp *et al.*, 1977; A.P. Brooke and G.F. McCracken, unpublished observations). *Vampyrum spectrum*, which is carnivorous and the largest bat in the neotropics, apparently forages alone, mostly on colonial roosting birds, but also on rodents and other bats (Vehrencamp *et al.*, 1977).

Saccopteryx leptura roost on the sides of trees in small groups. Group size in Trinidad ranged from one to five ($\bar{x}$ = 2.6) individuals (Bradbury and Emmons, 1974), whereas group size in Costa Rica ranged from two to nine ($\bar{x}$ = 4.6) individuals (Bradbury and Vehrencamp, 1976). At both sites, pairs consisting of a male and female were most common, suggesting that monogamous pairs are the basic social unit of this bat (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1976, 1977; McCracken, 1984). Groups larger than two appear to consist of two or more adult pairs or pairs with offspring which may be retained in the parental group for up to a year (Bradbury and Vehrencamp, 1976). This group configuration was maintained throughout the year at both sites, but some individuals shifted roosting trees. When larger groups split, the same male/female pairs typically remained together.

Roosting groups of *S. leptura* share colony-specific foraging areas. Most foraging was by solitary bats using separate but adjacent beats, but colony members also foraged in groups, and groups actively defended foraging areas from bats belonging to other colonies. Volant juveniles up to three months old sometimes foraged with their mothers (Bradbury and Vehrencamp, 1976).

Brosset (1982) reported on a population of *Hipposideros beatus* that roosted opportunistically in traps that were set in a grid to capture chevrotains. The regular dispersion of these roost sites over a small island suggested territoriality, and the same sites were occupied continuously by male/female pairs throughout a three year study. Marked pairs of *H. beatus* remained together throughout one year, but new pairs were formed at the beginning of each annual mating season. Yearlings associated in territories with adult pairs that were not their parents (Brosset, 1982).

Less information is available for *Pteropus samoensis, Taphozous peli* and *Rhinolophus sedulus*. *Pteropus samoensis* roosts and forages in pairs in primary rainforest. Repeated observations of single males and females roosting together, interacting at roost sites, and sharing the same foraging areas leads to the conclusion that they pair-bond (Cox, 1983; Pierson and Rainey, 1992). Similarly, monogamy is suspected in *R. sedulus* because it also typically roosts in pairs (Heller and Volleth, 1989; Heller *et al.*, 1993). Further evidence for monogamy in *R. sedulis* is provided by DNA fingerprint profiles that demonstrate an extremely high probability of paternity for an adult male that was captured roosting together with a lactating female and non-volant, nursing young (Heller *et al.*, 1993).

Monogamy implies that males can contribute to the survival of females or pups. The only evidence for male parental care in bats comes from *V. spectrum* and *L. frons*. In *V. spectrum*, at least one adult or older offspring remains in the roost with the most recent juvenile, while the other bats in the group forage. Foragers bring prey to the roost, and the energy content of these prey may exceed the needs of the solitary forager. Thus, it appears that both adults and the older offspring share in 'guarding' young and in provisioning the bats that remain in the roost (Vehrencamp *et al.*, 1977). Provisioning has been documented both in the wild, where a male brought prey to a juvenile in his roost (G.S. Wilkinson, personal observation), and in a captive breeding pair, where the male frequently provided food to the female that was caring for a pup (cited in Vehrencamp *et al.*, 1977).

In *L. frons*, the single juvenile remains closely associated with both parents at roost sites and on the foraging area for up to 50 days after young become volant (Vaughan and Vaughan, 1987). During this period, juveniles frequently huddle with and nurse from the mother at the roost site. The mother remains within a few meters of her young on the foraging grounds, the young frequently follows its mother on foraging flights, and she allows it to replace her at her roosting sites. Fewer interactions were observed between the juvenile and the male, but the male also allowed the young to take over his roosting sites (Vaughan and Vaughan, 1987).

The unusual foraging habits of *V. spectrum*, *L. frons*, and probably *C. cor*, appear to involve an extended period during which young must learn to forage. The extended period of parental involvement with the young, and, in the case of *V. spectrum*, the ability of the males to provision females and offspring, evidently select for monogamy in these bats (Vehrencamp *et al.*, 1977; Vaughan and Vaughan, 1987).

8.6 ECOLOGICAL DETERMINANTS OF MATING SYSTEMS IN BATS

While there has been substantial progress in describing bat mating systems, there has been considerably less progress toward understanding the ecological factors responsible for their evolution and maintenance. The conventional view holds that female dispersion patterns are primarily influenced by resource distribution while male dispersion patterns are primarily determined by female dispersion (Bradbury and Vehrencamp, 1977; Emlen and Oring, 1977). In mammals without paternal care, mating systems can be defined by what males defend, i.e. either females (female defense) or resources needed by females (resource defense). Leks are often considered a default mating system, adopted when males cannot defend either females or resources because resources are too finely distributed and females range too widely in unstable groups (Bradbury and Vehrencamp, 1977; Bradbury, 1981).

In bats, female defense polygyny has been attributed to *Phyllostomus hastatus* (McCracken and Bradbury, 1977, 1981) and *Tadarida pumila* (McWilliam, 1988). The similar mating systems of *Myotis bocagei* (Brosset, 1976), *Coleura afra* (McWilliam, 1987a), and *Noctilio leporinus* (Brooke, 1997) suggest that single males may defend stable groups of females in these species. Resource defense polygyny has been attributed to *Carollia perspicillata* (Williams, 1986), *Artibeus jamaicensis* (Morrison, 1979; Morrison and Handley, 1991), *Pipistrellus pipistrellus* (Gerell and Lundberg, 1985; Lundberg and Gerell, 1986), *Pipistrellus nathusii* (Gerell-Lundberg and Gerell, 1994) and several tent-roosting species of bats (e.g. *Artibeus jamaicensis*, *Artibeus cinereus* and *Uroderma bilobatum*, Kunz and McCracken, 1996; *Cynopterus sphinx*, Balasingh *et al.*, 1995; *Cynopterus horsefeldi*, Tan *et al.*, 1999) where preferred or limited roost sites may be defended from other males and used by groups of females. Resource defense polygyny has also been attributed to *Saccopteryx bilineata* (Bradbury and Vehrencamp, 1976,

1977) because males defend foraging areas utilized by multiple females, and to *Pipistrellus nanus*, where males may also defend foraging areas used by females (O'Shea, 1980).

These attempts to identify underlying ecological causes place important focus on understanding the evolution of diverse mating systems, but there are problems. One problem is the difficulty of inferring causation from correlation. Once females form groups at a fixed site, then males can either defend locations preferred by those groups or they can defend the groups directly. Related to this is the problem that the distribution of potential mates and their resources are linked, and both may be objects of defense (Ostfeld, 1987). While correlations between food dispersion, female dispersion, and mating systems have been documented in epomophorine (Bradbury, 1981) and emballonurid (Bradbury and Vehrencamp, 1977) bats, experimental evidence supporting causal factors influencing mating system variation is lacking for bats and for most other mammals (Davies, 1991). Lundberg and Gerell's (1986) experiments of adding new roost boxes to manipulate the resources available to *Pipistrellus pipistrellus* are a notable exception. These experiments appear to confirm resource defense polygyny in *P. pipistrellus*.

Some studies on bats have recognized the above problems. McWilliam (1988) suggested that the female defense mating system of *Tadarida pumila* may also involve resource defense because roosting sites are defendable. Kunz *et al.* (1983) suggested that harem polygyny may be best described as resource defense where *Artibeus jamaicensis* roost in limited available tree holes (Morrison, 1979; Morrison and Handley, 1991), but as female defense where this same species roosts in abundantly available erosion concavities in caves.

If mating systems are determined by what males can defend, it might be assumed that females have little opportunity to assess and choose mates. While female mate choice is clearly important in determining the extreme mating skew that occurs among lekking males (Bradbury, 1977b), leks are thought to result when males can defend neither females nor their resources. Indeed, it has been argued that polygynous territorial mating systems driven by female choice, such as have been proposed for birds (Verner, 1964; Orians, 1969), are inappropriate for most mammals because female mammals are often philopatric to their natal area, female groups often persist over seasons, and males often impose themselves on pre-existing female groups (Clutton-Brock, 1989).

These arguments clearly do not apply to many bats. As described above, seasonal male mating territories have been observed in many temperate cycle bats as well as several tropical species. In many of these species (e.g. *Pipistrellus pipistrellus*, *P. nathusi*, *P. nanus*, *Nyctalus noctula*, *Macrotus californicus*, *Tadarida brasiliensis*, *Saccopteryx bilineata*) males produce audible vocalizations which are often accompanied by aerial displays. *Saccopteryx bilineata* also differs from many other mammals in that females, not males, disperse from their site of birth (Bradbury and Vehrencamp, 1976). In all of these species, females do not form stable groups and frequently move between males during the mating season. While experimental work is needed to determine if females visit males to gain access to roosting sites or to preferred mates, these examples indicate that female choice is likely to be more important in determining male mating success in bats than in most groups of mammals.

Just as opportunities for female mate choice should be inversely related to female group stability, a male's opportunity to monopolize paternity within female groups should also be associated with the stability of female social groups. None the less, even in *Phyllostomus hastatus* which exhibits the highest level of female group fidelity known for bats, at least 10% of the offspring born to harem females are sired by males from outside of the harem (McCracken and Bradbury, 1977; 1981). Information on levels of polygyny and polyandry is lacking for almost all bats. However, the many examples of seasonal male mating territories, and the many examples of year-round social groups in which female group composition is unstable (Table 8.3), suggest that females mate with multiple males in many and probably most species of bats.

Multiple mating by females, as well as features of the reproductive physiology of bats (Racey, 1975; 1979; Racey and Entwistle, this volume) suggest that sperm competition is an important and underappreciated factor in the evolution of bat mating systems (Fenton, 1984; Hosken, 1997). Fenton (1984), Hoskin (1997), Crichton (this volume) and Racey and Entwistle (this volume) review the incidence of sperm storage in bats and consider the significance of sperm competition for the evolution of bat reproductive systems. Notably, the longevity of viable sperm correlates with the length of oestrus and female receptivity. Also, male testes mass correlates with the size of roosting or social groups over a wide range of bat taxa (Hosken, 1997). Testes mass is an index of the numbers of sperm produced, and group size an index of the risk of sperm competition (Hosken, 1997). It is likely that male mating success depends on sperm competition in *Myotis lucifugus* (Fenton, 1984; Watt and Fenton, 1995), and in banana leaf roosting *Pipistrellus nanus* (Happold and Happold, 1996). In one species, *N. noctula*, molecular studies have demonstrated that female reproductive tracts may contain sperm from up to five males, strongly supporting the significance of sperm competition (F. Mayer, personal communication). The role of sperm competition deserves major attention in future studies of bat mating systems.

The incidence of monogamy varies greatly among mammalian taxa, being the most common mating system in canids (Bekoff *et al.*, 1984) and known or suspected in 22% of primates species (Wrangham, 1987). The 18 possibly monogamous bat species comprise about 25% of the known mating systems in the Order (Table 8.3), so the frequency of monogamy in bats may be well above the mammalian average (Kleiman, 1977). Monogamy in other animals is associated with biparental care (Davies, 1991). Among bats, only *Vampyrum spectrum* and *Lavia frons* have been reported to exhibit male parental care. While these two species are similar to canids in being both monogamous and carnivorous, they differ in being monoestrous. Consequently, female reproductive rate is not obviously increased by male parental care. Alternative advantages of monogamy in bats might be that male parental care increases female survival, or that provisioning the young by males permits the female to resume reproduction more rapidly than would be possible if she were the sole source of nutrition for the offspring.

The recent discovery of male milk production in *Dyacopterus spadiceus* (Pteropodidae) (Francis *et al.*, 1994) demonstrates that some male bats lactate. However, male milk production could be due to factors such as phytoestrogen consumption, rather than an evolved participation in parental care. If male lactation allows biparental nursing, it is most likely to occur in monogamous species.

With the exceptions of *Vampyrum spectrum* and *Lavia frons*, the remaining examples of monogamy in bats (Table 8.3) have been inferred from observations of mixed sex pairs in species which feed on insects or fruit. While these observations are consistent with monogamy in which a male defends the range of a single female, other more complicated mating systems, such as sequential polyandry, cannot be excluded. Roost observations to determine the presence and extent of male parental care, tracking studies to determine overlap and persistence of male and female ranges, and molecular studies to determine paternity are all needed. Given the unusually high frequency of monogamy in bats as compared to other mammals, such studies are long overdue.

Only a few of the pre-1977 studies on mating systems in bats involved marking of individuals (Dwyer, 1970; Bradbury and Emmons, 1974; Bradbury, 1977a, b) or otherwise identifying individuals (e.g. '. . . by the patterns of bullet holes in the wing membranes', Nelson, 1965), thereby allowing observations of known individuals. There has been an increasing number of such studies since 1977, both for free-ranging and captive bats. Advances in radio-telemetry and night vision and low light observational techniques have all contributed greatly to documenting the mating systems of many bat species. We expect the next 20 years to provide even more information as these techniques are applied more widely.

Compared to work on other taxa, such as birds, research on bat mating systems has lagged considerably in the use of molecular gene markers. Although the first molecular analysis of a bat mating system dates from over 20 years ago (McCracken and Bradbury, 1977), there are still fewer than 10 such published studies on mating in bats. The advent of molecular markers based on DNA amplification (PCR) has eliminated many of the logistic problems (i.e. limited allelic diversity and the need for cryogenic storage of substantial blood or muscle samples) that were inherent to earlier applications based on allozymes (McCracken and Wilkinson, 1988). The development of hypervariable microsatellite markers now provides single-locus genetic markers that typically provide an order of magnitude more variability than is available with allozymes (Quellar *et al.*, 1993). Sufficient DNA for these analyses can be obtained from minimally invasive sampling of even the smallest bats, and samples can be stored indefinitely at room temperatures (Worthington Wilmer and Barratt, 1996). It is even possible to investigate multiple insemination from DNA analysis of sperm in female reproductive tracts (Mayer, 1995). Combined with detailed field studies, the immediate future will see an increasing emphasis on the use of these new molecular methods to resolve the big issues in bat mating systems. In our opinion, these issues are questions of apparent monogamy, the significance of multiple mating by females, and the roles of female choice and sperm competition.

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9

Life-history and Reproductive Strategies of Bats

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Reproductive Biology of Bats
0-12-195670-9

9.1 INTRODUCTION

The timing and frequency of reproduction are major determinants of life-history strategies, which have evolved to maximize lifetime reproductive output, and thus to maximize fitness in terms of an individual's genetic contribution to the next generation (Stearns, 1992). Life history traits do not develop independently, since trade-offs have been identified between different life-history characteristics such as life span, reproductive and mortality rates (Harvey and Zammuto, 1985; Partridge and Harvey, 1985; Read and Harvey, 1989; Promislow and Harvey, 1990). Therefore, sets of interrelated characteristics evolve, making it difficult to identify cause and effect.

Bats have distinctive life-history strategies for mammals of their size. Most small animals have evolved a 'live fast – die young' strategy (Promislow and Harvey, 1990), characterized by rapid reproduction and high mortality. In contrast, bats generally have a life history characterized by longevity with multiple reproductive events, low litter size and delayed onset of sexual maturity (Gaisler, 1989). Typically only a single young is produced (Racey, 1982), although twins are common in some species (Tuttle and Stevenson, 1982). The trend for smaller mammals to generally produce relatively larger young (Harvey, 1986) reaches its extreme in bats, in which the neonate may weigh up to 43% of maternal mass (Kurta and Kunz, 1987). Coupled with one of the lowest mass-specific foetal growth rates recorded within mammals (Frazer and Huggett, 1974), bats are characterized by long gestation lengths for mammals of their size (Racey, 1973a). This is followed by a relatively long period of lactation and post-natal care (Hayssen, 1993). Longer gestation, coupled with a shorter period of lactation, may reflect lower energetic investment per day in gestation, compared to the high costs of lactation, and may represent a strategy by which energetic investment in young is optimized in relation to seasonal food abundance. Alternatively, there may be advantages for extending gestation for the neuromuscular development of the young, thus enabling flight to coincide with weaning. Although larger young are likely to be precocial among mammals generally (Case, 1978; Martin and MacLarnon, 1985), bats do not fit easily within this categorization (Kurta and Kunz, 1987) since the degree of precocity appears to vary between different families. Nevertheless, all species of bats are totally dependent on maternal care for a period after birth (Kunz and Stern, 1995). Weaning occurs relatively abruptly, and in autumn females may roost away from their young (Burland, 1998), and may extend their foraging range beyond the vicinity of the roost (Clark *et al.*, 1993). However, there is at least one record of associations between mothers and young while foraging around the time of weaning (Brigham and Brigham, 1989).

It is not clear why bats should differ so greatly from similar-sized terrestrial mammals. However, it seems likely that the evolution of nocturnal flight may have been a key factor in the life histories observed. Life-history strategies presumably evolved in response to limitations imposed by morphology, phylogeny, demography and ecology (Sutherland *et al.*, 1986; Stearns, 1992). Two outcomes of flight may affect the evolution of life history characteristics – reduced mortality (Pomeroy, 1990) and large size at independence for young (Barclay, 1995).

Mortality patterns are a good predictor of life-history strategies across mammals (Harvey and Zammuto, 1985; Promislow and Harvey, 1990). The ability to fly may reduce predation and hence mortality rates, since both bats and birds have low mortality rates (Pomeroy, 1990). Recent advances in the understanding of mammalian life histories, particularly the optimality models of Charnov (1993) and Kozlowski and Weiner (1997), have suggested that mortality rates around maturity play a central role in life-history evolution. Both these models have received some empirical support from comparative studies across mammals (e.g. Purvis and Harvey, 1995, 1997) and are major contributions to our understanding of how life-history patterns have evolved. They focus on the importance of body size as an adaptation to life histories rather than assuming that life histories are themselves an adaptation to body size (Western and Ssemakula, 1982).

According to Charnov's (1993) model, selection acts to maximize an individual's lifetime reproductive success in the face of mortality rates imposed by the environment. Such success is optimized by altering the age and therefore size at which sexual maturity is reached. Individuals that are older and therefore larger at sexual maturity have more energy available to invest in offspring production per unit time. However, it takes time to grow large and this leads to a trade-off between the age and size at which maturity is reached and the likelihood of survival to maturity.

It is suggested that the covariation of birth rate and sexual maturity with mortality rates, independent of body size, can be explained by different species having different growth rates (Charnov, 1993). For example, the low birth rates, late sexual maturation and low mortality rates in primate species can be explained by their slow rate of growth. Charnov and Berrigan (1993) calculate a growth coefficient of 0.42 for primates, compared with a coefficient of 1 for other mammals (Charnov, 1993). Thus a primate will grow both itself and its offspring at less than half the speed of a similarly sized non-primate mammal. Bats, like primates, also have low birth rates, late sexual maturation and low mortalities relative to body size (Tuttle and Stevenson, 1982; Read and Harvey, 1989; Austad and Fischer, 1991). Charnov's (1993) model suggests that their later ages and larger sizes at maturity enable more energy to be diverted to offspring production per unit time in order to maximize their reproductive success. These later ages at maturity are possible because there is a high likelihood of survival to maturity through decreased vulnerability to predators associated with a volant and nocturnal lifestyle (Austad and Fischer, 1991). However, to some extent the evolution of flight in bats may have constrained the range of life-history patterns that are possible. Adult dimensions at weaning are not only desirable in terms of maximizing reproductive success (*sensu* Charnov, 1993) but are also a necessary constraint for flight to gain independence from the mother (Barclay, 1994, 1995).

What empirical support is there that Charnov's (1993) model explains the variation seen in bat life histories? The model predicts that bats as well as primates are characterized by slow rates of growth for their body size (of both themselves and their offspring). Figure 9.1 (from Jones, 1998) compares the relationship between the length of the juvenile period (measured between weaning and sexual maturation) and body size in bats, primates and other mammalian species. The growth coefficient (A) for the different groups can be calculated using the intercept of the regression line with the Y-axis where A = –ln intercept. Using these data the growth coefficients for primates is 0.65 as compared with 0.91 and 1.24 for bats and other mammals, respectively (Jones, 1998).

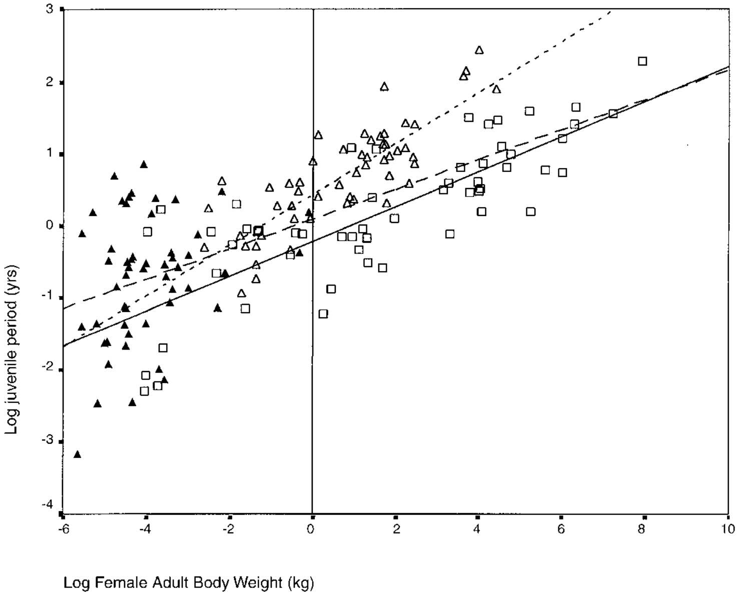

Figure 9.1 (From Jones, 1998). Comparison in mammals of the allometry of length of the juvenile period. Filled triangles represent bat species, open triangles primates and squares species from other mammalian orders. – – – – represents the regression line found in bats ($y = 0.21x+0.1$, $p = <0.05$, $r^2 = 0.07$, d.f. = 52); - - - - - in primates ($y = 0.35x+0.4$, $p = <0.001$, $r^2 = 0.69$, d.f. = 52, data from Ross and Jones, 1999) and ——— in other mammals ($y = 0.24x+-0.2$, $p = <0.001$, $r^2 = 0.64$, d.f. = 54, data from Purvis and Harvey, 1995).

9.2 COSTS OF REPRODUCTION

While the costs of reproduction in the short term relate to its energetic expense (Loudon and Racey, 1987, Gittleman and Thompson, 1988), in the longer term reproduction has costs in terms of individual body condition (Clutton-Brock *et al.*, 1989), and trade-offs with survival (Bell, 1980). Therefore, it would be expected that animals would be faced with decisions about when to reproduce, in relation to body condition, resource availability, ability to allocate energy to reproduction (Thompson, 1992), social context and future reproductive potential. For example, there may be advantages in timing births to correspond to peaks in food availability, both in ungulates (Clutton-Brock *et al.*, 1982), and in bats, where earlier birth affects both juvenile survival and fitness (Ransome, 1989). Within this context bats have evolved an unusual and diverse set of reproductive strategies, and consequently bat reproduction is more varied than in most other orders of mammals.

9.3 SEASONALITY AND REPRODUCTION

Because reproduction, particularly lactation, is energetically costly (Loudon and Racey, 1987), it would appear that food availability should be an important factor in determining the ability of females to allocate energy to reproduction (Thompson, 1992). Periods of optimal food availability determine the timing of reproduction (Bronson, 1985) and are referred to as the ultimate cause by Baker (1938). Under conditions where food is abundant throughout the year, animals may be able to initiate reproduction at any time. However, where food supplies vary markedly, animals may only be able to meet the costs of reproduction during periods of maximum food availability, and the success of a species may reflect its ability to produce young at a propitious time (Clarke, 1981). In unpredictable, unstable environments, animals often adopt an opportunistic strategy, responding to local changes in conditions, and breeding is initiated whenever conditions are suitable (Bronson and Heideman, 1994). However, in an environment dominated by seasonal changes in climate, where food availability fluctuates in parallel, certain periods of the year are generally more suitable than others for reproduction (Sadleir, 1969; Fretwell, 1972). Animals with a long gestation (such as bats), are unable to react quickly to short-term environmental fluctuations and may cue into predictable seasonal changes by which to optimize their time of reproduction (Heideman, this volume). Such species display single birth events, or series of births, within the propitious season, separated by periods of non-breeding during sub-optimal seasons (Clarke, 1981).

Most species of bats, both in temperate and tropical zones, reproduce seasonally (Racey, 1982; Heideman, 1995). Reproduction in many bats appears to be timed so that lactation, the most energetically expensive part of reproduction (Kurta *et al.*, 1989a), coincides with peak food availability (Racey, 1982), although in some species weaning may coincide with this peak (Fleming *et al.*, 1972). Such timing is most likely to maximize reproductive success (Bronson, 1985), but constrains reproduction to particular times of year and may thus reduce the frequency of breeding and annual reproductive output. However, some bat species may not be subject to the constraints of seasonal reproduction. Such exceptions include species inhabiting more stable environments, such as tropical rainforests, where food supply is more constant and shows less fluctuation in availability (Happold and Happold, 1990a). Additionally, species in seasonal environments which rely on an unvarying food source (such as the sanguivorous vampire bat *Desmondus rotundas*: Wimsatt and Trapido, 1952; Wilson, 1979) also show aseasonal patterns of reproduction.

9.3.1 Reproductive Seasonality in Temperate Zones

At temperate latitudes climate varies dramatically between winter and summer, and seasonality in reproduction of bats is pronounced (Tuttle and Stevenson, 1982). All temperate zone species are insectivorous, and climate, particularly temperature, directly affects insect availability (Taylor, 1963). In temperate latitudes, insect availability is seasonally constrained, with a clear peak during summer and a nadir in winter which cannot sustain prolonged bat activity. Insect numbers rise gradually in spring (March–April in northern latitudes) as temperatures increase, to peak in mid-summer (July/August) and thereafter decline through autumn (September/October). This pattern is mirrored in the southern summer. In temperate zones, reproductive activity of bats is thus tightly constrained by climate and insect availability (Wilson, 1979), with parturition occurring mid-summer, followed by rapid post-natal growth (Kunz and Stern, 1995), and reproductive quiescence during the winter months.

9.3.2 Seasonality in the Tropics

In some tropical environments (such as rainforest) food appears to be abundant year round, and some bat species display aseasonal patterns of reproduction (e.g. *Pipistrellus nanus*, Brosset, 1966; review in Happold and Happold, 1990b). However, although seasonal variations in temperature may not be extreme in most tropical regions they experience a greater or lesser degree of seasonality in rainfall patterns (Jackson, 1961). There is growing evidence that for the majority of insectivorous bats in the seasonal tropics, reproductive activity is associated with rainfall rather than temperature cycles (e.g. Ramakrishna and Rao, 1977; Wilson, 1979; O'Shea and Vaughan, 1980; Willig, 1985a; van der Merwe *et al.*, 1986, 1987; McWilliam, 1987a, 1988a,b; van der Merwe *et al.*, 1988; Happold and Happold, 1989, 1990a,b; Kofron and Chapman, 1994; Cumming and Bernard, 1997). Parturition generally takes place at the onset of the rains and lactation during the peak of the rainy season (Racey, 1982).

9.3.3 Insectivorous Species

It has previously been inferred that seasonal reproductive patterns in the tropics reflect changes in food availability (Racey, 1982). Indeed, evidence suggests that insect availability closely follows rainfall patterns and shows a marked increase associated with the onset of rains (Rautenbach *et al.*, 1988). A number of studies have demonstrated a peak in insect availability during the rainy season (Skutch, 1950; Janzen and Schoener, 1968; McWilliam, 1987c, 1988b; Mutere, 1973a;

Rautenbach *et al.*, 1988 – Figure 9.2; Happold and Happold, 1990a). These studies suggest a strong congruence between patterns of rainfall, insect abundance and reproductive events, and a direct correspondence between the three factors has now been demonstrated for some species (McWilliam, 1987c – Figure 9.3; McWilliam, 1988a; Vaughan and Vaughan, 1986).

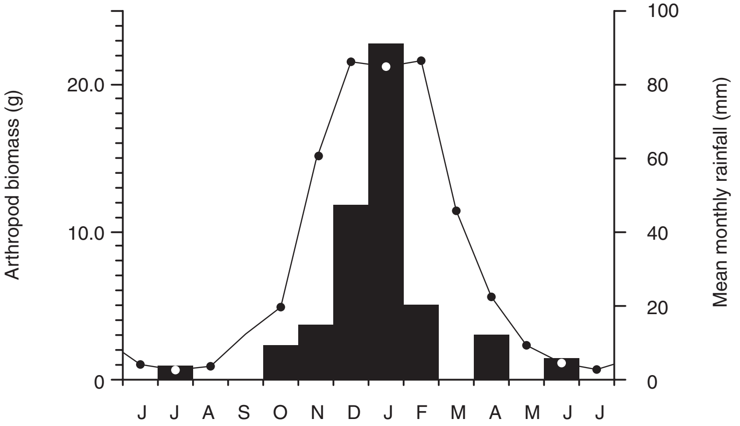

Figure 9.2 Variations in rainfall and arthropod biomass in South Africa (from Rautenbach *et al.*, 1988).

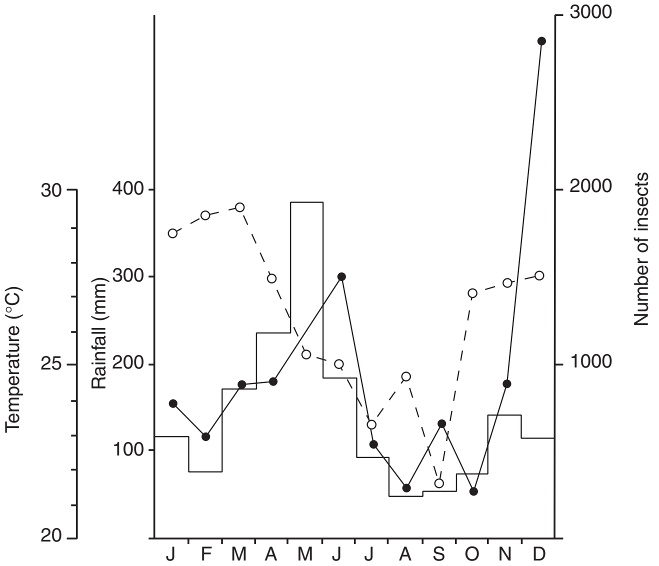

Figure 9.3 The relation between rainfall (– – – –, mm per month), food supply (●, monthly mean numbers of insects light-trapped per night) and temperature (○, monthly mean), averaged over 1978 and 1979 (from McWilliam, 1987c).

There is, however, some variation in the timing of reproduction in relation to rainfall between different species (Willig, 1985a; Happold and Happold, 1990a). This may reflect different dietary specialization and seasonal patterns of emergence of different insect groups. For instance, peak terrestrial insect availability appears to occur later than that of aerial insect availability (Rautenbach *et al.*, 1988) and species which feed predominantly on ground dwelling insects or other prey (e.g. *Lavia frons*, Vaughan and Vaughan, 1986) might be expected to reproduce later in the season than sympatric aerial hunters, if bats react directly to food availability.

9.3.4 Frugivores and Nectarivores

The seasonality of reproduction is well established in tropical frugivores (Heidemann, 1995). As in insectivores, frugivores display a correspondence between lactation and the peak of the rainy season (Mutere, 1967; Fleming, 1971; Okia, 1974; Thomas and Marshall, 1984; Willig, 1985b; Happold and Happold, 1990a). This may also reflect changes in food availability, since studies of fruiting phenologies indicate a general peak in fruit abundance during the rains (Janzen, 1967; Smythe, 1970; Daubenmire, 1972; Frankie *et al.*, 1974). However, factors beyond simple fruit abundance may be important for determining patterns, for example nutrient status or water content of fruit may vary with season. In some species, reproductive timing does not correlate well with rainfall, but instead appears to reflect fruiting of particular species, such as mangoes (e.g. *Cynopterus brachyotis*, Kofron, 1997).

While fruiting coincides with the rainy season (Janzen, 1967), flowering generally occurs during the preceding dry season, although in some cases it may also occur during the rains (Hepburn and Radloff, 1995). Reproductive patterns of obligate nectarivores appear in many cases to mirror the timing of flowering and, in contrast to other sympatric species, nectarivores may produce young during the dry season (e.g. *Glossophaga soricina*, Willig, 1985b; *Anoura geoffroyi*, Heideman *et al.*, 1992). Such a difference in the timing of reproduction emphasizes the importance of diet in determining reproductive timing, and suggests that food availability, rather than climatic factors, ultimately underlies the timing of reproductive events in tropical bat species.

9.3.5 Latitudinal and Local Variations in Seasonality

Since seasonality (of both climate and food supply) appears to be a key factor in determining the timing of reproduction, it might be predicted that local and latitudinal variations in climate and seasonality might be reflected in the timing of reproduction. A clear example of the effect of seasonality on temperate zone bats is seen in the direct asynchrony in reproductive events between boreal and austral cycles of bats in the two hemispheres (e.g. Anciaux de Faveaux, 1978, 1983; Happold and Happold, 1990a). Furthermore, it is well known that the timing of mating, length of gestation and time of birth vary with latitude (e.g. Dwyer, 1970; Schowalter *et al.*, 1979; Racey, 1982; Tuttle and Stevenson, 1982; Bernard, 1982a). Comparative studies have also revealed a correlation between latitude and other life history patterns. For example, Kunz and Stern (1995) reported faster post-natal growth rates at higher latitudes. Jones (1998) found a correlation between higher latitudes and shorter gestation lengths, faster prenatal growth rates, larger litter sizes, and lower annual fecundities. However, none of these relationships were supported when independent contrasts (Price, 1997) were used to control for phylogenetic inertia. At lower latitudes in the temperate zone, the variation in temperature, and presumably insect availability, between summer and winter is less extreme than at higher latitudes. At these lower latitudes the timing of reproduction may shift, with earlier arousal from hibernation and initiation of gestation, resulting in earlier birth (Racey, 1982). Indeed, in a study of *Myotis myotis* from southern Spain (37°N), the timing of reproduction had shifted dramatically, resulting in gestation during winter, and even winter births (Ibanez, 1997). Unfortunately, records of insect availability were not available, which would have helped to explain the underlying cause of this shift in reproductive timing. Similar latitudinal variations in timing have also been noted in tropical bats, although this situation may be complicated by associated changes in the reproductive pattern (e.g. release from monoestry to polyoestry with decreasing latitude in *Tadarida pumila*, Happold and Happold, 1989; see below).

9.3.6 Climate and Seasonal Reproduction

In general the proximate cues controlling the timing of reproduction in bats are not well understood (Heideman, this volume) although there is some evidence (Racey, 1978; Heideman and Bronson, 1994) that increasing photoperiod governs the onset of breeding as it does in many other groups of mammals and in birds (Follett, 1984). Under laboratory conditions of constant food availability and temperature, seasonal patterns of reproduction similar to those in wild populations are maintained for some years, but then break down (e.g. *Carollia perspicillata*, Laska, 1990), suggesting that some environmental cue is important in determining this cycle. It is often difficult to establish whether climatic factors are the most important proximate cues for reproduction in bats, since both climate and food availability are correlated. This is particularly the case in hibernators where spermatogenesis and pregnancy begin on arousal in spring when photoperiod, ambient temperature and insect food supply are all increasing. Experimental studies to separate these effects are rare (Racey, 1978). Temporal and spatial variations in the timing of reproduction in such tropical species as *Haplonycteris fischerii* may indicate some degree of adaptation to interannual and local variations in climate or food availability (Heideman, 1988). For bats, as in other mammals, the most important ultimate or evolutionary cause of the timing of reproduction is the availability of food to meet the energy demands of lactation and growth of the young (Racey, 1982) and the synchrony of reproduction with food availability rather than climate is recorded in at least one species (*Cynopterus brachyotis*, Kofron, 1997).

9.4 REPRODUCTIVE PATTERNS OF BATS

Bats show a range of annual patterns of reproduction, which vary in both the period of the year in which pregnancies occur, and their spacing, in relation to annual and seasonal climatic cycles, as well as the degree of synchrony of reproductive events within the population (Jerrett, 1979; Tuttle and Stevenson, 1982). Overall, the majority of bats studied to date, including all temperate zone species, are monoestrous, and thus have a single birth per year (Oxberry, 1979; Jerrett, 1979). However, a number of species of both micro- and megachiropterans are polyoestrous (reviewed by Jerrett, 1979). Indeed, it is suggested that polyoestry represents the ancestral form, from which monoestry arose in more seasonal zones (Bernard and Cumming, 1997). Recent studies suggest that much of the variation in both timing and in patterns of reproduction may be determined by the degree of climatic seasonality, and that the reproductive pattern of individual species varies across their geographic range (Happold and Happold, 1990a).

9.4.1 Temperate Zone Species

Temperate zone species (particularly vespertilionids and rhinolophids) are characterized by seasonal monoestry (Racey, 1982; Figure 9.4). Such species are subject to a summer season of insect availability, and an extensive period of winter torpor. Most reproductive activity, including gametogenesis, gestation and lactation, are compressed into the eight months of summer and autumn, and only a single breeding season is achieved each year (Tuttle and Stevenson, 1982). Gestation is initiated on arousal from hibernation in spring, and continues through early summer, with parturition occurring in midsummer. Lactation in mid and late summer is followed by folliculogenesis and fat accumulation, when body condition is improved prior to hibernation. Males undergo spermatogenesis during the summer months, and the testes regress fully in autumn as spermatozoa are released into the epididymides. Mating is initiated in autumn, and in many species also continues throughout winter (e.g. *Myotis lucifugus*, Thomas *et al.*, 1979; Racey

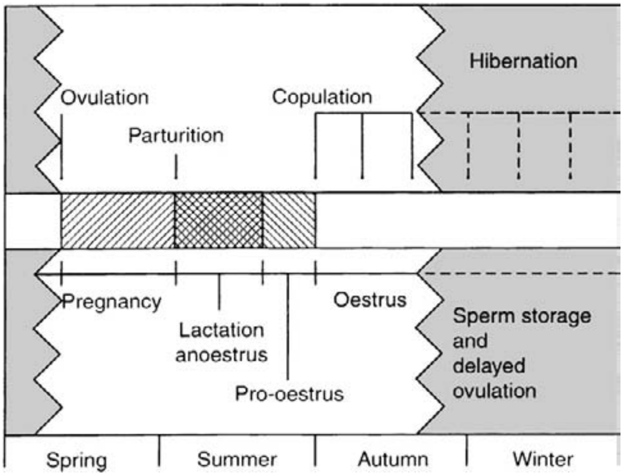

Figure 9.4 Female reproductive pattern in most hibernating bats (from Oxberry, 1979).

et al., 1987; Mendonca and Hopkins, 1997) using spermatozoa stored in the caudae epididymides, supported by continued low-level androgenesis which maintains accessory gland activity throughout hibernation (Racey and Tam, 1974; Racey, 1974b).

9.4.2 Tropical Species

Away from the extremely seasonal temperate regions, where insect food supply is limited for a large part of the year, a much wider range of reproductive patterns have been described. Although three main patterns were identified by Jerrett (1979) – seasonal monoestry, seasonal polyoestry and aseasonal polyoestry – this classification has been extended to describe 10 different patterns of reproduction observed among African bat species (Happold and Happold, 1990a; Figure 9.5).

1. *Restricted seasonal monoestry*. Females have a single litter in close synchrony during one climatic season.
2. *Extended seasonal monoestry*. Females have a single litter during one climatic season, but there is less synchrony of births.
3. *Aseasonal monoestry*. Females have a single litter but not in a particular season nor in synchrony with other individuals in the population.
4. *Seasonal bimodal polyoestry with postpartum oestrus*. Females have two litters per year in close synchrony, with the two birth periods linked to particular climatic seasons. The first birth is closely followed by a postpartum oestrus and thereafter females are simultaneously pregnant and lactating. There is a period of reproductive inactivity following the second birth.
5. *Seasonal bimodal polyoestry without postpartum oestrus*. Females have two litters a year born in synchrony and linked to climatic seasons. Neither birth period is followed by a postpartum oestrus.
6. *Continuous bimodal polyoestry with postpartum oestrus*. Each female has two litters and a gestation of five to six months, with litters born six months apart and each followed by a postpartum oestrus. Periods of parturition need not necessarily coincide with climatic seasons.
7. *Seasonal multimodal polyoestry with postpartum oestrus*. Each female has three or more litters a year, with postpartum oestrus after all but the last birth, which is followed by a period of reproductive inactivity until the start of the next year's breeding season. Females are in synchrony, and most births peak within a single climatic season.
8. *Continuous multimodal polyoestry with postpartum oestrus*. Each female has three or more litters a year with a postpartum oestrus and synchrony between females so that young are born together, but not necessarily related to season.
9. *Continuous multimodal polyoestry without postpartum oestrus*. Each female has three or more litters a year with no postpartum oestrus. There is some reproductive synchrony and birth peaks but births are not limited to a particular season and there is no extended period of reproductive inactivity.
10. *Aseasonal polyoestry*. Each female has two or more litters a year, but females are not in reproductive synchrony and young are not born in a single climatic season but in eight or more months of the year.

Happold and Happold (1990a) analysed the reproductive patterns of 55 bat species from eight families in Africa (Table 9.1). While the Microchiroptera, particularly the Molossidae, showed a wide variety in reproductive patterns, the Megachiroptera appeared more conservative, displaying only four different reproductive patterns. The apparent reproductive conservatism of fruit bats has been attributed to the lower annual fluctuations in fruit abundance relative to insect abundance, resulting in lower selection pressures to develop seasonal cycles (Happold and Happold, 1990a). However, initial reviews of neotropical fruit-eating phyllostomid bats suggest that they show a rather wider range of reproductive patterns compared to pteropodids (see Wilson, 1979). Although such investigations are not conclusive, they suggest that fruiting cycles may not be the only factor

Table 9.1 Reproductive patterns described within different families (adapted from Happold and Happold, 1990a)

Family	Pattern of reproduction	Example	Reference
Pteropodidae	● restricted seasonal monoestry	*Eidolon helvum*	Mutere, 1965
	● extended seasonal monoestry	*Rousettus aegyptiacus*	Smithers, 1983
	● continuous bimodal polyoestry with postpartum oestrus	*Epomophorus anurus*	Okia, 1974
	● aseasonal polyoestry	*Epomophorus wahlbergi*	Happold and Happold, 1990a
Rhinolophidae	● restricted seasonal monoestry	*Rhinolophus clivosus*	Bernard, 1983
	● extended seasonal monoestry	*Rhinolophus blasii*	Happold and Happold, 1990a
Emballonuridae	● seasonal bimodal polyoestry	*Taphozous mauritianus*	O'Shea and Vaughan, 1980
Nycteridae	● restricted seasonal monoestry	*Nycteris thebaica*	Bernard, 1982b
Megadermatidae	● restricted seasonal monoestry	*Lavia frons*	Vaughan and Vaughan, 1986
	● seasonal bimodal polyoestry	*Coleura afra*	McWilliam, 1987c
Hipposideridae	● restricted seasonal monoestry	*Hipposideros caffer*	Bernard and Meester, 1982
	● extended seasonal monoestry		
Vespertilionidae	● restricted seasonal monoestry	*Myotis tricolor*	Bernard, 1982a
	● aseasonal (monoestry?)	*Pipistrellus nanus*	Happold and Happold, 1990b
Molossidae	● restricted seasonal monoestry	*Otomops martiensseni*	Mutere, 1973a
	● seasonal bimodal polyoestry with postpartum oestrus	*Tadarida condylurus*	Happold and Happold, 1989
	● seasonal bimodal polyoestry without postpartum oestrus	*Tadarida condylurus*	Mutere, 1973b
	● continuous multimodal polyoestry with postpartum oestrus	*Tadarida pumila*	Happold and Happold, 1990a
	● continuous multimodal polyoestry without postpartum oestrus	*Tadarida pumila*	Mutere, 1973b
Phyllostomidae	● seasonal monoestry	*Leptonycteris nivalis*	Davis, 1966
	● aseasonal monoestry		
	● seasonal bimodal polyoestry with post-partum oestrus	*Artibeus jamaicensis*	Fleming, 1971
	● seasonal bimodal polyoestry	*Carollia perspicillata*	Heithaus *et al.*, 1975
	● continuous multimodal polyoestry	*Desmondus rotundus*	Wimsatt and Trapido, 1952

limiting the development of diverse reproductive patterns in pteropodids. Such reproductive inflexibility, along with a vegetarian diet, may be a factor constraining the Megachiroptera from exploiting diverse environments, in contrast to such reproductively variable families as the Molossidae (Happold and Happold, 1990a). It is also suggested that flight capabilities and thermal characteristics of the roost, as well as diet, may affect reproductive patterns particularly in the molossids, which at low temperate latitudes are often polyoestrous while other bats are monoestrous (Bernard and Cumming, 1997).

9.4.3 Latitude, Seasonality and Reproductive Patterns

The occurrence of varied reproductive patterns appears to be generally related to major differences in latitude (i.e. temperate v. tropical zones). However a gradual change from polyoestry to monoestry along a gradient of increasing latitude is also apparent among bat species within the African continent (Happold and Happold, 1990a; Vivier and van der Merwe, 1997; Bernard and Cumming, 1997). In the highly seasonal areas of South Africa, most species display restricted seasonal monoestry (e.g. *Nycteris thebaica*, Bernard, 1982b; *Myotis tricolor*, Bernard, 1982a), whereas up to five different reproductive patterns were recorded closer to the equator (Happold and Happold, 1990a). Bernard and Cumming (1997) identify a limit of 13 °N and 15 °S between which most Microchiroptera are either bimodally polyoestrous or aseasonal breeders. At higher latitudes seasonal monoestry, coupled with reproductive delays, is the norm for Microchiroptera, with the exception of the Molossidae (Bernard and Cumming, 1997).

Changes in reproductive patterns with latitude are also obvious within the Molossidae (Table 9.2). Molossids may therefore provide good models to examine reproductive lability (Happold and Happold, 1989, 1990a; Bernard and Tsita, 1995). Similar variations in reproductive patterns with latitude are evident in the genus *Myotis* (Bernard, 1982a). For example, while temperate zone species show a typical pattern of sperm storage and monoestry (e.g. *M. velifer*, Kunz, 1973), at

Table 9.2 Reproductive patterns described for species of Molossidae at different latitudes

Latitude	Species	Reproductive pattern	Reference
0 °S	*Tadarida pumila*	Aseasonal polyoestry	Mutere, 1973b
1–2 °S	*Otomops martiensseni*	Restricted seasonal monoestry	Mutere, 1973a
3 °S	*Molossus ater*	Aseasonal polyoestry	Marques, 1986
9 °S	*Tadarida pumila*	Seasonal multimodal polyoestry	McWilliam, 1987b
3–16 °S	*Neoplatymops mattogrossensis*	Restricted seasonal monoestry	Willig, 1985a
18 °N	*Molossus fortis*	Seasonal bimodal polyoestry	Krutzsch and Crichton, 1985
25 °S	*Tadarida pumila*	Seasonal multimodal polyoestry	van de Merwe *et al.*, 1986
33 °S	*Tadarida aegyptiaca*	Restricted seasonal monoestry	Bernard and Tsita, 1995
36 °S	*Mormopterus planiceps*	Restricted seasonal monoestry and sperm storage	Crichton and Krutzsch, 1987

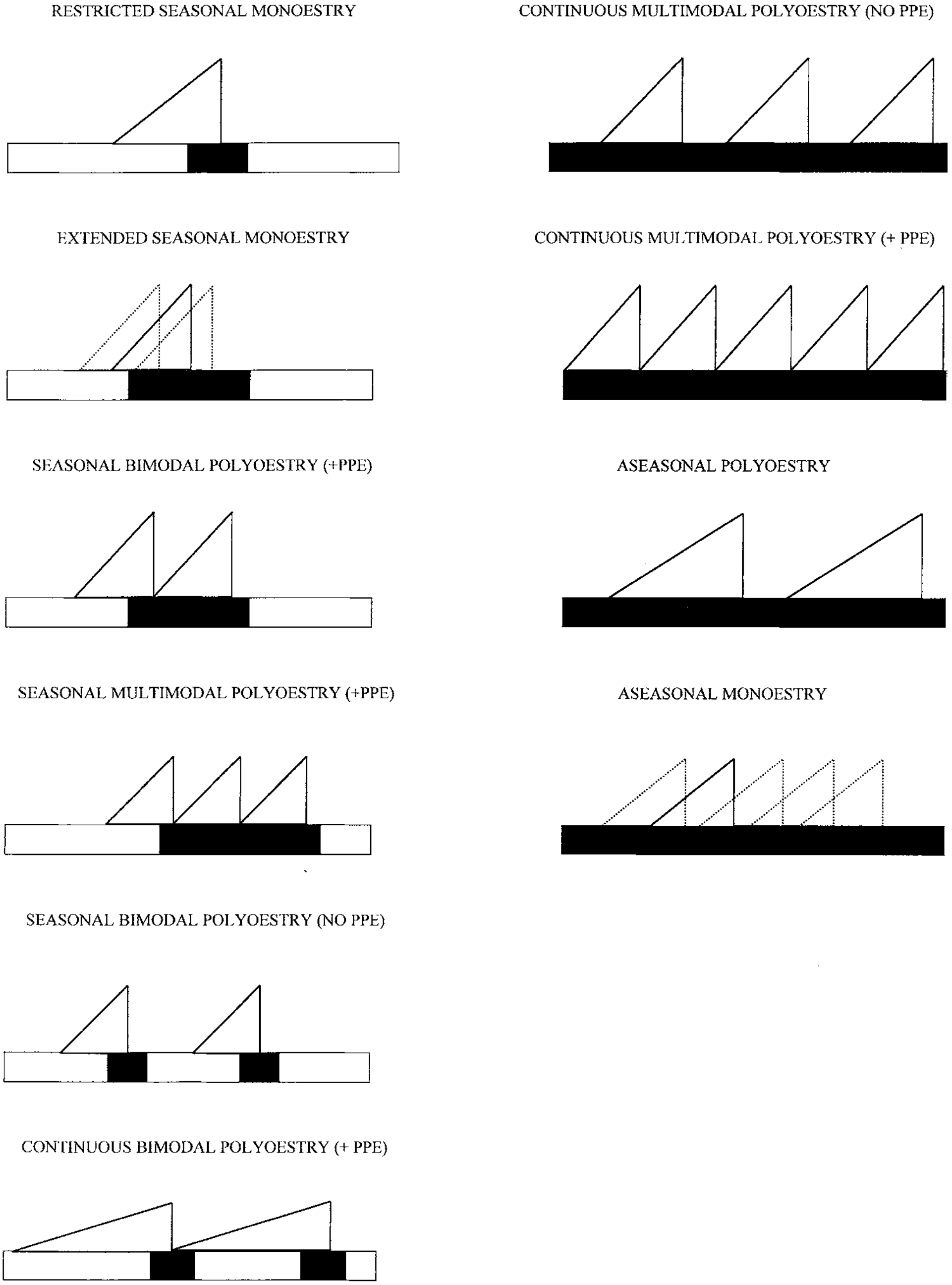

Figure 9.5 Associations of different reproductive patterns, with differing temporal patterns of rainfall. PPE = post-partum oestrus; black bar indicates rainfall. (Diagram after Happold and Happold, 1990a.)

intermediate latitudes, species display intermediate patterns (e.g. *M. austroriparius*, Rice, 1957; *M. albescens*, Myers, 1977) and polyoestry has been recorded in tropical regions (e.g. *M. nigricans*, Wilson and Findley, 1970; Myers, 1977). The underlying factor determining latitudinal variation in reproductive patterns appears to be differences in the degree in seasonality of climate – particularly in rainfall patterns, and corresponding food availability (Figure 9.5).

Variations in reproduction also reflect local differences in patterns of rainfall. The pattern of rainfall, and the severity of intervening dry seasons, in different geographic regions seem to underlie the reproductive patterns found in bats (see Figure 9.5). In many cases there are clear relationships between bimodal births and bimodality of rainfall (*Micropteropus pusillus*, Thomas and Marshall, 1984), monoestry in short unimodal rains (e.g. *Pipistrellus nanus*, Happold and Happold, 1990a, b), and bimodal or multimodal polyoestry with longer periods of unimodal rainfall (e.g. *Epomops buettikoferi*, Kofron and Chapman, 1994). The variability in reproductive patterns reported within regions may result from the ability of individual species to exploit differences in the duration of rainfall, and to become polyoestrous in longer rainy seasons. In particular, a species' response is likely to depend on gestation length and the ability to undergo postpartum oestrus (Bernard and Tsita, 1995). Thus closely related species may display different reproductive patterns in areas of sympatry. For example *Tadarida pumila* is able to complete three pregnancies within the wet season, whereas the longer gestation length in *T. condylurus* precludes more than two births within the same period (Happold and Happold, 1989). General relationships between body size and gestation length (Hayssen and Kunz, 1996; Read and Harvey, 1989) may underlie the tendency for larger bats to have one breeding period, while smaller species have two (Okia, 1987), and may restrict breeding patterns in larger bats, including megachiropterans.

9.4.4 Intraspecific Flexibility in Reproductive Patterns

Lability in reproductive patterns with climate are also clear within species. Intraspecific variation has been reported, not just in the timing of reproduction, but also in the periodicity of reproduction in different environments and across the geographic range of the species (Wilson, 1979; Thomas and Marshall, 1984; Happold and Happold, 1989, 1990a). It is therefore often impossible to characterize a specific pattern of reproduction within species with a wide distribution (Bernard and Cumming, 1997). For example, in *Tadarida condylurus* the interval between consecutive gestation periods appears to vary in response to latitudinal and regional patterns of rainfall (Happold and Happold, 1989), resulting in continued congruence between lactation and peak rainfall in different areas (Figure 9.5). *Tadarida pumila* responds to increasing latitude by adjusting both the number of reproductive cycles and the interval between them, through the use of a postpartum oestrus (Vivier and van der Merwe, 1997). At more seasonal latitudes two breeding cycles of *Tadarida aegyptiaca* can only be accomplished if the second pregnancy follows immediately on the first one, and even then the length of pregnancy may result in sub-optimal timing of the second period of parturition, which may affect survival of young (Vivier and van der Merwe, 1997).

9.4.5 Male Reproductive Patterns

Spermatogenesis in bats, in common with other mammal groups, is highly seasonal at temperate latitudes (Clarke, 1981). The timing of seasonal cycles of male reproduction is not simply a consequence of the timing of female cycles, but also appears to correspond directly to climatic seasonality and food availability (e.g. McWilliam, 1987c). Seasonal variation in spermatogenesis involves profound involution of the seminiferous tubules in autumn (and in some species migration of the testes up the inguinal canal) followed by recrudescence the following spring (Racey and Tam, 1974; Racey, 1974b; Gustafson, 1979; Krutzsch and Crichton, 1987). Although androgenesis and secondary reproductive function is maintained during winter in temperate zone bats (Racey and Tam, 1974; Racey, 1974b), spermatogenesis cannot proceed during periods when the resting metabolic rate (RMR) is persistently depressed and torpor is extended (Kenagy and Trombulak, 1986), leading to a dissociation between spermatogenesis and hibernation (Wimsatt, 1969). One exception is *Rhinopoma kinneari*, in which spermatogenesis has been reported during hibernation, although in this species seasonal torpor is shallow and frequently interrupted (Anand Kumar, 1965). Even during summer, it has been suggested that daily torpor may retard spermatogenesis (e.g. Kurta and Kunz, 1988, Entwistle *et al.*, 1998).

At temperate latitudes, spermatogenesis corresponds with the peak in insect availability during midsummer, and may be affected by body condition (Speakman and Racey, 1986), and foraging opportunities (Entwistle, 1994) or advanced experimentally by simulating the increased temperature and food supply of an early spring (Racey, 1972, 1978). Similar patterns have also been noted in tropical species, where spermatogenesis corresponds to periods of rainfall and high food availability (Jones, 1972; McWilliam, 1987c, 1988a, b) and the number of spermatogenic peaks corresponds with the modality of rainfall (e.g. Mutere, 1973a; McWilliam, 1987c), suggesting that food supply may be a key factor in determining the timing of reproductive cycles in males. Furthermore, even in tropical areas spermatogenesis may be interrupted or suppressed during the coldest or driest months when bats resort to daily torpor (Jolly and Blackshaw, 1987). Nevertheless, the role of photoperiod as a proximate cue of reproduction in male bats cannot be discounted (Heideman, this volume) although clear experimental evidence of its importance is forthcoming in only one species, *Antrozous pallidus* (Beasley and Zucker, 1984).

In temperate zones, spermatogenesis is completed during late summer and there is a close correspondence between reproductive cycles of males and females (Krutzsch, 1979; Bernard and Cumming, 1997), which show similar flexibility and geographic variation (Happold and Happold, 1989; Vivier and van der Merwe, 1996; Bernard and Cumming, 1997). Where females are monoestrous, males show a single, limited peak in spermatogenesis (e.g. *Pipistrellus nanus* at 2 °S, O'Shea, 1980). However in one monoestrous species (i.e. *Taphozous hildegardeae*, McWilliam, 1988a) a secondary nonfunctional spermatogenetic cycle may represent a relict characteristic from an ancestral polyoestry. Two peaks of androgenesis are inferred in *Taphozous hildegardeae* and McWilliam (1988a) has suggested that the second peak of androgenesis is a vestige from a previous bimodal reproductive pattern, and has been retained as a consequence of year-round harem maintenance in this species. Where bimodal polyoestry occurs, males typically display two restricted peaks in spermatogenesis (e.g. *Tadarida condylurus*, Vivier and van der Merwe, 1996). Where females show multi-modal polyoestry or aseasonal reproduction, males show an extended period of spermatogenesis (e.g. *Myotis albescens*, Myers 1977), or may be fertile throughout the year (e.g. *Myotis nigricans*, Myers 1977; *Haplonycteris fischeri*, Heideman, 1989). Such patterns may reflect aseasonal breeding patterns of females (e.g. *Epomophorus wahlbergi*, Sowler, 1983), or social factors (Vivier and van der Merwe, 1996), such as the need for harem defence (e.g. *Myotis bocagei*, Brosset, 1976) or year-round territoriality (*Epomops buettikoferi*, Thomas and Marshall, 1984).

Compared with other mammals of comparable size, spermatogenesis in temperate zone bats is prolonged. Three to four months elapse between the first signs of growth of the seminiferous tubules in late spring and release of spermatozoa from the tubules in late summer and the subsequent filling of the epididymis with mature spermatozoa (Racey, 1974b; Racey and Tam, 1974). In contrast, the duration of spermatogenesis varies from 35 to 74 days in a range of mammals from laboratory mice to man respectively (Monesi, 1972).

The reasons for the extended duration of spermatogenesis in bats may be the energy costs involved in growth of the testes and accessory glands. Bat testes increase in mass during spermatogenesis by up to forty times (Racey 1974b, Racey and Tam, 1974), and at maturity testes and accessory glands may achieve a joint mass of the same order of magnitude as term foetuses which also grow slowly (Racey, 1973a). Whatever the reason, the duration of spermatogenesis must be a major factor in the evolution of reproductive delays in temperate zone bats. Wimsatt (1969) showed that several species of non-volant hibernators were able to complete male and female reproductive cycles in the same year only because males produced spermatozoa much earlier than bats.

9.5 REPRODUCTIVE DELAYS

A number of unusual reproductive cycles are represented among the Chiroptera, and these often incorporate some form of reproductive delay. Reproductive delays interrupt the reproductive cycle, and thereby extend the period between mating and parturition, either by increasing the length of gestation or by delaying conception. Three mechanisms of obligate reproductive delay have been described in the Chiroptera: delayed fertilization combined with sperm storage, delayed implantation and delayed development (Oxberry, 1979; Racey, 1982). In addition, environmentally mediated facultative delays occur during gestation in temperate zone and subtropical species (Racey, 1969, 1973a; Racey and Swift, 1981; Bernard, 1994). However, reproductive delays are not confined to temperate zone species, and occur in tropical vespertilionids, as well as in families with a predominantly tropical distribution.

9.5.1 Delayed Fertilization

Delayed fertilization is the most common reproductive delay mechanism described in bats (Oxberry, 1979; Racey, 1979). Delayed fertilization and sperm storage occur in almost all hibernating Rhinolophidae and Vespertilionidae, with the exception of the genus *Miniopterus* (e.g. *Rhinolophus ferrumequinum*, Matthews, 1937; *Nyctalus noctula*, Racey, 1974b; *Pipistrellus hesperus*, Krutzsch, 1975; *Chalinolobus gouldii*, Kitchener, 1975; *Eptesicus vulturnus*, Tidemann, 1993; *Myotis tricolor*, Bernard, 1982a). These species display a clear disassociation between male and female gamete production. Spermatogenesis occurs during summer, and (in most examples) by autumn the testes have regressed and the caudae epididymides are full of stored spermatozoa. Mating is initiated in autumn, after young are weaned and following a short period of anoestrus in females. Spermatozoa from these inseminations are stored within the female reproductive tract, but also continue to be stored throughout winter in the caudae epididymides of males (Racey, 1975; Gustafson, 1979). The maintenance of androgenesis and accessory gland function in males enables mating behaviour to continue during winter in some species (e.g. Strelkov, 1962; Racey, 1973b; Thomas *et al.*, 1979; Racey *et al.*, 1987). Ovulation occurs on arousal from hibernation (Wimsatt, 1969), and the egg is fertilized by one of the spermatozoa stored within the female. Thereafter gestation proceeds normally until the young are born mid-summer (Oxberry, 1979). Such a reproductive cycle, characterized by the retention of fertility of spermatozoa after periods of prolonged storage by male and female bats, is unique among mammals (Figure 9.4).

Within females, spermatozoa are stored in specific sites including the oviduct, utero-tubal junction and uterus (Racey, 1979). Stored spermatozoa appear closely packed and the heads show an intimate relationship with the epithelium of the reproductive tract (Crichton, this volume; Racey and Potts, 1970; Racey, 1975; Racey *et al.*, 1975; Racey, 1979; Andreuccetti *et al.*, 1984; Racey *et al.*, 1987). Spermatozoa retain their fertilizing ability for the duration of hibernation (for up to 10 months, depending on the species and environmental conditions; Racey, 1973b). Where a vaginal plug of female origin is produced in autumn, further matings are restricted and sperm stored throughout the winter are responsible for fertilization in spring (e.g. *Pipistrellus pipistrellus* and *Nyctalus noctula*, Racey, 1979). Other species continue to mate throughout winter and spring (e.g. Pearson *et al.*, 1952; Strelkov, 1962; Thomas *et al.*, 1979).

Although most examples of sperm storage are from vespertilionids and rhinolophids, a similar phenomenon has now been recorded in a temperate zone molossid, *Mormopterus planiceps*. In this species spermatozoa are stored within the uteri and oviducts for two months prior to ovulation (Crichton and Krutzsch, 1987) and males also show sperm storage and an associated extension in secondary reproductive function (Krutzsch and Crichton, 1987). Such a pattern is otherwise unique among the molossids studied to date. Nor is sperm storage a uniquely temperate zone phenomenon (Jerrett, 1979). Sperm storage by both sexes has been reported from non-hibernating tropical species, although the periods for sperm storage recorded (one to three months) are considerably less than those reported in hibernating temperate zone species, and the duration of fertilizing ability is consequently reduced (Racey, 1979). A number of tropical species are reported to display sperm storage – these include vespertilionids (including several species of *Pipistrellus*) and one species of pteropodid (*Macroglossus minimus*, Hood and Smith, 1989) (Table 9.3). Furthermore, it is not clear whether sperm storage or multiple matings (with prolonged receptivity) explains the interval of days or weeks between the time of first insemination and the time of fertilization recorded in a number of species (e.g. *Molossus ater* Rasweiler, 1987; *Molossus fortis*, Krutzsch and Crichton, 1985; *Rhinopoma hardwickei*, Karim and Banerjee, 1989). Interestingly, sperm storage by females has not been reported from any tropical rhinolophids or hipposiderids, and instead males and females show greater synchrony in reproductive events. Although sperm storage has been demonstrated unequivocally in the tropical vespertilionids *Tylonycteris pachypus* and *T. robustula* at 3 °N in Malaysia, where females were isolated prior to ovulation (Medway, 1972; Racey *et al.*, 1975), other forms of evidence for sperm storage are not as conclusive, and some reports of sperm storage in other tropical bats may require further validation. For example, an interval between the initiation of mating and ovulation (e.g. *Lasiurus ega*, Myers, 1977) may represent an extended mating season with repeated inseminations. Furthermore, tight packing and

Table 9.3 Tropical species for which sperm storage in females has been described or inferred.

Species	Latitude	Site of storage	Duration (months)	Evidence	Reference
Pipistrellus ceylonicus	21 °N	Oviduct	1	Isolation of females prior to ovulation	Gopalakrishna and Madhavan, 1971; Racey, 1979
Tylonycteris pachypus	3 °N	Oviduct	<1	Isolation of females; sperm-epithelium relationship	Medway, 1972; Racey *et al.*, 1975
Tylonycteris robustula	3 °N	Oviduct	<1	Isolation of females; sperm-epithelium relationship	Medway, 1972; Racey *et al.*, 1975
Lasiurus ega	22–25 °S	–	–	Interval between first mating and ovulation	Myers, 1977
Eptesicus furinalis	22–25 °S	–	–	Interval between first mating and ovulation	Myers, 1977
Myotis albescens	22–24 °S	–	–	Interval between first mating and ovulation	Myers, 1977
Scotophilus heathi	21 °N and 25 °N	Utero-tubal junction	ca.2	Interval between first mating and ovulation; orientation/contact of spermatozoa	Krishna and Dominic, 1978; Gopalakrishna and Madhavan, 1978
Pipistrellus mimus	25 °N	–	–	Interval between first mating and ovulation	Krishna, 1985
Macroglossus minimus	8 ° S	Uterus	–	Orientation/contact of spermatozoa	Hood and Smith, 1989
Pipistrellus nanus	15 °S	Utero-tubal junction	<2.5	Orientation of sperm; interval between first mating and ovulation	Bernard *et al.*, 1997

orientation of spermatozoa towards the secretory epthelium in the uterus (e.g. *Macroglossus minimus*, Hood and Smith, 1989) is not in itself evidence for sperm storage, but may result from the movements of spermatozoa within a confined space since similar alignments of spermatozoa are found in species which do not store sperm for prolonged periods (Racey, 1975).

In several cases sperm storage by tropical bats has been reported from regions which display a degree of climatic seasonality, and from species capable of heterothermy (e.g. *Tylonycteris pachypus*, Medway, 1973; Racey *et al.*, 1975). It is possible that such reproductive delays may be correlated with periods of daily torpor during cooler dry seasons when food availability is reduced. For example, male *Pipistrellus nanus* at 15 °S store sperm for six weeks in the epididymis while remaining torpid during the day in the cool season, and then, after mating, sperm is stored by females for a further 2.5 months prior to ovulation at the start of hot dry season (Bernard *et al.*, 1997). At 24 °S, in South Africa the typical temperate zone pattern of sperm storage is evident in *Pipistrellus rusticus* (van der Merwe and Rautenbach, 1990). In this respect, a possible gradation in the use of sperm storage between tropical and temperate latitudes may be evident in response to environmental conditions, and the degree of dysynchrony in gametogenesis by the two sexes. This may be reflected in inferred differences in the duration of sperm storage with latitude (e.g. *Myotis myotis*, Ibanez, 1997). Similar differences and lability in the timing of ovulation have been reported from different latitudes for *Myotis austroriparius*, with ovulation being delayed until spring in northerly populations (Rice, 1957).

An alternative pattern of sperm storage is reported in sub-tropical latitudes, where spermatogenesis is completed by autumn, but mating is postponed until spring, leaving the burden of sperm storage to fall on the males (e.g. *R. capensis* at 33 °S, Bernard, 1985, 1988; *Taphozous georgianus* at 24 °S, Jolly and Blackshaw, 1987; *Rhinolophus megaphyllus* at 27–32 °S, Krutzsch *et al.*, 1992), although sperm storage by females and delayed fertilization are evident in some congeners at the same latitudes (e.g. *Rhinolophus clivosus* at 30 °S, Bernard, 1983).

9.5.2 Delayed Implantation

Although delayed implantation occurs in a range of mammalian taxa and is particularly common in carnivores (Clarke, 1981; Sandell, 1990) it is relatively rare in bats, but has been recorded in several species from both temperate and tropical regions (Table 9.4). In *Miniopterus schreibersii*, the most commonly studied species displaying delayed implantation, the reproductive cycle is in many ways similar to other temperate zone vespertilionids (Oxberry, 1979; Figure 9.6). Males undergo spermatogenesis in summer, and mating takes place in autumn once the young have been weaned. Males may continue to store sperm, although accessory glands regress, and the fertilizing ability of spermatozoa declines. However, females ovulate in autumn and the egg is fertilized immediately. Development of the blastocyst then proceeds but is retarded before implantation. The blastocyst remains in diapause during hibernation and only implants once the bat arouses in spring. Development then proceeds normally and young are born midsummer. However, the extent to which delayed implantation is an obligate part of the

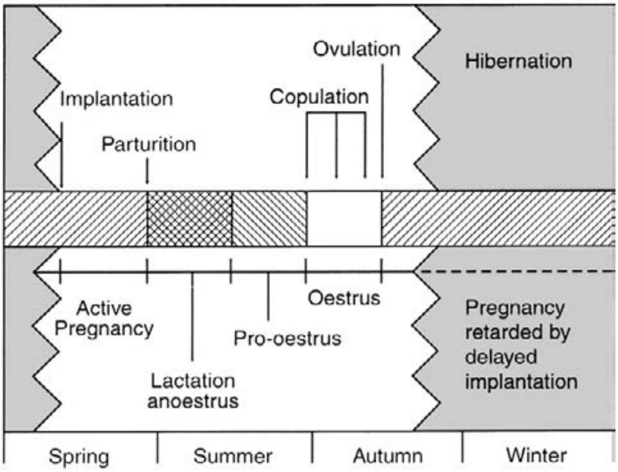

Figure 9.6 Female reproductive pattern in hibernating bats showing delayed implantation (from Oxberry, 1979).

reproductive cycle, under neuroendocrine control, rather than being a passive response to environmental conditions (Peyre and Herlant, 1963, 1967), remains to be established by experiments in which those conditions are manipulated. The only evidence of neuroendocrine control of delayed implantation is that of Bernard and Bojarski (1994) whose treatment of *M. schreibersii* with exogenous prolactin suggested that this was the most important pituitary hormone involved in implantation.

Delayed implantation is not solely a temperate zone phenomenon, but is maintained in *M. schreibersii* in the sub-tropics (Bernard *et al.*, 1996), and has been reported in other miniopterine bats in both temperate and tropical regions (*M. australis* at 28 °S, Richardson, 1977; *M. minor* at 4 °S, McWilliam, 1988b). Pre-implantation delays have also been inferred in other tropical non-miniopterine vespertilionids including *Scotophilus heathi* (Krishna and Dominic, 1978) and *Rhinolophus rouxi*, where blastocysts appeared to remain unimplanted for between 40 and 45 days (Ramakrishna and Rao, 1977). Pre-implantation delay in another non-hibernating species *Scotophilus borbonicus* differs in that development is retarded (but not arrested) for three months during winter at 22 °S (van de Merwe *et al.*, 1988). Delayed implantation has also been reported from tropical lineages including the Pteropodidae (*Eidolon helvum,* Mutere, 1965; *Cynopterus brachyotis* and *C. minutus*, Kofron, 1997) and Phyllostomidae (*Artibeus jamaicensis*, Fleming, 1971). However, in *Artibeus jamaicensis* delayed implantation appears to be facultative, and only occurs during the second of the two annual pregnancies, resulting in the separation of the two births and the correspondence of both peaks of lactation with peaks in rainfall (Fleming, 1971). It is not entirely clear whether this delay occurs pre- or post-implantation, but it appears to be a lactational delay (Sadleir, 1969).

Table 9.4 Pre- and post-implantation developmental delays described in bats

Species	Type of delay	Reference
Macrotus californicus	Delayed development	Bradshaw, 1961
Artibeus jamaicensis	Delayed development	Fleming, 1971; J.J. Rasweiler (pers. comm.)
Natalus stramineus	Delayed development (implicated)	Mitchell, 1965
Carollia perspicillata	Delayed development	Rasweiler and Badwaik, 1997
Hipposideros caffer	Development delayed	Bernard and Meester, 1982; Bernard, 1985
Rhinolophus rouxi	Pre-implantation delay	Ramakrishna and Rao, 1977
Taphozous longimanus	Facultative delay	Krishna and Dominic, 1982
Cynopterus sphinx	Pre/post-implantation	Krishna and Dominic, 1983
Haplonycteris fischeri	Delayed development	Heideman, 1989
Cynopterus brachyotis and *C. minutus*	Pre-implantation delay	Kofron, 1997
Scotophilus borbonicus	Retarded development	van der Merwe *et al.*, 1988
Eidolon helvum	Delayed development*	Mutere, 1965, 1967; J.J. Rasweiler (pers. comm.)
Miniopterus schreibersii	Pre-implantation and facultative delay	Crichton *et al.*, 1989
M. minor	Pre-implantation delay	McWilliam, 1988b
M. australis	Pre-implantation delay	Richardson, 1977
Otopteropus cartilagonodus	Delayed development (implicated)	Heideman *et al.*, 1993
Ptenochirus jagori	Facultative delayed development in primigravida	Heideman and Powell, 1998

* The figure in Mutere (1967) shows a large and well-developed trophoblast characteristic of a post-implantation embryo. Shrinkage during fixation had caused the yolk sac to separate from Reichert's membrane and so the blastocyst appears to be free floating.

9.5.3 Variation in Delayed Implantation in *Miniopterus schreibersii*

A number of authors have noted the apparent plasticity in timing of reproductive events in *M. schreibersii* (Dwyer, 1963; Richardson, 1977; Happold and Happold, 1990a; Bernard *et al.*, 1996). Variations have been recorded in the timing of reproductive events, the duration of pre-implantation delays, and in some cases in the presence of a subsequent post-implantation delay (Table 9.5). Within Africa, a short increase in the duration of delayed implantation with increasing latitude has been described (Bernard *et al.*, 1996).

A difference is apparent in the total period of post implantation embryonic growth (gestation period minus total delay) between temperate zone populations in northern and southern hemispheres (Table 9.5), suggesting that these populations may have diverged. In addition, a secondary post-implantation developmental delay has been reported from Japan at 32°N (Kimura and Uchida, 1983) and from Australia at 37°S (Crichton *et al.*, 1989). In a Japanese population of *M. schreibersii*, delayed implantation lasting 60 days was followed by delayed development lasting two months (Kimura and Uchida, 1983). Experiments demonstrated that the duration of post-implantation delay was reduced under conditions of elevated temperatures and abundant food, by the same period as their

Table 9.5 Duration, in months, of delayed implantation and delayed development in *Miniopterus schreibersii* at different latitudes

Latitude	Country	Delayed implantation	Delayed development	Total delay	Gestation length	Period of foetal growth	Reference
45°N	France	5	–	5	10	5	Peyre and Herlant, 1967
32°N	Japan	2	3	5	8.5	3.5	Kimura and Uchida, 1983
17°N	India	–		0	4	4	Gopalakrishna *et al.*, 1986
11°S	Africa	3		3	6–7	3–4	Anciaux de Faveaux, 1977
18°S	Africa	2–3		2–3	6–7	4	Bernard *et al.*, 1996
26°S	Africa	4		4	8	4	van der Merwe, 1986
28°S	Australia	2.5–3	?	2.5–3	7	4–4.5	Richardson, 1977
30°S	Africa	4		4	7.5–8	3.5–4	Bernard, 1980
33°S	Africa	3–3.5		3–3.5	7	3.5–4	Bernard, 1994
37°S	Australia	3	1.5	4.5	7	2.5	Crichton *et al.*, 1989

application (Uchida *et al.*, 1984). The period of post-implantation delayed development in *M. schreibersii* therefore appears to be a facultative response to low temperature and food availability, and is analogous to the climatic delays of gestation reported in other vespertilionids (Racey, 1982). Year-to-year variations in gestation length, corresponding to rainfall, have also been reported from South Africa (Bernard, 1994). Reproductive flexibility with latitude, as displayed by *Miniopterus schreibersii*, has been proposed as one reason to explain the extensive geographic range and apparent success of this species (Dwyer, 1963; Bernard *et al.*, 1996).

9.5.4 Delayed Development

Delayed development is the least common form of reproductive delay found in bats, and has not been described in other mammalian groups (Crichton *et al.*, 1990) (Table 9.4). This phenomenon was first described from the semi-hibernate *Macrotus californicus* in which autumn mating is followed immediately by ovulation (Bradshaw, 1961, 1962). The blastocyst then implants superficially, grows only to the primitive streak stage (Bradshaw, 1961, 1962; Bleier, 1975), and enters a period of retarded growth. This delayed early embryogenesis lasts for 4.5 months, and is followed by a 3.5-month period of embryogenesis at a normal rate (Crichton *et al.*, 1990). These delays are not a facultative response to low temperatures or food shortage but are an obligate part of development in this species (Burns *et al.*, 1972, 1981).

Similar delays also occur in other non-hibernating species such as *Hipposideros caffer*, which does however undergo short periods of torpor (Bernard and Meester, 1982). Developmental delay has also been described in the megachiropteran species, *Haplonycteris fischeri* (Heideman, 1989). As a result of eight months of retarded embryonic growth, followed by a period of 3.5 months of normal growth, this species displays a total gestation length of 11.5 months – the longest described in bats. Inter-individual variation in the length of delay was apparent, and it is suggested that a normal rate of embryonic growth is initiated by some common environmental cue (Heideman, 1989).

9.5.5 Facultative Delays

In temperate zones, particularly at higher latitudes, bats become torpid, i.e. reversibly lower their metabolic rate throughout the summer breeding season (Eisentraut, 1937; Pearson *et al.*, 1952; Saint Girons *et al.*, 1969; Ransome, 1973), apparently in response to cool conditions and the associated decrease in food availability (Kurta, 1986). Since torpor has a non-specific action (Wilde *et al.*, 1999) all metabolic-dependent processes are affected, including foetal growth, and pregnancy is thus extended at low temperatures (Racey, 1969). A clear association has been demonstrated experimentally between the duration of torpor in the absence of food, and the extension of pregnancy by the same period (Racey, 1973a). Even when food is available and consumed, sustained low temperatures (ca. 5 °C) may still delay birth (Racey, 1973a). Such experiments indicate that these developmental delays are environmentally mediated, rather than obligate and under neuroendocrine control. Such facultative responses to climate enable bats to vary their energy expenditure in relation to a variable environment, and so reduce the risks of starvation during temporary deteriorations in climatic conditions.

Observations from field studies demonstrate such facultative delays in natural populations. Variations in gestation length directly parallel differences in temperature and rainfall between subsequent years (*Pipistrellus pipistrellus*, Racey and Swift, 1981; *Eptesicus nilssoni*, Rydell, 1989; *Antrozous pallidus*, Lewis, 1993). In addition, similar patterns have been found in *Eptesicus fuscus* where prolonged periods of torpor correspond with extensions of gestation length (Audet and Fenton, 1988). Similar environmentally mediated delays are reported in *Miniopterus schreibersii* (Kimura and Uchida, 1983; Bernard, 1994), and may also occur in tropical species – for example, gestation length in *Taphozous longimanus* is reportedly longer in the cooler season (Krishna and Dominic, 1982).

A facultative post-implantation delay in embryonic development of up to five months occurs in *Ptenochirus jagori* reproducing for the first time (Heideman and Powell, 1998). As a result young females give birth once in their first year, whereas adult females, which showed no evidence of such a delay, gave birth twice a year.

9.6 ADAPTIVE SIGNIFICANCE OF REPRODUCTIVE DELAYS

All bat species of temperate zones, and a number in the tropics, display some form of delay in their reproductive cycle. Although the low metabolic activity during hibernation may be an important mechanism underlying the extension of reproductive delays, it is insufficient to explain the evolution and maintenance of reproductive delay across bat species. The adaptive significance of reproductive delays needs to be considered, as does the occurrence of delays in tropical species. A number of suggestions for the adaptive significance of reproductive delays are discussed below.

9.6.1 Heterothermy and Reproductive Delays

The first studies of reproductive delay were carried out on temperate zone species which store sperm over winter. Such studies suggested there was a direct functional relationship between such delays and torpor (e.g. Wimsatt, 1960, 1969). Low metabolic rates during seasonal torpor were considered to be incompatible with the continuation of reproductive processes in females, thus interrupting the reproductive cycle for the duration of hibernation, either pre- or post-ovulation (Wimsatt, 1960, 1969). Torpor was also considered to be important for sperm storage, reducing loss or degradation of spermatozoa (Racey, 1973b), and inhibiting ovulation (Wimsatt, 1969), and hence limiting the geographical distribution of the bat species concerned (McNab, 1974). However, the presence of sperm storage in tropical species and lineages of bats, and other non-hibernating mammals, suggests that torpor is not an absolute prerequisite for this phenomenon. Furthermore, a period of hibernation itself does not necessitate the reproductive delays observed, since other hibernating animals complete a reproductive cycle during the summer months (e.g. Wimsatt, 1969).

However, hibernation does clearly affect reproductive delays. The persistence of fertilizing ability of spermatozoa is much lower in tropical species which store sperm (Racey, 1979), and the duration of storage often corresponds to latitudinal variation in the duration of hibernation. Indeed, even in tropical species there may be a correspondence between sperm storage and periods when daily torpor occurs (e.g. Bernard *et al.*, 1997), and the extent of winter hibernation has been proposed as a factor underlying the evolution of different forms of reproductive delay in bats (Bernard, 1989). Bernard (1989) suggests that within southern Africa, in those species undergoing early onset of torpor in autumn and subsequent deep hibernation in winter, ovulation is inhibited until spring, requiring sperm storage during this period (as in most temperate zone vespertilionids and rhinolophids). In contrast, in species which display a shallow and frequently interrupted period of hibernation, ovulation is less likely to be inhibited, and instead delays occur during embryonic growth (either pre- or post-implantation, e.g. *Miniopterus schreibersii*, Bernard *et al.*, 1996) or mating may be postponed, and spermatozoa stored by males (e.g. *Rhinolophus capensis*, Bernard, 1985).

9.6.2 Seasonal Constraints on Reproduction

In most mammals, mating, ovulation and gestation follow closely without interruption (Bronson, 1985). Similar patterns may occur in most tropical and subtropical bat species, whereas in most temperate zone bats autumnal mating is separated from summer gestation. It has been argued that reproductive delays result from the inability of bats in temperate zones to complete all reproductive activities (from mating to weaning) within the short period of food availability of summer (Bernard, 1989). The slow foetal growth rates of bats, and the consequent length of gestation, limits the time available during summer to complete lactation, weaning and recovery of maternal body condition after lactation. Early initiation of gestation may therefore be essential to ensure that lactation coincides with the peak in insect availability, and that weaning is completed and mothers and young have time to accumulate fat prior to hibernation. Furthermore, early birth dates for young have been positively correlated with their future survival (Ransome, 1989). Mating in spring would delay the initiation of gestation, which would compromise the optimal timing of lactation, and the completion of reproduction and fat accumulation before autumn. Furthermore, the poor body condition of bats emerging from hibernation in spring, coupled with low food availability and high potential competition at this time of year, may be incompatible with the formation of mating assemblages at this time. Instead, mating in autumn ensures that ovulation and fertilization can occur immediately upon arousal from hibernation in spring, and that gestation is initiated without further delay. In some species, the mating season is extended through hibernation and into spring (Strelkov, 1962). These later matings may top up existing reservoirs of stored sperm, but may also ensure that all females are inseminated, particularly in well-dispersed species. In such highly dispersed populations, females thus rely on an extended mating season and sperm storage to guarantee fertilization, since induced ovulation has not been reported in bats.

At sub-tropical latitudes an intermediate pattern has been described, with storage of sperm by males over winter and mating delayed until spring (*Rhinolophus capensis*, Bernard, 1985; *Taphozous georgianus*, Jolly and Blackshaw, 1987) thus releasing males from the constraint of completing an entire reproductive cycle (from initiation of spermatogenesis to copulation) that would be necessary during a short temperate summer (Bernard, 1989). Seasonality in tropical regions may similarly favour a separation of mating and gestation, where favourable seasons are short and gestation must be initiated quickly to ensure optimal timing of reproduction. As in temperate zones, early mating may allow an extended mating season, and thus increases the chances that all females in a dispersed population are inseminated.

9.6.3 Timing of Male and Female Cycles

Seasonality in food resources appears to determine the optimal season for gametogenesis in both males and females. However, different selection pressures on the two sexes may result in a dysynchrony in optimal reproductive timings. In females, lactation is generally timed to coincide with the peak in insect availability during summer (Racey, 1982). In males, spermatogenesis also occurs during periods of food availability (McWilliam, 1988a), and is generally restricted to summer months in temperate zone species, and during the rains in tropical species.

The need for males to undergo gametogenesis during the season of optimal climate or food availability may be a factor driving the disassociation between gametogenesis and subsequent reproductive events both within and between sexes

(Jolly and Blackshaw, 1987; Bernard and Cumming, 1997). Thus spermatozoa are produced only during periods of food availability, and must be stored until they can fertilize ova that will result in young produced early in the following season. All mammals have the ability to store spermatozoa in their caudae epididymides for varying periods of time (Hamilton, 1977), and storing sperm for relatively short periods may have provided pre-adaptations from which more prolonged storage, including storage by females, could evolve. Bernard and Cumming (1997) thus suggested that the evolution of prolonged sperm storage by bats did not involve the appearance of a new phenomenon but rather the perfection of an existing capability.

9.6.4 Mate Selection

It has been suggested that reproductive delays may evolve when the optima for the timing of both parturition and mating differ (Sandell, 1990; Birkhead and Møller, 1993). The need for bats to undergo lactation during the summer peak in food availability is clear. It has been suggested that females can increase their fitness by selecting high quality mates (Bateson, 1983; Partridge and Halliday, 1984), and if this is the case then mating should take place at a time when possibilities for female choice or mate competition are greatest. It has been argued that the time of high food availability would also be the best time for mating, since it enables females to assess males while they are in good body condition, and differences in their relative quality are clear (Sandell, 1990). Thus mating following fat accumulation in autumn would facilitate mate selection in a way that would not be possible in spring, when low body condition would make it less easy for females to discriminate between good and poor quality males (Sandell, 1990). However, this explanation would not satisfactorily explain all occurrences of developmental delay in bats. In those species which exhibit sperm storage without any vaginal plug of either male or female origin and thus continue to mate beyond autumn (Racey, 1975), the occurrence of hibernal rape (Thomas *et al.*, 1979) and spring matings (Strelkov, 1962; Philips and Inwards, 1985) would counter any advantage of mate selection the previous autumn. Nor does it explain the apparent lack of mate selectivity in some species (Wai-Ping and Fenton, 1988), or the absence of reproductive delays in those species with the most obvious sexually selected traits, such as the epaulettes of *Epomophorus wahlbergi*, and highly competitive mating behaviour (Bernard and Cumming, 1997).

9.6.5 Sperm Competition

Bats which store sperm over winter fulfil all the prerequisites for sperm competition (Fenton, 1984; Hosken, 1997). Multiple matings increase the chances of females receiving viable spermatozoa, and sperm competition could act as an indirect mechanism for mate choice (Birkhead and Møller, 1993) since 'higher quality' spermatozoa may be more likely to fertilize the ova (Madsen *et al.*, 1992). There is no evidence that sperm competition actually takes place in bats, or whether instead sperm from the last mating are more likely to achieve fertilization (last in, first out hypothesis; Birkhead, 1988). The adaptive significance of sperm storage relative to reproductive delays observed in bats is unclear, although an identified relationship between testis mass and group size might imply higher risks of sperm competition in larger groups (Hosken, 1997). In addition, genetic analyses have described multiple paternity (Mayer, 1995) and skewed individual fertilization success in males (Watt and Fenton, 1995), which may indicate that sperm competition does occur.

9.6.6 Synchrony

In bats, reproductive delays may provide the basis for birth synchrony, when a common environmental cue stimulates the overwintering follice to ovulate (Racey, 1979, 1982). For example, synchronous implantation in *Miniopterus schreibersii* resulted in synchronous births (Bernard, 1994). Reproductive synchrony may provide advantages for survival of young by diluting predation risk (Ims, 1990a,b), and through reducing thermoregulatory costs by formation of larger clusters (Rutberg, 1984; Rachlow and Bowyer, 1991; Bunnell, 1982). Other studies suggest that synchrony may be an outcome of common reproductive timing in relation to food availability, although it may be further tightened by predation risk (Rutberg, 1987). Reproductive synchrony may be achieved through synchronized oestrus and mating (Berger, 1992), and will occur in the absence of reproductive delays in social animals. Although many bat species do show a degree of reproductive synchrony (Tuttle and Stevenson, 1982), reproductive delays in bats do not necessarily result in synchrony (e.g. *Mormopterus planiceps*, Crichton and Krutzsch, 1987). It is suggested that synchrony may be more common at the lower temperatures associated with higher latitudes (Bunnell, 1982). In bats, it has been suggested that species experiencing cooler temperatures show more highly restricted birth periods (e.g. Tuttle and Stevenson, 1982; Philips and Inwards, 1985). Although reproductive synchrony has been invoked as a possible selection pressure for the evolution of reproductive delays (Racey, 1979), in general synchrony would only be advantageous within colonial species (Bernard and Cumming, 1997). Synchrony may instead be an outcome of the seasonal constraints and common cues which optimize the timing of reproduction in bats.

9.6.7 The Evolution of Reproductive Delays

Bats are thought to have evolved in the tropics (Koopman, 1970), and reproductive delays occur in both obligate tropical lineages and in hibernating species of temperate latitudes. It has therefore been suggested that reproductive delays are an ancestral characteristic, rather than a secondary characteristic which evolved under temperate conditions, in lineages which then reinvaded the tropics (Bernard and Cumming, 1997). However, in other groups reproductive delays are found mainly in those species occupying the temperate zone (e.g. Rhinolophidae). Even in tropical regions there is some association between the occurrence of sperm storage and of daily torpor during the cool dry season (Racey, 1979; Bernard *et al.*, 1997). Indeed it has been suggested that heterothermy evolved in the tropics, as a response to seasonally fluctuating insect densities (McNab, 1969). If reproductive delay mechanisms and heterothermy are both considered ancestral characteristics, they may have provided bats with reproductive flexibility, and such characteristics may have been fundamental to expansion of bats into sub-tropical and temperate zones. Given the unique life-history strategy and costs of reproduction in a small volant mammal, relying on an unpredictable food source, a reproductive delay may have been an ancestral characteristic that coevolved with flight.

It is possible to identify a number of potential selective advantages that might favour the development of reproductive delays. In a seasonal tropical environment, lactation would be timed to coincide with maximal food availability, and spermatogenesis would also occur during times of relative food abundance coinciding with the rains. If separate peaks in food availability occur, asynchrony may develop in the timing of spermatogenesis and that of ovulation, with spermatozoa stored by males (Bernard and Cumming, 1997). Sperm storage for relatively short periods has been reported in other mammal species (e.g. dog, Doak *et al.*, 1967; hare, Martinet and Raynaud, 1974), and there is nothing intrinsically unlikely about the evolution of sperm storage in female mammals. In bats, where males store sperm for periods prior to ovulation, there may be advantages for females also to adopt this role.

Sperm storage, and other reproductive delays, would enable gestation to begin as soon as food supply improves (Bernard and Cumming, 1997), would act to separate pregnancies (which in turn would extend the lactational delays described by Sadlier, 1969) or could extend the mating season and ensure inseminations in dispersed populations. Alternatively, reproductive delays might have evolved to promote reproductive synchrony when females mated over an extended period (Racey, 1979), or to provide a basis for indirect mate selection through sperm competition, although these explanations are considered unlikely by some authors (see also Bernard and Cumming, 1997).

During the invasion of temperate zones, greater seasonality would have resulted in an interval equivalent to the period of hibernation between the optimal time for spermatogenesis and parturition. Although originally the burden of sperm storage may have fallen predominantly on males, females may have gained an advantage by storing sperm for longer periods, since this ensures that gestation could be initiated as soon as hibernation ended. The lower temperatures and metabolic rate during hibernation may be important factors in facilitating sperm storage for extended periods of time, and in inhibiting ovulation until spring arousal. Continued sperm storage by males may provide advantages of later matings with uninseminated females, and also of topping up stored sperm reservoirs since the most recently inseminated sperm may have a better chance of fertilizing (Birkhead, 1988). In other lineages where hibernation of females is relatively short, ovulation may not be inhibited by shallow and intermittent torpor and the delay may occur at a later stage of pre- or post-implantation development (Bernard, 1989). The importance of phylogeny and differing selection pressures may underlie the occurrence of several different mechanisms for reproductive delay in species with overlapping ranges (Bernard, 1989; Krutzsch *et al.*, 1992). Evolutionary advantages associated with sperm selection do not always explain the evolution of reproductive delays during embryonic development, and it is possible that reproductive delays evolved separately in disparate chiropteran lineages (Bernard and Cumming, 1997).

9.7 WHAT LIMITS REPRODUCTION?

Ultimately, the need for young to reach adult size to allow flight prior to weaning may constrain reproduction. However, there are factors obviously limiting the timing of reproduction with regard to season, and the geographic limits, and habitats available, to breeding populations. What are the proximate limits placed on reproductive outputs in bats? Temporal and spatial associations between food availability and reproduction have led to suggestions that this is a key factor limiting reproduction in bats and hence that climatic and geographic variation in food availability determines the distribution and population size of bats. Since reproduction is energetically expensive, limits to reproduction are frequently attributed to energy limitation. However, other nutritional factors, such as calcium and nitrogen, have also been considered to limit reproduction in bats.

9.7.1 Calcium

Calcium is the most important mineral component of bone. Since young bats are not weaned until they achieve close to adult skeletal dimensions, mothers must provide almost all skeletal requirements of calcium for their young (Barclay, 1994, 1995). Since the diet of many bat species does not appear to be high in calcium (e.g., Keeler and Studier, 1992), this may require the mother to ingest more food than is required for energy alone, or else to adopt a strategy by which calcium can be gained in higher proportions by other means (Barclay, 1994). Thus, calcium rather than energy may dictate the food requirements of bats, and hence limit reproduction. Few extraneous sources of calcium are available to bats. Instead, calcium depletion during reproduction is clear from reported decreases in maternal skeletal mass during this period (Kwiecinski *et al.*, 1987). This suggests that the calcium requirements may limit the number of young that bats can produce. Bernard and Davison (1996) showed that bone calcium was lowest in *Miniopterus schreibersii* during lactation and suggested that calcium availability played a major role in the evolution of the timing of reproduction in this species.

9.7.2 Nitrogen

Nitrogen (or protein) availability may be limited for species eating protein-poor food such as fruit (Thomas, 1984). In such cases bats need to ingest more food than is required to meet energy needs alone, in order to satisfy the demands for protein (Thomas, 1984; Steller, 1986). Strategies such as extraction of juice from fruits without ingestion may increase food passage rate (Thomas, 1984). However, frugivores may supplement their diet with items which are relatively protein rich, such as insects (Courts, 1997) and pollen, and leaves with high nitrogen content (Kunz and Diaz, 1995). Indeed, for one nectarivore, consumption of pollen meets protein requirements sooner than energy requirements can be met by nectar (Law, 1994).

9.7.3 Energy

In mammals, energy costs are elevated during reproduction and are highest during lactation (Gittleman and Thompson, 1988; Thompson, 1992), and a similar pattern of energy requirements is described in bats (Kurta *et al.*, 1989a; Speakman and Racey, 1987). The most commonly cited factor limiting reproduction in bats is energy (e.g. McNab, 1982). Reproduction is energetically expensive, and its costs appear to be relatively higher in smaller animals (Hanwell and Peaker, 1977). Energetic costs of reproduction in bats have been estimated by a number of techniques, both from laboratory studies and from free-ranging animals (Kunz, 1980; Kurta *et al.*, 1987, 1989a, b, 1990; Racey and Speakman, 1987; Speakman and Racey, 1987). All such studies have demonstrated increasing energy requirements throughout reproduction. Although the costs of reproduction remain relatively low during pregnancy, they increase dramatically through lactation when they are estimated to be 50% greater than those in pregnant animals (Kurta *et al.*, 1989a). Costs associated with reproduction include not only the direct costs of production (of the young and of milk supply), but also increased costs of maintenance and of flight during pregnancy, and the needs of neonates for thermoregulation and other forms of maternal care.

Studies have demonstrated that energy may be a limiting factor to both individual body condition and population size, compared to other nutrients including nitrogen (e.g. Law, 1992, 1995). The increased costs of reproduction may place a particular burden on bats. Bats have an energetically expensive mode of locomotion which during lactation accounts for 66% of energy requirements (Kurta *et al.*, 1989a) and their small size incurs proportionately higher thermoregulatory costs. Furthermore, for many temperate zone insectivorous species, the ability to meet these requirements may be limited by a feeding period restricted to the hours of darkness coupled with a temporally and spatially unpredictable food supply, which is directly affected by climatic conditions (Taylor, 1963). A number of strategies appear to have evolved to enable reproduction to proceed in the face of such limitations, and these are discussed further below.

9.7.4 Strategies to Overcome Energetic Limits on Breeding

Mammals have evolved a number of strategies to meet the increased costs of reproduction, particularly those associated with lactation (reviewed by Racey and Speakman, 1987). These fall into three main groups: use of fat reserves, increased intake or reduction in energy expenditure through a reduction in other functions.

9.7.5 Storage of Fat Reserves

Accumulation of fat reserves to support the additional costs of reproduction is common in larger mammal species (Gittleman and Thompson, 1988), but uncommon among smaller species. Fat reserves in small mammals may be limited due to increased maintenance costs (Millar, 1978), and because a relatively large foetal size limits the space available for fat reserves (Kurta *et al.*, 1990). It has been estimated that the typical body fat stored by bats would support the costs of lactation for one (Kurta *et al.*, 1990) to three days (Racey and Speakman, 1987). Although, some stores are obviously used, given that maternal body mass decreases through lactation, fat reserves in bats are insufficient to meet increased energetic demands.

9.7.6 Increased Energy Intake

An obvious way for animals to meet increased energy demands is to increase food intake (e.g. Millar, 1978; Gittleman and Thompson, 1988). This is reflected in changes in foraging behaviour through pregnancy and lactation. Laboratory studies demonstrate an increase in food consumption in bats between pregnancy and lactation (e.g. Kunz, 1974; Anthony and Kunz, 1977). However, the ability of free-living bats to increase their food intake may be limited both by the duration of the night in summer, the need to return to the roost to suckle and keep young warm, and by the associated costs of flight. Flight is the greatest component of the energy budget of bats, even during lactation (Kurta *et al.*, 1989). Hence increasing the duration of foraging increases the costs of locomotion, and net energy gain is proportionately lowered, so bats should only extend foraging when insect availability makes such an extension energetically profitable (Rydell, 1989). Changes in the behaviour of females during reproduction appear to indicate increased energy intake. Differences in diet between males and females (Belwood and Fenton, 1976) and changes in foraging patterns during lactation have been described (e.g. *Pipistrellus pipistrellus*, Racey and Swift, 1985; *Lasiurus cinereus*, Barclay, 1989; *Eptesicus nilssoni*, Rydell, 1993). For example, in *Eptesicus nilssoni* foraging time doubled between pregnancy and lactation (Rydell, 1993). However, others have found no differences in overall foraging duration during this period (Racey and Swift, 1985; Entwistle *et al.*, 1996; Wilkinson and Barclay, 1997). At northern latitudes the short night and lower insect densities may limit the net benefit of extending foraging.

9.7.7 Compensation (Compensated Respiration)

Bats may increase the energy available for the increased costs of reproduction by reducing the energy expenditure on other functions. Such energetic compensation may occur through behavioral changes (e.g. decreased locomotion) or through physiological changes, reducing the energy required for maintenance. Bats appear to reduce their energy budgets during pregnancy and lactation both by reducing the energetic cost of thermoregulation through selection of warm environments when homeothermic, or by forgoing active thermoregulation and entering torpor.

9.7.8. Compensation Through Torpor

Allowing the body temperature to fall and enter a state of torpor, in which metabolic rates are substantially reduced, can greatly reduce daily energy requirements (Kurta, 1986). Bats frequently enter torpor for short periods throughout summer (Racey *et al.*, 1987), often in response to cooler, wetter or windier weather (Audet and Fenton, 1988; Lewis, 1993; Entwistle, 1994). Low temperatures result in both reduced insect availability and increased costs of homeothermy. Bats continue to enter torpor during pregnancy and lactation, although the extent of use appears to vary with the stage of reproduction. Experiments using doubly labelled water indicated that *Plecotus auritus* appeared to use torpor during early and mid pregnancy, and during early lactation, but rarely used torpor in late pregnancy or in late lactation (Speakman and Racey, 1987). However, direct assessments inside roosts showed a decrease in the occurrence of torpor throughout late pregnancy and lactation (Entwistle, 1994). Similar patterns in the use of torpor during pregnancy and lactation have been found in field studies of *Eptesicus fuscus* (Audet and Fenton, 1988), and in laboratory studies of *Myotis lucifugus* (Kurta, 1990).

There may be a substantial cost to the use of torpor during reproduction. Torpor interrupts prenatal growth thus increasing the duration of gestation (Racey, 1973a), and since all metabolic processes are affected by torpor, milk production is likely to be substantially reduced during periods of torpor (Wilde *et al.*, 1995, 1999). Torpor is also likely to directly affect young, which although they have functional brown adipose tissue (Trayhurn *et al.*, 1991) appear to be incapable of thermoregulation early in life (Noll, 1979; Kunz, 1987; Kurta and Kunz, 1987), and later in development it is likely that periods spent in torpor may slow post-natal growth. Significant differences in growth rate of young in different thermal environments have been demonstrated (Tuttle, 1975). Such delays throughout pregnancy and lactation may result in later weaning dates and smaller young, both of which affect juvenile survival (Ransome, 1989). Furthermore, late weaning is also likely to reduce maternal survival, since the period available to assimilate fat prior to winter hibernation would be reduced. The higher risks of torpor for female reproductive success, appear to be reflected in a minimal use of torpor (e.g. Audet and Fenton, 1988), especially when compared to males (e.g. Hamilton and Barclay, 1994; Grinevitch *et al.*, 1995). Females may instead choose to remain homeothermic, but reduce associated energetic costs by selecting the warmest possible environment.

9.7.9 Selection of Warm Environments

The costs of thermoregulation when bats remain homeothermic can be minimized by reducing the differential between body temperature and ambient conditions (Lyman, 1970). Bats achieve this both through selection of warm roost environments (Kunz, 1982; Entwistle *et al.*, 1997) and through clustering with other individuals, which increases local temperature (McDonald *et al.*, 1990) and decreases energy expenditure (Roverund and Chappell, 1991). It is predicted that breeding females may gain a greater advantage than males from remaining homeothermic, and avoiding torpor. Differences are apparent in some species in the temperature preferenda and use of clustering between males and females during summer (e.g. *Myotis lucifugus*, Fenton, 1970) but not in others (e.g. *Plecotus auritus*, Entwistle *et al.*, 1998). Warmer roost temperatures may also have important consequences for the growth of young, resulting in faster pre- and post-natal growth rates (Tuttle, 1975; Tuttle and Stevenson, 1982; Kunz, 1982; Hoying and Kunz, 1998), and thus may affect survival (Racey, 1982).

The requirement for warm roost sites in which to breed may be an important factor determining the distribution and success of bats (Humphrey, 1975; Bell *et al.*, 1989). The need for predictably warm sites, where other bats are present with which to cluster, may also be an important factor underlying patterns of roost philopatry in many species (Thompson, 1992; Entwistle, 1994; Lewis, 1995), and hence in the development of long-term associations and groups.

9.7.10 Resorption, Abortion and Abandonment of Young

Where food availability is insufficient to support a continued pregnancy, a trade-off is necessary between maternal survival and associated future reproductive potential and the loss of the current offspring (Wasser and Barash, 1983). In such conditions, abortion or abandonment may increase lifetime reproductive output (Clutton-Brock *et al.*, 1989), especially in species with long reproductive lifespans. In bats, abortion or post-implantation resorption of embryos occurs in a number of species (e.g. Wimsatt, 1945; Pearson *et al.*, 1952; Myers, 1977). The occurrence of reproductive failure has been directly linked to climatic conditions and to previous body condition in one species of bat (*Plecotus auritus*, Stebbings, 1976), and facultative resorption or abortion, rather than failure of fertilization, may account for relatively high levels of non-breeding reported in some populations subject to climatic extremes. A higher incidence of non-breeding within colonies of *Antrozous pallidus* was associated with lower spring temperatures (Lewis, 1993). In periods of inclement weather during lactation, abandonment of young sometimes occurs (R.E. Stebbings, personal communication).

9.7.11 Geographical Limits to Reproduction

Food available to bats is likely to vary geographically with regard to latitude, local climatic conditions and local habitat availability. Bats show distinct limits in their distributions, particularly of breeding populations, which may be directly related to climate (Law, 1994). This is reflected in differing latitudinal and regional distributions of species, and in differences in the distributions of males and females (Stutz and Haffner, 1985–86), resulting from their different energy requirements (Thomas, 1988; Barclay, 1991). Although limits to breeding are most commonly described in relation to temperature, the distribution of breeding populations may also be affected by rainfall patterns (e.g. Grindal *et al.*, 1992), and similarly by altitude (Richards, 1989).

Studies in northern temperate zones have examined populations close to the edge of their range (e.g. Racey *et al.*, 1987; Rydell *et al.*, 1994). Latitudinal variation in reproductive parameters is apparent at the population level with populations at higher latitudes displaying lower rates of breeding, later birth, less incidence of twinning and delayed sexual maturity (Schowalter *et al.*, 1979; Racey, 1982). However, at the species level Jones (1998) found that higher latitudes were significantly correlated with shorter gestation lengths, faster prenatal growth rates and larger litter sizes. However, none of these relationships were supported when independent contrasts were used to control for phylogenetic inertia. Latitudinal limits to breeding may result from lower temperatures affecting both thermoregulatory costs and the proportion of nights on which bats can feed, shorter night length, shorter summer, less variety in habitat and the absence of some insect groups (Kunz, 1982; Racey, 1982; Lewis, 1993). At high latitudes, bats are likely to spend longer in torpor, often resulting in later birth (Racey, 1982). Increased use of torpor may also underlie latitudinal patterns of body size which contradict Bergmann's rule, since smaller individuals have been reported at higher latitudes (e.g. *Rhinolophus ferrumequinum*, Krýstufek, 1993).

Different species show different responses to latitude, with a reduction in the number of bat species with latitude (see Racey *et al.*, 1987; Findley, 1993). The geographical breeding limits of species are likely to be related to their ecology (Lewis, 1993). A range of factors including body size, degree of dietary specialization, roost type and availability, species-specific threshold for torpor, colony size and emergence time are likely to affect the geographical limits of particular species (Figure 9.7).

Even in tropical and sub-tropical regions, seasonality may affect food abundance through the year, leading to periods of low food availability and daily torpor and highly seasonal reproduction (e.g. *Pipistrellus nanus*, Happold and Happold, 1990b; Bernard *et al.*, 1997). However, some species are able to follow local and regional changes in food availability through migrations (e.g. Thomas, 1983; Law, 1993; Fleming *et al.*, 1993).

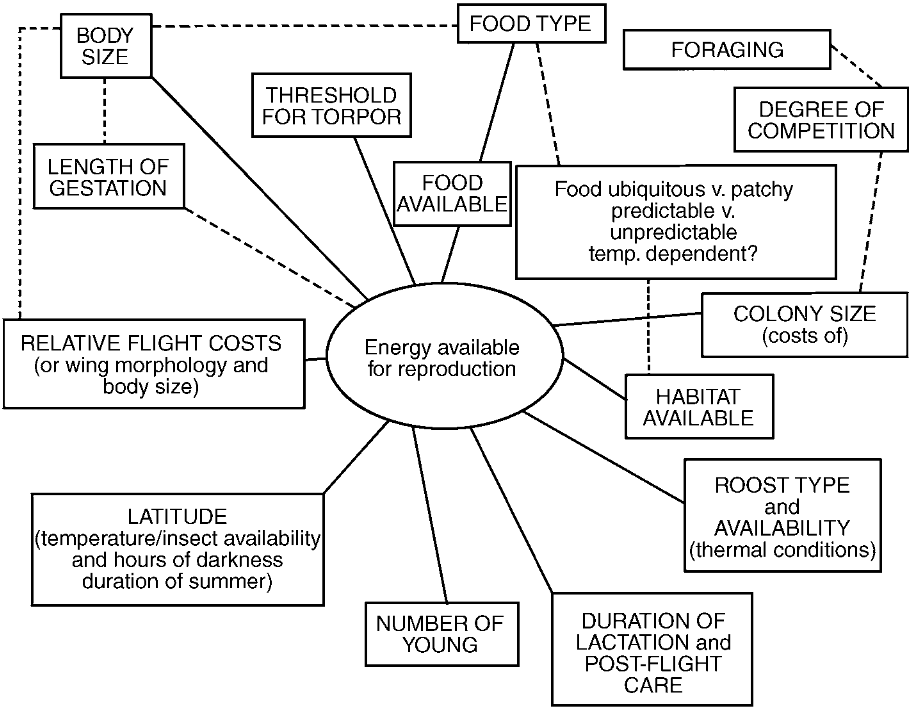

Figure 9.7 Indicative diagram of factors likely to affect energy available for reproduction, which may in turn affect distribution of different bat species.

9.8 LIMITS TO REPRODUCTIVE OUTPUT

9.8.1 Limits to Annual Reproductive Output

The annual production of a population is limited by a number of factors including the number of pregnancies, the size of litters and the proportion of the population which is breeding. The bat species showing the highest reproductive output is *Pipistrellus mimus*, which produces up to six young in any year as a result of multiple births of twins (Suthakar Isaac *et al.*, 1994).

9.8.2 Number of Pregnancies

The number of pregnancies appears to be initially determined by environmental factors including the degree of seasonality and period of food availability, but for some species appears phylogenetically constrained.

9.8.3 Size of Litters

Most species of bats are monotocous (Tuttle and Stevenson, 1982; Racey, 1982). Polytocy is recorded occasionally in temperate latitudes, with an increased frequency of twinning at lower latitudes (Tuttle and Stevenson, 1982). Several sub-tropical species are typically polytocous (e.g. *Nycticeius schlieffenii*, van de Merwe and Rautenbach, 1987; *Eptesicus capensis*, van der Merwe, 1990). Polytocy is common in vespertilionids, but has also been recorded in phyllostomids, hipposiderids and pteropodids (see Happold and Happold, 1990a). The size of litters may be limited by the constraints of flight (Myers, 1978), and by the demands of lactation given that offspring are suckled until they reach adult size (Barclay, 1994, 1995). The capacity for multiple ovulations has been recorded in many species although this is rarely reflected in litter size, as excess embryos are resorbed or aborted after implantation (Wimsatt, 1945; Myers, 1977; van der Merwe and Rautenbach, 1987, 1990; Rasweiler, 1988). Such overproduction may facilitate increased litter size in good conditions, or may provide insurance against failure of fertilization and development (Rasweiler, 1988). Jones (1998) found limited evidence that polytocy is more common in smaller species (e.g. *Pipistrellus nanus*, Happold and Happold, 1990b; *P. rusticus*, van der Merwe and Rautenbach, 1990; *P. mimus*, Suthakar Isaac *et al.*, 1994) where the capacity to enter torpor at higher ambient temperatures (McNab, 1974) may help to counter the increased energetic requirements of feeding larger litters. In addition, as a result of power–mass relationships, smaller species are capable of carrying relatively larger masses associated with multiple young (Aldridge and Brigham, 1988).

9.8.4 Reproductive Patterns and Polytocy

There is some evidence that across species there is a tendency for those showing monoestry to produce multiple young while polyoestrous species generally produce single young (Jones, 1998). This suggests that some limitation on total reproductive output exists (energetic or otherwise), but this can be allocated in different ways. Few cases are recorded of a polyoestrous species generally producing multiple young (e.g. *Pipistrellus mimus*, Suthakar Isaac *et al.*, 1994). Latitudinal variation in reproductive pattern may also affect the occurrence of polytocy. Under highly seasonal conditions *Pipistrellus nanus* displays monoestry with polytocy (twinning), but aseasonal monoestry or polyoestry together with monotocy in more stable conditions (Happold and Happold, 1990b).

9.8.5 Reproductive Failure

Although some studies indicate a high proportion of breeding females among bat colonies (e.g. Humphrey and Cope, 1970; Humphrey and Kunz, 1976; Racey, 1982) others show relatively low breeding success (Grindal *et al.*, 1992; Entwistle, 1994). Across different populations the proportion of breeding females in a species may vary dramatically, often in response to climate (Grindal *et al.*, 1992; Lewis, 1993), and between years (Lewis, 1993; Entwistle, 1994). Failure to breed may result from failure of fertilization, or more commonly from resorption or abortion of embryos in response to harsh conditions during pregnancy (Wimsatt, 1945). Such reproductive failure may have a selective advantage when considered in the light of potential future reproductive potential (Wasser and Barash, 1983), especially if reproductive efficiency changes with age.

9.8.6 Lifetime Reproductive Output

Lifetime reproductive output is a function of annual reproductive output, length of reproductive lifespan (determined by time of sexual maturity and longevity), and inter-annual patterns of reproduction.

9.8.7 Time to Sexual Maturity

Many bat species show some delay in the onset of sexual maturity in females (Tuttle and Stevenson, 1982), which may be as long as five years (e.g. *Rhinolophus ferrumequinum*, Ransome, 1995). Only a proportion of the young of some species (e.g. *Nyctalus noctula*) achieve sexual maturity in the year of their birth (Kleiman and Racey, 1968), whereas the fact that almost all females of species such as *Pipistrellus pipistrellus* are found to be inseminated during winter implies that they achieved sexual maturity at three months of age (Racey, 1974a). However, in the majority of species, males and females undergo sexual maturity at one or two years of age (Tuttle and Stevenson, 1982; Racey, 1982). However, even within a species, time to sexual maturity varies geographically (e.g. *Tadarida pumila*, Happold and Happold, 1989), and is typically delayed at higher latitudes (e.g. *Myotis lucifugus*, Schowalter *et al.*, 1979; *Plecotus auritus*, Entwistle, 1994). It is predicted that long-lived species with low natural mortality may increase lifetime reproductive output by delaying the onset of sexual maturity (Harvey and Zammuto, 1985; Partridge and Harvey, 1985; Charnov, 1993). Indeed, delayed sexual maturity is associated with higher life expectancy in *Rhinolophus ferrumequinum* (Ransome, 1995). The age of reproduction may also affect reproductive efficiency and investment, and older bats are more likely to produce larger litters (Tuttle and Stevenson, 1982).

9.8.8 Longevity

Recorded longevity in bats is much greater than in non-flying mammals of similar size (Tuttle and Stevenson, 1982). For example *Plecotus auritus* has been recaptured 30 years after initial banding (Lehmann *et al.*, 1992) and *Rhinolophus ferrumequinum* after 26 years (Ransome, 1991). Most species appear to breed regularly, and there are no reports of species breeding in alternate years. Climatic factors appear to determine whether or not bats breed in any year (e.g. Lewis, 1993), rather than whether they produced young in the preceding year.

9.8.9 Implications for Population Growth and Dynamics

Lifetime reproductive outputs within a population are the major determinant for the growth or decline of that population and vary dramatically from species to species. Species with high productivity appear to be able to over-produce thus allowing for population growth (*Pipistrellus mimus*, Suthakar Isaac *et al.*, 1994; *Tadarida pumila*, McWilliam, 1987b). In other species such as *Plecotus auritus*, productivity is low in northern Scotland, and over a lifetime the population appears not to replace itself, suggesting that it may be declining (Entwistle, 1994). Reproductive strategies appear to vary with environmental determinants, particularly the degree of seasonality. Some emballonurid species in stable environments are polyoestrous and share delayed sexual maturity and high adult survival. In contrast, those species inhabiting more seasonal environments produce a single young which undergoes rapid sexual maturity and shows lower adult survival (e.g. Bradbury and Vehrencamp, 1977).

Other factors may, however, also affect the reproductive output of a species. These include a range of impacts associated with the presence of humans. In particular breeding success and adult survival may be affected by the availability of suitable roosts to establish breeding colonies, stress resulting from interference, pollutants and pesticides, and reduction of foraging habitats (Stebbings, 1988). Furthermore, a number of factors directly increase mortality in bats – for instance direct persecution, disturbance at hibernacula and effects of wood treatment chemicals (Racey and Swift, 1986).

In this way the fragile balance of the life history of bat species may be disrupted, leading to population declines and local extinctions. Because of their restricted annual reproductive rate, bat populations take a relatively long time to recover from population losses associated with human activities. This slow population growth rate is a factor that compounds the identified threats to bat populations, and an understanding of what limits population growth in different bat species may be an important tool in the development of appropriate conservation strategies. Indeed, those species with strategies most adapted to high adult survival, with delayed onset of sexual maturity (e.g. *Rhinolophus ferrumequinum*) may be most at risk from a changing environment and increased risks of mortality.

ACKNOWLEDGMENTS

We are grateful to R.T.F. Bernard and J.J. Rasweiler for helpful comments on earlier drafts. We are particularly indebted to K.E. Jones for her contribution and for allowing us to use an unpublished figure from her thesis.

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10

Parental Care and Postnatal Growth in the Chiroptera

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10.1 INTRODUCTION

How parents influence the fitness of offspring has generated a large body of empirical and theoretical literature. The importance of parental care in determining reproductive success in bats has received less attention compared with other aspects of reproduction. Efforts to integrate research on mother–infant recognition, lactation, non-nutritional care and postnatal growth have largely been descriptive. In this chapter we review the available literature on parental care and postnatal growth in bats and invite others to further investigate how these traits vary among species and how parental effort can be adjusted to the variation in costs and benefits to both parents and offspring. We follow Clutton-Brock (1991) in defining parental care as ‘any form of parental behavior that appears likely to increase the fitness of parents and offspring.’

Reproductive Biology of Bats
0-12-195670-9

Interspecific and intraspecific variation in parental care in mammals can largely be linked to differences in the amount of energy and nutrients allocated to offspring in the form of milk, but non-nutritional care also may influence this variation. These differences may be associated with several factors, including phylogeny, diet, offspring gender, litter size, climate, habitat and risk of predation. Depending on the relative availability of food, environmental changes may increase or reduce efforts of parental expenditure in offspring (Clutton-Brock, 1991). In this review we examine patterns of parental effort in bats and summarize factors that appear to influence patterns of postnatal growth in the Chiroptera in an effort to determine how parents contribute to the development of their young.

10.2 PARENTAL CARE

In bats, as in most other mammals, mothers provide a continuum of protection to their offspring from gestation to weaning. In the foetal period, the mother's womb protects the foetus from many environmental stimuli and strongly affects the biochemical and thermal environment in which the offspring develops. After birth, the mother's direct biochemical influences are limited to the milk that she supplies. In addition, non-nutritional care may be provided and can include sensory stimulation, thermal influence, and pup retrieval and transport.

Maternal interactions with a developing infant can be divided into two major phases. The first phase of interactions occurs during pregnancy when hormones and antibodies regulate placental nutrients, oxygen and waste exchange with the foetus. The second phase occurs when the mother influences the newborn through major sensory systems (tactile, olfactory, thermal, auditory and visual) and the biochemical transfer of nutrients and energy in the form of milk. Combinations of these interactions are evident during the developmental period and are manifested by unique characteristics and the quality of parental care exhibited by each species.

Few studies have focused directly on parental care in free-ranging bats. Notable exceptions include studies by Nelson (1965), Bradbury and Vehrencamp (1977a,b), Thomson *et al.* (1985), McCracken and Gustin (1991), and Wilkinson (1985, 1992a). In this chapter we review various parent–infant interactions including nutritional and non-nutritional forms of care. We discuss nutritional investment that mothers make in young bats, largely in the context of milk production and yield. In addition, we describe mammary form and function in bats, suckling behavior, regurgitation (a special case of maternal care in *Desmodus*), and the incidence of spontaneous secretion of milk by males. Acoustic communication and its role in mother–pup recognition and reunions has received moderate attention, but few studies have investigated the role of spatial, olfactory, visual and tactile cues in these contexts.

Most newborn bats are functionally altricial at birth; they are naked and have a limited ability to regulate their body temperature. An important aspect of parental care in bats is the selection and maintenance of a thermal environment by the mother that facilitates rapid growth and development of young. Acoustic and olfactory responses are important for promoting mother–infant recognition, tactile interactions are important for the development of appropriate social and locomotor functions, and suckling behavior is obviously essential for the transfer of milk. Alloparental behavior, when it occurs, may facilitate the development of parenting skills, cohesion of groups, and socialization (McKenna, 1981; Kunz *et al.*, 1994a). Retrieval of a pup that has fallen from its roost is a relatively common occurrence in bats, and because this behavior also involves transporting young in flight, maternal effort may be high in some species because most young bats are unable to fly until they reach 90 per cent of adult wing dimensions (Kunz, 1987; Barclay, 1995). For species that regularly carry their young during foraging bouts, or transport them to alternate roosts, the increased energetic costs of flight associated with this behavior may be substantial.

In most species of mammals, including bats, the primary care-givers are females. However, in some species, males assume a more active role. For example, paternal care may involve the construction and/or defense of roosts or the defense of female groups (see McCracken and Wilkinson, this volume). Recent evidence suggests that males of at least one species (*Dyacopterus spadecius*) maintains active mammary tissue at certain times of year (Francis *et al.*, 1994). This finding suggests that nutritional care by males may be possible, but there is no current evidence that pups are suckled by these males.

10.2.1 Sensory Interactions and Mother–Infant Recognition

Bats that roost in large aggregations appear to rely on a combination of spatial memory, acoustic, olfactory, tactile and/or visual cues to identify young or to communicate with conspecifics. Early observations on bats suggest that females which form large aggregations suckled young indiscriminately (*Tadarida brasiliensis*, Davis *et al.*, 1962; *Miniopterus australis* and *M. schreibersii*, Brosset, 1962b). However, more recent studies on these and other species have shown that nearly all female bats selectively suckle their own infants (*Antrozous pallidus*, Brown, 1976; *Desmodus rotundus*, Schmidt, 1972; *Eptesicus fuscus*, Davis *et al.*, 1968; *Myotis velifer*, Kunz, 1973; *Pipistrellus pipistrellus*, Bishop *et al.*, 1992; *Corynorhinus rafinesquei*, Pearson *et al.*, 1952; *Rhinolophus condylura*, Kulzer, 1962; but see *Nycticeius humeralis*, Wilkinson, 1992a, and *Tadarida brasiliensis*, McCracken, 1984). Selective nursing requires individual recognition, which ultimately is based on some form of communication.

Although most animals rely on one form of communication over others (Scott, 1968), female bats may use several cues to locate and identify their dependent young. Females of some species initially rely on spatial memory for locating their pups, but subsequently use audition and olfactory cues for individual recognition (Gustin and McCracken, 1987). In these and similar situations, pups are accepted and allowed to suckle if recognized (De Fanis and Jones, 1995b).

Mother–offspring interactions may occur well past the onset of weaning in some species (Bradbury, 1977a). Although we are unaware of any experimental evidence to support this claim, recognition of weaned young appears to occur in *Desmodus rotundus* (Wilkinson, 1988), *Myotis adversus* (Dwyer, 1970), *Pteropus vampyrus*, and *P. hypomelanus* (W.R. Hood, personal observation). For species of bats that are relatively long-lived (Austed and Fischer, 1991) and highly philopatric (Lewis, 1995), we would expect roost mates to develop some form of individual recognition. The best candidates for long-term recognition include species that form small female groups based on matrilineal relationships. These and similar associations suggest that recognition cues are either committed to long-term memory or are genetically based on sensory cues similar to their own—so-called phenotypic matching (Beecher, 1982). Recognition of young by fathers has not been demonstrated in bats, although we would expect paternal recognition to occur in monogamous species, where males participate in the care of young (e.g., *Lavia frons*, Vaughan and Vaughan, 1987; *Cardioderma cor*, Vaughan, 1976; and *Vampyrum spectrum*, Vehrencamp *et al.*, 1977) and where kin recognition serves as a mechanism to avoid inbreeding (Krebs and Davies, 1991).

Spatial memory

Spatial memory appears to be the principal sensory cue used by females for roost recognition (Bradbury, 1977a; Mueller and Mueller, 1979). Spatial perception and memory may be important for species that leave their young in different roost locations at night, especially in those species that roost in large aggregations (Bradbury, 1977a; Mueller and Mueller, 1979). In *Tadarida brasiliensis*, lactating females associate with pups during scheduled nursing bouts, but they typically aggregate with other adults when they are not nursing their young. In an experiment to test for spatial memory in *T. brasiliensis*, McCracken (1993) marked females and removed their pups from known roosting areas in a maternity cave housing approximately seven million adults. Females returned to these roosting areas within the cave two or three times each night, and searched for their young which had been temporarily removed from the cave. Results from this experiment provided support for the spatial-memory hypothesis, because visual, olfactory, and acoustic stimuli were absent. Use of spatial memory for locating young also has been suggested for *Lasiurus cinereus* (Koehler and Barclay, 1988) and *Hipposideros speoris* (Habersetzer and Marimuthu, 1986).

Acoustic stimuli

Acoustic communication between females and their young is continually refined from birth until weaning, especially as the neonatal laryngo-nasal junction develops (Matsumura, 1979), hearing is established in young (Brown *et al.*, 1978), and pups learn adult vocalizations and echolocation calls (e.g., Matsumura, 1981; Jones *et al.*, 1992, Jones *et al.*, 1993; Masters *et al.*, 1995; Moss *et al.*, 1997). Young bats of several species are able to utter sharp, metallic ‘chirps’ or ‘squeaks’ during (*Nyctalus noctula*, Kleiman, 1969) or within hours of birth (*Corynorhinus rafinesquei*, Pearson *et al.*, 1952). *Tadarida brasiliensis* pups and their mothers vocalize almost continuously following parturition, presumably allowing each to learn the vocalizations of the other (Gelfand and McCracken, 1986). These single- and double-note vocalizations are termed ‘isolation calls’ (Gould, 1971; Schmidt, 1972; Brown, 1976). Newborn *Antrozous pallidus* emit an almost continuous series of isolation calls if separated from their mother (Brown, 1976). Similarly, pups of *Eptesicus fuscus* less than two weeks of age squeak continuously until they are retrieved following a fall to the floor (Davis *et al.*, 1968).

The segmented nature of isolation calls appears to help mothers locate their young at least in captive situations (Davis, 1970). Isolation calls of *Tadarida brasiliensis* pups were distinguished by their mothers (60.4% classified correctly) in experimental trials, suggesting that similar recognition occurs in free-ranging populations (Gelfand and McCracken, 1986). In several species, pups produce individually distinctive calls that appear to attract mothers when they become separated (e.g., *Phyllostomus hastatus*, Rother and Schmidt, 1985; *T. brasiliensis*, Gelfand and McCracken, 1986; *Pipistrellus pipistrellus*, Jones *et al.*, 1991; *Nycticeius humeralis*, Scherrer and Wilkinson, 1993; *Plecotus auritus*, De Fanis and Jones, 1995a).

Directive calls (i.e., attractive calls, Matsumura, 1981) are often emitted by mothers as they search for their young. They can be distinguished by pups at distances up to one meter in extremely dense clusters of *T. brasiliensis* (Balcombe and McCracken, 1992). These directive calls are stereotyped and statistically discernible among individuals (Balcombe and McCracken, 1992), a characteristic considered to be highly suitable for effective communication.

Vocal communication is also likely to be important when pups first become volant. A young *Saccopteryx leptura* was observed making short sallies from its roost during the day, and after a short period the mother emitted several vocalizations, stimulating her pup to return to the roost (Bradbury and Emmons, 1974). As the pup approached its mother and climbed upon her, both mother and pup emitted audible vocalizations, presumably verifying their identities. O'Shea and Vaughan (1977) reported 'rallying' calls in *Antrozous pallidus*, which presumably promotes contact between mother and pups upon return of the mother to the roost.

Vocal communication between mothers and pups appears to be most complex early in the postnatal period. Infant bats which become separated from their mothers emit continuous broad-band attractive calls, and mothers respond with 'lead signals' (i.e., directive calls, Brown, 1976; antiphonal calls, Matsumura and Uchida, 1975; and search calls, Nelson, 1964). During the production of mutual, alternating signaling in *Rhinolophus ferrumequinum*, the acoustic character and pattern of infant vocalizations gradually changes until the high-intensity call precisely overlaps the call of its mother. Contact calls emitted by both mother and infant also appear to establish identity and facilitate mother–pup reunions in *Pteropus* (Nelson, 1965), *Eptesicus fuscus* (Gould, 1971), *Desmodus rotundus* (Schmidt, 1972), *Antrozous pallidus* (Vaughan and O'Shea, 1976), and *R. ferrumequinum* (Matsumura, 1981).

Acoustic recognition of offspring by mothers, and mothers by pups, has been verified experimentally in *Myotis lucifugus* and *Tadarida brasiliensis.* Turner *et al.* (1972) placed *Myotis* pups individually in a Y-maze and allowed a choice between its mother and another lactating female, and pups selected their own mothers in 75% of the tests. Despite the high error rate and overlap in duration of isolation calls of different infants, vocal cues may aid a mother in locating her own infant in a large maternity colony, with final recognition based on other cues such as olfaction, as in *Rousettus aegyptiacus* (Kulzer, 1961) and *Tadarida brasiliensis* (Gustin and McCracken, 1987). Balcombe (1990) evaluated call recognition by lactating female *T. brasiliensis*, and when they were presented with calls of their pup and an unrelated pup, the mothers showed a significant preference for calls from their own young. Similar observations were reported for *P. pipistrellus* (De Fanis and Jones, 1996).

In some species, isolation calls appear to have both genetic and learned components. Acoustic learning appears to be important in the development of isolation calls in *Phyllostomus discolor* (Esser and Schmidt, 1989). Mothers produce directive calls that are unique, especially in their patterns of frequency modulation. Isolation calls of pups gradually change to resemble the directive calls of their mothers, as mothers and pups exchange isolation and directive calls. In a series of experiments, Esser (1994) demonstrated that when pups of *P. discolor* were isolated from conspecifics at birth and hand reared, individuals that were subjected to playback calls of their mothers subsequently produced calls with more frequency minima and maxima than control pups which received no acoustic stimulation. Scherrer and Wilkinson (1993) argued that the isolation calls of *Nycticeius humeralis* had a strong genetic component, because pups were unable to learn isolation calls from mothers who did not emit equivalent sounds. However, both young and adult members of social groups in *P. hastatus* appear to learn calls that help coordinate foraging movements of other members in the same social group (Boughman, 1998).

Echolocation calls may be used by some species for individual recognition, but these are probably less important in mother–pup interactions. Individual variation in FM calls has been described for several species of bats, including *Eptesicus fuscus* (Schnitzler *et al.*, 1987; Thomas *et al.*, 1987; Brigham *et al.*, 1989; Masters *et al.*, 1991, 1995; Fenton, 1994; Obrist, 1995; but see Rasmuson and Barclay, 1992) and *Myotis lucifugus* (Thomson *et al.*, 1985). Echolocation calls produced by adult *E. fuscus* were highly repeatable (>70%), compared to young (12 to 80%) but self-consistency increased with age (Masters *et al.*, 1995). In *Pipistrellus pipistrellus*, echolocation calls of pups were correctly discriminated in 82% of the trials by six-day old infants and by 91% in 15-day old infants (Jones *et al.*, 1991). Scherrer and Wilkinson (1993) found a high degree of repeatability in the calls of young *Nycticeius humeralis* (44 to 94%). Significant variation in echolocation calls exists among family members (38%) in *E. fuscus*, suggesting a genetic basis to vocal signatures (Masters *et al.*, 1995).

The characteristics of echolocation calls of young bats also appear to change with age, where the frequencies of young bats are typically lower than older individuals (*Rhinolophus hipposideros*, Jones *et al.*, 1992; *R. ferrumequinum*, Jones and Ransome, 1993; *Asellia tridens*, Jones *et al.*, 1993; *Myotis daubentonii*, Jones and Kokurewicz, 1994; and *M. lucifugus*, Pearl and Fenton, 1996; Moss *et al.*, 1997). Jones and Ransome (1993) demonstrated that the resting frequencies (RF) emitted by young *R. ferrumequinum* are highly correlated with the RF calls of their mothers – older females raise young with lower RFs that do younger females. Thus, age-related changes in echolocation calls of bats appear to have both genetic and learned components (Jones and Ransome, 1993; Masters *et al.*, 1995).

Females of *Hipposideros speoris* respond to the early FM calls of infants, but apparently are unable to recognize individuals. Because calls of pups are dynamic, females apparently are unable to rely on a single acoustic cue for pup recognition. Alternatively, females may resort to other forms of communication (Jones *et al.*, 1991), or may respond to a changing template of their offspring's vocal signature.

Individual isolation calls may be more ambiguous, and individual recognition may be less important in solitary species (Koehler and Barclay, 1988).

Olfactory stimuli

Several investigators have suggested that female bats recognize their own young using olfaction, although few studies have been conducted to test this hypothesis. The presence of odoriferous glands in females and their young, observations of maternal or mutual sniffing and apparent scent marking have been reported for several species. Females of *Nycticeius humeralis* possess a submandibular gland that increases in size by nearly 25% within three days of parturition. Immediately before departing to forage, females apparently used this gland to mark the face of young bats less than or equal to two weeks of age (Watkins and Shump, 1981). Females of *Pteropus giganteus* have specialized glands in the tarsal, oral angle, muzzle and perineal regions. Mothers may groom their infants in these regions, a behavior which may be important for sharing odors and for establishing mother–infant bonds (Jayaprakash and Alexander, 1993). Specialized skin glands become hypertrophied during lactation in *Megaderma lyra* (Begum and Alexander, 1993), and females of *Tadarida brasiliensis* actively produce odoriferous secretions from their muzzle and touch their chins to their own pup several times when selecting them and while nursing (Gustin and McCracken, 1987). Odor profiles of subaxial secretions from individual *Noctilio leporinus* are unique (Brooke, 1994), but the role of these secretions in mother–pup recognition has not been determined.

Female bats of several species may initially locate their infants using auditory cues, but final recognition appears to be based on olfaction. Young of *Nycticeius humeralis* produce audible vocalizations when females return to their roost after foraging, but females apparently do not recognize their infants until olfactory cues are tested by sniffing the facial region of putative offspring (Watkins and Shump, 1981). This behavior has also been observed in *Plecotus auritus*, although whether the female sniffed a specific region is unclear (De Fanis and Jones, 1995a). When females of *Pipistrellus pipistrellus* return to their roost, they move through the cluster of young bats, moving their heads, apparently sniffing for their young (Rakhmatulina, 1972). De Fanis and Jones (1995b) demonstrated that adult females of *P. pipistrellus* were able to discriminate between the odors of females from their own and different colonies. Individual recognition was also apparent, but females needed more time to recognize odors of individuals from their own colony than females from different colonies. Upon their return from foraging, females of *Pteropus poliocephalus* typically circle the camp where young pups are roosting, exchange calls with these infants, and finally land near their own young. Females sniff the chest of pups and either accept their own by opening one or both wings, or reject the pup by pushing it away using their thumb and wing (Nelson, 1965). Although olfaction is extremely acute in *Desmodus rotundus* (Schmidt, 1973), no studies have examined the role of odors in individual recognition (Wilkinson, 1985).

Only one study has unambiguously demonstrated that mothers recognize their own young using olfaction. Gustin and McCracken (1987) designed a double-blind test where lactating females of *Tadarida brasiliensis* were allowed to choose between the odor of their offspring and that of a randomly chosen, conspecific pup. Females showed a preference for the odor of their pup over that of a randomly chosen pup, and showed a significant preference for their own muzzle odor over that of a randomly chosen female. These observations suggest that females may use olfaction for phenotypic matching in pup recognition. Newborn pups, one to three days old, showed a preference for the scent of their mothers over a randomly chosen female, but this form of recognition was apparently abandoned by day four (Loughry and McCracken, 1991). Although *T. brasiliensis* pups recognized the auditory directive calls of females (Balcombe and McCracken, 1992), pups apparently shifted from olfactory to auditory modes once the auditory system began to mature.

Several studies have shown that olfactory recognition is important in insectivorous bats, although we expect that olfactory recognition is even stronger in frugivorous, omnivorous and sanguivorous species. Olfactory bulbs of bats with the latter food habits are more highly developed than those of insectivorous species (Mann, 1960; Stephan and Pirlot, 1970). The vomeronasal organ, an important site for olfactory and gustatory sensation, is rudimentary in many bats but is well developed in phyllostomids, a family which consists largely of frugivorous and nectarivorous species (Bhatnagar, 1980). Although highly developed olfaction undoubtedly contributes to the location of food, the assumption that olfaction should play an important role in individual recognition is reasonable for these and related taxa, as has been suggested for frugivorous megachiropterans such as *Rousettus aegyptiacus* and *Pteropus* spp. (Kulzer, 1958; Nelson, 1965).

Visual stimuli

Virtually nothing has been published on the interactions of mothers and pups based on visual stimuli. Most reports of visual communication involve interactions between conspecific roost mates associated with group formation, courtship, and mating displays. Considering the relatively well-developed visual system of bats (Suthers, 1970), we would expect mothers and pups to communicate visually whenever possible. Some species, especially foliage-roosting pteropodids and phyllostomids and some emballonurids, exhibit a rich array of visual displays, often produced independently of vocal and auditory signals. These include postural changes, wing flicking, wing shaking, hovering flight, baring of teeth, ‘boxing,’ head jerking, and erection of specialized hair patches (Fenton, 1985). Responses of bats to visual signals may be accompanied or followed by vocal or olfactory signals, but interpretation of visual signals can be ambiguous. For example, if a threat posture is accompanied by an audible vocalization or release of an odoriferous compound, response of the recipient may be to the combination of signals, not only to one. In this manner each signal may reinforce the other, leading to an appropriate response by the recipient. Given the apparent occurrence of visual signaling among adult bats, it seems likely that mothers and pups should also rely on visual stimuli for recognition.

Tactile stimuli and grooming

Some types of tactile stimulation provided by the mother may be important for stimulating urination and defecation in bat pups, as reported for other mammals (see Hofer, 1981). Repeated tactile stimulation also may reduce the level of behavioral reactivity as has been observed in some terrestrial mammals (Matthews, 1969; Gubernick and Klopfer, 1981). In highly gregarious species of bats, there is often a high level of tactile stimulation among roost mates. To what extent such tactile stimulation is directed toward specific roost mates, including infants, or is simply a consequence of contact clustering remains to be clarified. In maternity colonies of *Myotis lucifugus* and *Pipistrellus subflavus*, adult females groom their young before departing on evening foraging bouts and again upon return from feeding (Burnett and August, 1981; Winchell and Kunz, 1996). Whether this type of tactile stimulation facilitates gut and bladder emptying of pups, reduces metabolic rates, or lowers parasite loads invites further study. Contact clustering does reduce metabolic rates in some young and adult bats (*M. thysanodes*, O'Farrell and Studier, 1973; *Tadarida brasiliensis*, Herreid, 1967; *Antrozous pallidus*, Trune and Slobotchikoff, 1976; *M. lucifugus*, Kurta *et al.*, 1987), but whether this physiological response is a consequence of reduced behavioral anxiety or reflects other factors remains to be established.

10.2.2 Lactation, Milk and Related Nutritional Effort

Mammals are unique in their ability to produce milk from specialized mammary glands. Production of milk is generally considered the most costly aspect of mammalian reproduction (Millar, 1977; Oftedal, 1985; Gittleman and Oftedal, 1987), and likely has the most important influence on reproductive success (Maynard Smith, 1977; Pond, 1977; Daly, 1979). Milk composition and output are generally more closely correlated with suckling behavior and diet than with phylogeny (Ben Shaul, 1962). Energetic requirements of lactation scale negatively with increasing body size, and thus lactation is most costly in small mammals (Hanwell and Peaker, 1977; Oftedal, 1985). Since bats are a diverse group, with a wide range of life histories, dietary specializations, and body sizes (Kunz, 1982; Hill and Smith, 1984; Kunz and Pierson, 1994), interspecific comparisons among the Chiroptera should provide additional insight into how these variables influence parental effort.

Mammary glands, nipples and false nipples

Mammary glands of lactating females can usually be recognized by the whitish tissue that lies directly beneath the skin (Racey, 1988; Kunz *et al.*, 1996). These glands are typically flattened, disk-shaped structures that extend from the mid-thoracic to the axillary region. Over the course of a female's annual life cycle, mammary glands progress from being undeveloped during the non-breeding season to well developed at peak lactation (Heideman, 1988). Mass of mammary glands can be substantial, as the emptied mammary glands of lactating *Tadarida brasiliensis* average 8% of a female's post-absorptive body mass (Davis *et al.*, 1962). Mammary glands may account for an even greater percentage of a female's body mass in some pteropodids such as *Cynopterus brachyotis* (Dobson, 1878).

All female bats possess at least one pair of thoracic nipples, generally located in the anteriolateral pectoral position (Figure 10.1). Only two vespertilionid genera.

Figure 10.1 Lactating female Dayak fruit bat, *Dyacopterus spadecius* (Pteropodidae), showing an attached pup (left) and a well-developed nipple (right). Note the axillary position of the exposed nipple, which is characteristic of most bats. Photo by Charles M. Francis.

Lasiurus and *Otonycteris*, have two pairs of functional nipples. This extra pair of nipples is found in species where litter sizes are greater than two (Nowak, 1994). As in other mammals, the condition of nipples is an important criterion for parity, as relative nipple size can be used to distinguish parous and multiparous females from non-parous females (Racey, 1988).

Nipple morphology has been used to assess reproductive status of both microchiropterans (Ryberg, 1947; Sluiter, 1954; Sluiter and Bowman, 1951; Pearson *et al.*, 1952; Racey, 1974; Baage, 1977) and megachiropterans (Ryberg, 1947; Heideman, 1988; Francis *et al.*, 1994), but interpretations of reproductive status based on nipple size alone can be ambiguous (Racey, 1988). Distinguishing between primiparous and nulliparous females outside the breeding season is often difficult. In microchiropterans, nipples of nulliparous females, and sometimes primiparous females, are rudimentary and often give rise to tufts of hair, whereas nipples of older parous females are typically cornified and blackish or yellowish in color, with few or no hairs (Pearson *et al.*, 1952; Sluiter, 1954; Racey, 1974, 1988; Baage, 1977). Based on a combination of criteria, including relative nipple size, presence or absence of tufts of hairs on the nipple, and degree of nipple cornification, the assignment of females to relative stages of lactation and post-lactation may be possible (Heideman, 1988; Kunz *et al.*, 1996).

With the exception of a few species in which males reportedly lactate or have hypertrophied mammary tissue (Dobson, 1878; Ryberg, 1947; Francis *et al.*, 1994), males typically have rudimentary nipples and the mammary glands are absent or inconspicuous. The histomorphology of mammary tissue in lactating males of *Dyacopterus spadiceus* is indistinguishable from that of lactating females. Although the nipples of lactating females and males are darkened and cornified, the nipples of lactating males are considerably smaller than those of females (Francis *et al.*, 1994).

Some bats posses a pair of 'holdfast' nipples in the ventral, posterior-medial region, near the genitalia (Simmons, 1993). These 'false nipples' are present in all species of the families Craseonycteridae, Megadermatidae, Rhinopomatidae and Rhinolophidae, where they are present ubiquitously in females but occur inconsistently in males. A lactiferous function of false nipples has been confirmed in some species of Rhinolophidae and Rhinopomidae based on the expression of milk and/or the characteristic texture, color, and ductal branching of mammary tissue (Simmons, 1993). Pubic nipples appear to act primarily as holdfasts for infants (Simmons, 1993). Young bats in the Rhinolophidae and Rhinopomidae adopt unusual postures when attached to pubic nipples in which the pup's head is positioned posteriorly on the female's ventral surface and their legs are wrapped around the neck of their mother (Gaur and Shahrohk, 1989; Duangkhae, 1990; Vaughan and Vaughan, 1987; Simmons, 1993).

Nursing behavior

Early studies described nursing behavior in *Tadarida brasiliensis* and include such statements as 'mothers apparently act as one large dairy herd delivering milk passively to the first aggressive customer' (Davis *et al.*, 1962) and in *Miniopterus schreibersii* that 'suckling of young is not an individual but a communal business' (Brosset, 1962b). However, more recent studies suggest that non-offspring nursing does not occur in bats (e.g., *Antrozous pallidus*, Davis, 1969; Brown, 1976; *Eptesicus fuscus*, Davis *et al.*, 1968; *Myotis lucifugus*, *M. thysanodes*, *M. velifer*, *Corynorhinus rafinesquei*, Pearson *et al.*, 1952; Twente, 1955; *Pipistrellus pipistrellus*, Hughes *et al.*, 1989; Bishop *et al.*, 1992, and *Rousettus aegyptiacus*, Kulzer, 1962) except in cases of mistaken identity (*Tadarida brasiliensis*, McCracken, 1984), when the opportunity for reciprocity is high (*Nycticeius humeralis*, Wilkinson, 1992a), or when maternal mortality is high among captive individuals (*Pipistrellus pipistrellus*, Eales *et al.*, 1988).

Young bats may attempt to suckle indiscriminately, as in *Pipistrellus pipistrellus*, *Myotis velifer*, and *Tadarida brasiliensis* (Hughes *et al.*, 1989; Twente, 1955; McCracken and Gustin, 1991). This behavior can lead to non-offspring suckling if the identity of young is mistaken (McCracken, 1984) or when opportunity for reciprocation is high (Trivers, 1971). Non-offspring nursing was observed in 18% of all suckling bouts in *Nycticeius humeralis* when young were greater than two weeks of age (Watkins and Shump, 1981; Wilkinson, 1992a), but this percentage is greater than expected if non-offspring nursing occurred randomly (Wilkinson, 1992a). Watkins and Shump (1981) suggested that after two weeks of age, selective nursing may be too energetically expensive for mothers. Wilkinson (1992a) found that females of *N. humeralis* did not nurse matrilineal kin preferentially, but selectively nursed young females, and 73% of observed incidents of non-offspring nursing involved females whose pups had already begun to forage.

Wilkinson (1992b) postulated that female-biased communal nursing in *N. humeralis* gives both an immediate and delayed benefit to lactating females which experience variable hunting success, assuming that roostmates exchange information about feeding and roosting sites. He also suggested that communal nursing may increase with matrilineal colony size and thus give the immediate energetic benefit of mass reduction to those females with milk in their ducts and by giving them an opportunity to offload the milk prior to their next feeding bout. In *Tadarida brasiliensis*, non-offspring nursing occurs in approximately 17% of the females (McCracken, 1984), but because of large colony size and lack of group stability (Villa and Cockrum, 1962; Constantine, 1967; Cockrum, 1969), the evolution of communal nursing through kin selection or an opportunity for reciprocity is unlikely (McCracken and Gustin, 1991).

No cases of nursing have been observed where mutual auditory and olfactory exchanges were absent, making 'milk dumping' by *N. humeralis*, as suggested by Watkins and Shump (1981), unlikely. Instead, non-offspring nursing may be attributed largely to parental mistakes and milk stealing by young (McCracken and Gustin, 1991). Milk theft or 'adoptive' nursing may increase when animals are disturbed by human observers (Fogden, 1971).

Communal nursing appears to be more common in captive situations (*Macrotus californicus*, Gould, 1977; *Desmodus rotundus*, Schmidt, 1978, Mills, 1980; *Pipistrellus pipistrellus*, Eales *et al.*, 1988). In these instances, communal nursing situations may be more frequent because opportunities for reciprocation are higher. Although communal nursing has been documented for *D. rotundus* in captivity, this behavior has not been observed in the wild (Schmidt, 1972; Wilkinson, 1988). Individuals of *D. rotundus* regurgitate blood to starved roostmates (Wilkinson, 1984), but regurgitation is less energetically expensive than milk production. In a captive colony of *P. pipistrellus* (Eales *et al.*, 1988), five of eleven pups suckled from two females. These authors suggested that this behavior may have been associated with high infant mortality and the need for the mothers who lost their young to offload excess milk.

In many species of bats, newborn pups are attached almost continuously to their mothers, and appear to suckle on demand during both day- and night-roosting periods. In a few species, pups during their first few weeks of life may remain attached to their mothers while they are on nightly foraging bouts, as in *Carollia perspicillata* (Pine, 1972; Bradbury, 1977a), *Artibeus lituratus* and *Glossophaga soricina* (Tamsitt and Valdivieso, 1963), *Pteropus poliocephalus* (Bartholomew *et al.*, 1964), *Choeronycteris mexicana* (Mumford and Zimmerman, 1964), and *Desmodus rotundus* (Schmidt and Manske, 1973). In other species, suckling is not constant, but instead milk is provisioned to pups on a regular schedule. In *Myotis thysanodes* (O'Farrell and Studier, 1973), *Miniopterus schreibersii* (Brosset, 1962b), *Miniopterus australis* and *M. pusillus* (= *macrocneme*; Hill, 1983; Sanborn and Nicholson, 1950), *Macrotus waterhousii* (Goodwin, 1970), and *Tadarida brasiliensis* (Davis *et al.*, 1962; Pagels and Jones, 1974; McCracken and Gustin, 1991; Kunz *et al.,* 1995b), females leave their offspring in a cluster with other pups and roost apart with other females during the day. Typically, females find and suckle their young soon after they return from foraging and again in the late afternoon.

In some species of bats, energy density of milk may be closely linked to suckling frequency. Kunz *et al.* (1995b) found that energy density of milk in *Tadarida brasiliensis*, a bat that nurses its young on a schedule following each of two nightly foraging periods, is higher than that of *Myotis velifer* and *M. lucifugus*, both of which allow their young to remain attached to their nipple throughout the day. Notwithstanding, the amount of time a pup remains attached to its mother's nipple throughout the day does not imply that milk is produced in constant quantities. Further studies are needed to verify whether a bat's suckling schedule is correlated with its mother's milk composition. In most mammals the suckling schedule of females is not a reliable index of milk energy (Oftedal, 1984).

Mother–offspring conflict during the latter stages of lactation appears to be rare in bats, and mothers and their young of several species are known to forage together near or shortly after the onset of nutritional independence. Only one study has described overt mother–offspring conflict at the time of weaning. In the monogamous species, the young of *Lavia frons* were not weaned until nearly three weeks after they began to forage. During this period, females were occasionally observed 'grappling' and fending off young that were trying to suckle (Vaughan and Vaughan, 1986). Hughes *et al.* (1989) described females of *Pipistrellus pipistrellus* as being indifferent to the approach of their young as they reached weaning age, although active rejection was not observed.

Overt conflict between mother and young may not be common among bats. Mothers and their young have been observed or captured while flying together at the onset of weaning in some species: *Antrozous pallidus* (Bateman and Vaughan, 1974), *Cardioderma cor* (Vaughan, 1976), *Eptesicus fuscus* (Brigham and Brigham, 1989), *Lavia frons* (Vaughan and Vaughan, 1987), *Noctilio albiventris* (Brown *et al.*, 1983), and *Saccopteryx leptura* (Bradbury, 1977a). During their first year, juvenile *Desmodus rotundus* often feed from the same wound as their mothers (Wilkinson, 1985).

Milk composition and intake by young

Milk composition and milk energy intake by young bats, as indices of maternal effort, have been investigated in relatively few species. Both terrestrial and marine mammals exhibit considerable interspecific variation in both quality and quantity of milk produced (Oftedal, 1984, 1985; Oftedal and Iverson, 1995). Studies that have examined the nutritional requirements of female mammals and their young during lactation have largely focused on females and the proximate composition of milk. Few studies have explored the possibility that nutritional factors (e.g., minerals) other than energy and nutrients derived from fat, protein, and carbohydrates may influence postnatal growth of young bats (Barclay, 1994, 1995).

(a) Macronutrient composition. For species of bats in which the proximate composition of milk has been reported (Kunz *et al.*, 1983, 1995b; Oftedal and Iverson, 1995; Messer and Parry-Jones, 1997; Stern *et al.*, 1997; Korine and Arad,

Table 10.1 Composition of bat milks (all nutrient values are expressed as a per cent composition)

Taxa and Stage of Lactation (if given)	Wild or Captive[a]	Diet[b]	Dry Matter (%)	Fat (%)	Protein (%)[c]	Carbohydrate (%)	Citrate (%)	Ash (%)	Energy (kJ/g)	Sample Size[d]	Ref.[c]
Megachiroptera											
Pteropodidae											
Epomophorus wahlbergi	W,C	F	12.0	3.5	4.2	4.0	–	–	3.0[g]	1 (13)	10
Pteropus hypomelanus											
early	C	F	16.5	7.6	2.5	6.0	–	–	3.8	8–11	1
late			18.8	9.4	2.9	5.6	–	–	4.6	14–18	
Pteropus poliocephalus											
early	C	F	11.2	2.0	2.5	6.2	–	0.5	2.4[g]	15	8
mid			10.8	1.7	2.4	6.3	–	0.4	2.3	31	
late			11.1	1.9	2.8	5.9	–	0.5	2.4	21	
early to mid	W	F	12.7	2.2	3.6	6.4	–	0.6	2.8	1–14	8
Pteropus pumilus											
early	C	F	17.2	8.1	2.7	5.7	–	–	4.2	13	1
late			21.9	9.7	2.9	6.2	–	–	5.0	6–9	
Pteropus rodricensis											
early	C	F	18.9	8.9	3.1	6.2	–	–	4.6	4–7	1
late			22.3	8.3	3.5	6.8	–	–	5.9	5–10	
Pteropus vampyrus											
early	C	F	16.2	6.3	3.1	5.8	–	–	3.3	16–17	1
late			17.4	7.9	2.6	5.5	–	–	4.6	7	
Rousettus aegyptiacus											
early	W	F	–	2.7	2.2	5.7	–	–	2.5	1 (14)	3
mid			–	7.1	2.3	6.1	–	–	4.3	2 (23)	
late			–	9.0	2.9	5.5	–	–	5.1	2, 6	
Microchiroptera											
Molossidae											
Tadarida brasiliensis	W	I	34.4	18.9	11.1	3.7	–	0.7	10.5[g]	NG	2
	W	I	25.3[f]	16.3	C: 3.0 W: 3.2	2.8	0.2	–	8.2[g]	2 (10)	7
early	W	I	29.1	17.3	8.3	3.6	–	–	9.0	11 (64)	5
peak			36.5	25.8	7.7	3.4	–	–	12.1	21 (71)	
Phyllostomatidae											
Artibeus cinereus	W	F	30.8[f]	23.0	C: 0.6 W: 3.4	3.8	0.1	–	10.4[g]	1 (4)	7

Artibeus jamaicensis	W	F	30.6[f]	18.6	C: 1.1 W: 3.6	7.3	0.1	–	9.4[g]	2	7
peak	C	F	17.8	9.0	3.6	6.1	–	–	5.3[g]	21	9
Carollia perspicillata	W	F	–	–	C: ~7	4.1	0.2	–	–	1 (2)	7
Glossophaga soricina	W	N	11.0[f]	5.2	C: 1.1 W: 0.8	3.9	0.1	–	3.1[g]	2 (12)	7
Leptonycteris sanborni	W	N	12.1	1.7	4.4	5.4	0.2	0.6	2.6[g]	NG	2
			27.6[f]	18.5	C: 2.5 W: 1.8	4.8	–	–	8.9[g]	2 (13)	7
Phyllostomus hastatus											
early (10 d)	W	F,I	19.7	9.1	7.8	4.0	–	–	5.9	31–32	11
mid (40 d)			25.7	13.0	9.1	4.0	–	–	7.7		
late (70 d)			31.7	16.9	9.7	4.0	–	–	9.5		
Vampyrodes caraccioli	W	F	36.2[f]	29.0	C: 0.8 W: 2.3	4.1	0.1	–	12.5[g]	2	7
Vespertilionidae											
Eptesicus fuscus	W	I	25.1[f]	16.4	6.2	2.5	–	–	8.4	4 (12)	4
Myotis lucifugus	W	I	16.4[f]	6.0	C: 3.8 W: 3.5	3.1	0.2	–	4.6[g]	2 (9)	7
early	W	I	25.2[f]	13.1	8.9	3.2	–	–	7.7	4 (7)	4
mid			23.3[f]	14.3	5.8	3.2	–	–	6.9	6 (15)	
late			24.6[f]	12.4	8.7	3.5	–	–	7.5	3 (10)	
early	W	I	26.5	12.4	9.5	3.9	–	–	7.4	4 (60)	5
peak			27.1	15.8	8.5	4.0	–	–	8.6	3 (42)	
Myotis velifer											
early	W	I	25.4	11.6	9.2	4.0	–	–	7.1	3 (28)	5
peak			32.4	19.9	10.7	4.4	–	–	10.6	3 (11)	
Myotis thysanodes	W	I	40.5	17.9	12.1	3.4	–	1.6	10.4[g]	1	6

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[a] Indicates whether bats were wild caught (W) or in captivity (C) prior to milking.

[b] Indicates typical diet of species. Diets include insects (I), fruit (F), nectar (N), and blood (B). Note: Captive bats often receive nutrient supplements, however, published reports generally do not give a detailed description of the animal's diet.

[c] When casein and whey proteins are presented rather than crude protein, each component is labeled C for casein and W for whey.

[d] Samples from individual bats were pooled for analysis. Values in parentheses are number of bats milked, if different from sample size. All analyses were not performed for some samples. In this case, sample size is presented as a range. NG indicates sample size is not given.

[e] References: 1. W.R Hood and T.A. Kunz, unpublished data; 2. Huibregtse, 1966; 3. Korine and Arad, 1998; 4. Kunz *et al.*, 1983; 5. Kunz *et al.*, 1995b; 6. Jenness, 1974; 7. Jenness and Studier, 1976; 8. Messer and Parry-Jones, 1997; 9. Oftedal and Iverson, 1995; 10. Quicke *et al.*, 1984; 11. Stern, 1995.

[f] Dry matter content is predicted from the sum of the fat, protein, and carbohydrate components.

[g] Energy content calculated from the energy equivalents of fat (38 kJ/g), protein (17 kJ/g), and carbohydrate (17 kJ/g). In some cases these values were presented by the authors in kcal/g. Values are converted to kJ/g (1 kcal = 4.184 kJ) to facilitate comparison with other studies.

1998; W.R Hood and T.A. Kunz, unpublished; Table 10.1), the milk from insectivorous species generally has higher dry matter, fat, and protein content than from omnivorous or frugivorous species (Kunz and Stern, 1995; Table 10.1). Some of this variation may reflect species-specific differences in maternal diet. The relatively low fat, protein and dry matter content of milk of frugivorous species is consistent with this hypothesis given the relatively low fat and protein content of available fruit (Morrison, 1980; Fleming, 1988; Kunz and Diaz, 1995). Insectivorous bats produce milk with percentages of fat and protein which often exceed values reported for other small mammals, whereas carbohydrate content of milk is similar to that of other mammals (Jenness, 1974; Oftedal, 1984; Oftedal and Iverson, 1995). These findings also are consistent with Blaxter's (1961) hypothesis that small mammals produce milk of higher energy content than most large mammals.

For many species of bats, either the stage of lactation was not reported or sample sizes are too small to make meaningful interspecific comparisons. Jenness and Studier (1976) suggested that dry matter and fat content of the milk of some frugivorous phyllostomids (e.g., *Artibeus cinereus*, *Vampyrodes caraccioli* and *Leptonycteris sanborni*) may be similar to that of insectivorous vespertilionids and molossids (but see Huibregtse, 1966). However, sample sizes in the two latter studies were inadequate to be considered representative of these species. This problem is further highlighted by comparing the results of Jenness and Studier (1976) for *Artibeus jamaicensis* (n = 2) with that of Oftedal and Iverson (1995; n = 21). The fat composition of milk at peak lactation reported by Oftedal and Taft (unpublished data, cited in Oftedal and Iverson, 1995) is half the value reported by Jenness and Studier (1976). Thus, based on a larger sample size, the values reported in Oftedal and Iverson are considered more representative, even though values derived from the latter study were from captive bats, and those from Jenness and Studier (1976), and Huibregtse (1966) from free-ranging populations. The nutritional plane of captive bats may differ from free-ranging bats (Studier and Wilson, 1979), although Messer and Parry-Jones (1997) found no significant differences between the milk composition of wild and captive *Pteropus poliocephalus*.

(b) Fatty acid composition. Fatty acids in bat milk have been identified for 13 species (Table 10.2) and, as in other mammals, the lipid fraction is composed almost entirely of triglycerides (Davies *et al.*, 1983; Jenness, 1985). Fatty acids which esterify to form triglycerides may originate from the diet or be synthesized *de novo* in the mammary gland (Iverson and Oftedal, 1995). The balance between *de novo* and dietary fatty acids found in milk can vary according to stage of lactation and diet (Iverson and Oftedal, 1995). Dietary and milk fatty acids for a given stage of lactation have not been reported for the milk of any bat species. Concentrations of linoleic (18 : 2) and linolenic (18 : 3) in insectivorous bats are higher than has been reported for other terrestrial mammals. The concentrations of these two fatty acids are also high in insectivorous marsupials and thus may reflect their insectivorous diet (Iverson and Oftedal, 1995). Fatty acid signatures of milk have been used to identify dietary items and dietary shifts in pinnipeds (Iverson, 1993; Iverson *et al.*, 1997), but whether such signatures ultimately prove useful for identifying dietary shifts in bats remains to be determined.

Table 10.2 Fatty acid composition of bat milks. All fatty acids expressed as a percentage of total fat composition. Fatty acids are expressed as length of fatty acid : number of double bonds

Taxa	Wild or Captive[a]	Diet[b]	10:0	12:0	14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3	Other[c]	Ref.[d]
Microchiroptera															
Molossidae															
Tadarida brasiliensis	W	I	trace	trace	3.0	1.2	23.8	1.9	0.4	5.6	49.8	14.3	–	–	4
Phyllostomidae															
Artibeus cinereus	W	F	–	–	7.5	–	38.0	6.5	–	5.2	41.0	0.4	–	2.0	2
Artibeus jamaicensis	W	F	–	–	5.6	–	34.6	6.6	–	5.4	44.2	3.4	2.5	2.4	2
Carollia perspicillata	W	F	–	–	9.4	–	32.6	7.6	–	2.5	24.9	3.5	–	0.4	2
Diphylla ecaudata	W	B	–	–	4.0	–	37.2	6.6	–	4.9	43.1	4.2	–	–	2
Glossophaga soricina	W	N	–	0.2	12.0	–	37.4	10.7	–	2.2	–	2.1	–	1.2	2
Leptonycteris sanborni	W	N	trace	2.5	21.0	1.9	40.9	6.6	0.2	3.3	23.4	–	–	–	4
			–	2.0	25.6	–	31.2	11.4	–	2.1	24.9	0.6	1.8	2.3	2
Vampyrodes caraccioli	W	F	–	–	6.0	–	30.2	2.9	–	5.4	33.5	6.3	15.9	–	2
Vespertilionidae															
Eptesicus fuscus	W	I	–	3.9	1.5	–	21.5	8.3	–	7.4	37.9	15.7	2.1	1.9	3
Myotis lucifugus	W	I	–	3.8	4.6	–	22.6	18.6	–	6.5	22.8	10.6	7.2	3.2	1
	W	I	–	4.1	3.3	–	21.0	11.6	–	5.4	24.7	12.0	11.0	7.0	3
Myotis thysanodes	W	I	–	0.3	0.9	–	18.7	7.5	–	4.0	36.0	22.2	9.3	0.5	1

[a] Indicates whether bats were wild caught (W) or in captivity (C) prior to milking.

[b] Indicates typical diet of species. Diets include insects (I), fruit (F), nectar (N), and blood (B). Note: Captive bats often receive nutrient supplements, however, published reports generally do not give a detailed description of the animal's diet.

[c] Other fatty acids include those fatty acids not measured directly. This column may include fatty acids described by other investigators.

[d] References: 1. Glass and Jenness, 1971; 2. Jenness and Studier, 1976; 3. Kunz *et al.*, 1983; 4. Stull *et al.*, 1966.

Table 10.3 Mineral composition of bat milks. All mineral values expressed as ml/g dry mass (g)

Species	Wild or Captive[a]	Diet[b]	Na$^+$	K$^+$	Ca^{2+}	Mg^{2+}	Fe	P	Sample Size[c]	Ref.[d]
Megachiroptera										
Pteropodidae										
Cynopterus horsefeldi	W	F	7.9	11.6	9.0	0.3	0.3	–	1	3
Dyacopterus spadiceus	W	F	3.0	16.8	14.3	0.4	0.3	–	1	3
Megaerops ecaudatus	W	F	5.7	25.7	20.5	0.7	0.5	–	1	3
Pteropus hypomelanus	C	F	–	6.2	8.3	0.6	–	6.4	19	2
Pteropus vampyrus	C	F	–	5.3	7.6	1.2	–	7.3	13	2
Microchiroptera										
Molossidae										
Tadarida brasiliensis	W	I	1.5	3.5	6.1	0.5	0.3	–	56	3, 4
Phyllostomidae										
Carollia brevicauda	W	F	2.5	33.1	3.8	0.6	–	–	1 (2)	3
Carollia castanea	W	F	1.6	26.1	2.5	0.4	–	–	9 (11)	3
Carollia perspicillata	W	F	1.9	28.4	2.3	0.3	–	–	4 (7)	3
Phyllostomus hastatus	W	F, I	10.2	5.5	8.5	0.6	–	–	1	3
Vespertilionidae										
Eptesicus fuscus	W	I	–	6.2	8.8	1.2	–	3.6	5 (49)	1

[a] Indicates whether bats were wild caught (W) or in captivity (C) prior to milking.

[b] Indicates typical diet of species. Diets include insects (I), fruit (F), nectar (N), and blood (B). Note: Captive bats often receive nutrient supplements, however, published reports generally do not give a detailed description of the animal's diet.

[c] References: 1. W.R. Hood, unpublished data; 2. I.W.R. Hood *et al.*, unpublished data; 3. Studier *et al.*, 1995; 4. Studier and Kunz, 1995.

(c) Mineral composition. Mineral composition of bat milk has only been determined for two insectivorous (Studier and Kunz, 1995), eight frugivorous (Studier *et al.*, 1995; W.R. Hood and T.H. Kunz, unpublished), and one omnivorous species (Stern *et al.*, 1997) (Table 10.3). Moreover, stage of lactation and variation in mineral content of milk during the lactation period has only been reported for one insectivorous species, *Tadarida brasiliensis* (Studier and Kunz, 1995) and one omnivorous species, *Phyllostomus hastatus* (Stern *et al.*, 1997). Concentration of calcium, magnesium and sodium in the milk of *T. brasiliensis* declines over the course of lactation, whereas, potassium, nitrogen, and iron remain constant (Studier and Kunz, 1995). Similarly, both magnesium and calcium content decreased from birth until weaning in *P. hastatus*, but no change was observed in sodium (Stern *et al.*, 1997). Potassium, nitrogen and iron did not change over the course of lactation in *P. hastatus* (Stern *et al.*, 1997). Calcium, magnesium and potassium, however, did not vary over the course of lactation in captive *Pteropus hypomelanus* and *P. vampyrus* (W.R. Hood and T.H. Kunz, unpublished), although this may reflect a uniform diet. Sample sizes were small and stage of lactation was not reported for the frugivorous phyllostomids evaluated by Studier *et al.* (1995), thus a comparison among dietary habits could not be made. Notwithstanding, preliminary analyses suggest that the potassium content of milk in frugivorous species is generally higher than in omnivorous and insectivorous taxa (W.R. Hood and T.H. Kunz, unpublished).

Available evidence suggests that females of insectivorous bats may be severely calcium limited during lactation (Kwiecinski *et al.*, 1987; Studier *et al.*, 1991; Keeler and Studier, 1992). Young bats, as in other mammals, remain nutritionally dependent on their mothers until they are able to feed independently (Barclay, 1994; Kunz, 1987; Pond, 1977). While young of other mammals are weaned at nearly 40% of adult body mass, young bats are not weaned until they reach about 71% of adult body mass (Barclay, 1994, 1995). This extension in the duration of dependency occurs because young bats are unable to fly and feed on their own until they have reached adult dimensions (Kunz, 1987; Barclay, 1994, 1995), and mineralization of wing bones is almost complete (Swartz *et al.*, 1994; Papadimitriou *et al.*, 1996).

Bone is the primary reservoir of calcium within the body, and resorption of calcium from bone typically occurs when the diet does not meet current demands. Calcium demands are especially high during gestation and lactation, when females are faced with the additional burden of providing nutrients for foetal and neonatal development. Kwiecinski *et al.* (1987) found that bone density in females of *Myotis lucifugus* is lower during late gestation and lactation than at other times, suggesting that active bone resorption occurs at these times. In addition, based on foraging times and attack rates on insects, Aldridge and Brigham (1991) found that lactating females of *Eptesicus fuscus* foraged for a longer time than was expected to meet energetic demands, suggesting that dietary habits may be constrained by requirements other than energy. In contrast, calcium content of lactating females of *Miniopterus schreibersii* was not significantly different from those during the non-reproductive period (Bernard and Davison, 1995). Calcium absorption from bone can occur during lactation even in well nourished humans (Sowers *et al.*, 1993). Animals that experience bone loss during lactation may not remain in a state of mineral stress. At least in humans, bone density recovers quickly following weaning, and even individuals that experience a subsequent pregnancy are not at risk for substantial bone loss (Sowers *et al.*, 1995).

(d) Milk yield of mothers and intake by young. An essential aspect of understanding nutritional and energetic requirements of lactation is a reliable estimate of milk yield. Milk yield has only been measured directly in four insectivorous and one omnivorous species (Table 10.4). Based on isotope dilution, Kunz (1987) reported milk energy yield at peak lactation in *Myotis lucifugus* and *Eptesicus fuscus* as 18.5 and 22.87 kJ per day, respectively. These values were similar to those predicted from allometric equations of Hanwell and Peaker (1977) and Oftedal (1984). Using estimates of milk composition from Kunz *et al.* (1983) and data on metabolic rates of pups, Kurta *et al.* (1989, 1990) predicted milk energy yield at peak lactation in *Myotis lucifugus* and *Eptesicus fuscus* to be 19.3 and 29.7 kJ per day, respectively.

Predicted values for milk yield (Figure 10.4) are based on both the metabolic mass of females and their young, as described by Oftedal (1984). Females of *N. humeralis* which give birth to twins reportedly produce up to one-half their body mass in milk per day, and milk production apparently increases even further when litter size is three (Steele, 1991). By contrast, milk output in *E. fuscus* females as predicted by the Oftedal (1984) equation is less than the predicted values (Kunz, 1987). Values of milk yield reported for captive *P. auritus* (McLean, 1995) are nearly two times higher than predicted from Oftedal's (1984) equation.

At least two species of bats that give birth to singletons produce more milk than predicted from both allometry and litter size. Stern (1995) found that free-ranging *Phyllostomus hastatus* produced a maximum of 15.7 ml per day of milk at peak lactation. This value is comparable to estimates of milk production, based on body size, for rodents that produce more than one young (Kenagy *et al.*, 1990; Rogowitz and McClure, 1995). At peak lactation, *Tadarida brasiliensis* has a milk energy yield three times predicted values (T.H. Kunz, unpublished).

Regurgitation: special case in Desmodus rotundus

In addition to provisioning pups with milk, females of *Desmodus rotundus* regurgitate blood to their young. Within minutes of birth this blood may inoculate the digestive tract of pups with symbiotic bacteria (Müller *et al.*, 1980). However, females do not routinely feed blood meals to their young until they are about three months old, after which this behavior continues until the onset of weaning at approximately seven months (Schmidt and Manske, 1973; Wilkinson, 1984, 1988).

During 400 hours of focal observations on the behavior of free-ranging *Desmodus rotundus*, Wilkinson (1984) reported 110 bouts of regurgitation, of which 75% occurred between mothers and their dependent young. The other 25% were between females and non-dependent young, relatives, and unrelated individuals. Thus, food sharing in *D. rotundus* appears to be associated with the degree of relatedness and the opportunity for reciprocation (Wilkinson, 1984). In this species, females belong to small, stable social groups of kin and non-kin, where opportunities for

Table 10.4 Milk yield in bats based on isotope dilution. Predicted values are based on Oftedal (1984)

Taxa	Wild or Captive[a]	Diet[b]	Litter Size	Mean Body Mass (g)	Milk Yield			Ref.[c]
					Measured (ml/day)	Measured (kJ/day)	Predicted (kJ/day)	
Phyllostomidae								
Phyllostomus hastatus[d]	W	F,I	1	87.6[e]	15.7	112.6	109.3	4
Vespertilionidae								
Eptesicus fuscus[d]	W	I	2	20.2[e]	–	45.7	62.7	1
Myotis lucifugus[d]	W	I	1	7.3[e]	–	19.0	15.1	1
Nycticeius humeralis[d]	C	I	2.2	10.2[e]	4.5	37.2	32.2	3
Plecotus auritus	C	I	1	7.1[e]	2.6	22.3–23.2	13.4	2

[a] Indicates whether bats were wild caught (W) or in captivity (C) prior to milking.

[b] Indicates typical diet of species. Diets include insects (I), fruit (F), nectar (N), and blood (B). Note: Captive bats often receive nutrient supplements, however, published reports generally do not give a detailed description of the animal's diet.

[c] References: 1. Kunz, 1987; 2. McLean, 1995; 3. Steele, 1991; 4. Stern, 1995.

[d] Value for milk output at peak lactation.

[e] Virginia Hayssen, personal communication.

reciprocation are high. Females that do not obtain a nightly blood meal are more likely to be fed by a female who has recently received a meal from another member of the group.

10.2.3 Non-nutritional Care

Thermal influences

The evolution of membranous wings allows bats to exploit an aerial niche for the acquisition of food, but the increased surface area gained with the evolution of flight also increases passive thermal conductance during flight and in roosting situations (Ochoa and Kunz, 1999). Increased thermal conductance lowers the ability of bats to maintain euthermy, and thus may increase the energetic demands of thermoregulation or alternatively lead to torpor and reduced energy expenditure. For bats, as in other altricial mammals, females often help maintain the euthermic body temperature of their young by sharing body heat through conduction and radiation. This thermal influence is directly proportional to the amount of time a mother spends in contact with its young.

Because newborn bats are altricial, often lacking hair and with a limited ability to maintain euthermic body temperatures (Fujita, 1986; Kurta and Kunz, 1987), they often depend on their mothers for heat. McLean and Speakman (1997) found that for *Plecotus auritus*, physical contact between mothers and pups declined as the pups increased in age. At ambient temperatures of 10 and 20°C, newborn *Myotis lucifugus* were unable to maintain their body temperature when they roosted alone (Fujita, 1986). However, when these pups were allowed to form clusters they maintained elevated body temperatures for longer periods at these same ambient temperatures.

A decrease in body temperature in lactating females can adversely affect milk protein synthesis and output by females (Wilde *et al.*, 1999), and indirectly the growth rates of pups (Hoying and Kunz, 1998). Because torpor reduces the general level of activity in mammals, this may adversely affect the maturation of the brain, internal organs, and muscles and lead to subsequent motor defects and reduced feeding efficiency (Gubernick and Klopfer, 1981).

Rapid postnatal growth and survival of young bats is enhanced when females select roosting environments that near thermal neutrality or form dense clusters that promote efficient heat transfer to the pups. Thus, the gregarious nature of young bats in many species also promotes conductive heat exchange which is expected to facilitate digestion and improve assimilation efficiency (Twente, 1955; Davis *et al.*, 1962; Kunz, 1974), and facilitates rapid postnatal growth (Pearson *et al.*, 1952; Dwyer and Hamilton-Smith, 1965; Kunz, 1973; Tuttle, 1975).

Ambient temperatures in the thermoneutral zone of bats not only provide favorable conditions for postnatal growth (Tuttle and Stevenson, 1982; Kunz and Stern, 1995), but also reduce thermal stress to lactating females (Huibregtse, 1966). High energetic costs experienced by females during lactation in *M. lucifugus* and *M. thysanodes* exceed levels that would allow bats to physiologically regulate their body temperature (Studier *et al.*, 1973). Not unlike poikilotherms, bats may maintain their body temperatures behaviorally. Clustering of bats modifies the microclimate of the maternity roost by maintaining optimum roost temperatures (Licht and Leitner, 1967; Kunz, 1973).

Many temperate zone bats form maternity colonies ranging from a few dozen up to several million individuals (e.g., *Eptesicus fuscus*, Davis *et al.*, 1968; *Miniopterus schreibersii*, Dwyer, 1963, respectively). Within these colonies, mother–pup pairs aggregate in dense clusters, and maintain their body temperatures greater than those which roost singly (Twente, 1955). Mean rates of oxygen consumption are lower in clusters than in individuals at ambient temperatures between 15–35°C, and percentage mass loss is also decreased giving cluster members a metabolic advantage (Herreid, 1967; Trune and Slobodchikoff, 1976).

Bats and other small mammals often enter torpor when ambient temperatures are too low to maintain an elevated body temperature or when food resources are scarce (Wang and Wolowyk, 1988; Thomas, 1995; Hoying and Kunz, 1998). Because torpor slows physiological processes, including those required for milk production (Wilde *et al.*, 1999), this form of energy conservation can be disadvantageous during lactation. Some species, however, remain euthermic during lactation, and apparently do not enter torpor at this time; these include *Antrozous pallidus* and *Myotis yumanensis* (Licht and Leitner, 1967), *Tadarida brasiliensis* (Herreid, 1967; Licht and Leitner, 1967), *Miniopterus schreibersii* (Dwyer, 1964), *Myotis nigricans* (Wilson, 1971), *Lavia frons* (Vaughan, 1977), *Macrotus californicus* (Bradshaw, 1962). Others, however, enter torpor when ambient temperatures are low and food resources are scarce – *Eptesicus fuscus* (Burnett and Kunz, 1982; Audet and Fenton, 1988; Hamilton and Barclay, 1994; Grinevitch *et al.*, 1995), *Myotis lucifugus* (Studier and O'Farrell, 1972; Burnett and Kunz, 1982), *Myotis thysanodes* (Studier and O'Farrell, 1972), *Pipistrellus subflavus* (Hoying and Kunz, 1998). Hamilton and Barclay (1994) examined differential use of torpor by males and females of *Eptesicus fuscus* during the reproductive season, and found that torpor is less frequent in females when foraging conditions are poor (Grinevitch *et al.*, 1995).

Pup retrieval and transport

Transport of young bats by mothers while foraging has been reported for several frugivorous and nectarivorous species (e.g., *Carollia perspicillata*, Pine, 1972; Bradbury, 1977a; *Artibeus lituratus* and *Glossophaga soricina*, Tamsitt and Valdivieso, 1963; *Pteropus poliocephalus*, Bartholomew *et al.*, 1964; *Choeronycteris mexicana*, Mumford and Zimmerman, 1964; *Desmodus rotundus*, Schmidt and Manske, 1973), but this behavior is uncommon or rare among most small insectivorous species (*Pipistrellus pipistrellus*, Rakhmatulina, 1972; *Myotis velifer*, Kunz, 1974; *Myotis lucifugus*, Kunz and Anthony, 1996; *Hipposideros caffer*, Brosset, 1969; *Tadarida brasiliensis*, Davis *et al.*, 1962; and *Nycticeius humeralis*, Watkins and Shump, 1981). Exceptions among the insectivorous species include *Taphozous perforatus* and *Taphozous melanopogon* (Brosset, 1962a).

Transport of a pup during the lactation period is expected to increase a female's wing loading, decrease maneuverability and foraging efficiency (Norberg, 1987; Hayssen and Kunz, 1996), and increase energy expenditure (Hughes and Rayner, 1993). Since most insectivorous species seek moving prey, and many ingest up to 30% of their pre-feeding body mass during a single feeding bout (Kunz, 1974; Anthony and Kunz, 1977; Kunz *et al.*, 1995a), the additional load of transporting young bats in flight is expected to reduce the ability of a mother to feed efficiently. A small insectivorous bat, transporting one or two relatively large pups on prolonged foraging bouts, probably could not meet their daily energy demands while carrying pups.

Females of several species of bats have been observed or captured while carrying their non-volant young (Barbour and Davis, 1969; Fenton, 1969; Davis, 1970; Ansell, 1986; Baumgarten and Vieira, 1994), and some species actually carry volant young to foraging areas (Marimuthu, 1988; Radhamani *et al.*, 1990). Whether these females were transporting pups to alternative roosts in response to disturbance while on foraging bouts, is unknown in most situations. If predation is reduced by moving pups to alternative roosts, as in *Pteropus poliocephalus* (Nelson, 1965), *Saccopteryx bilineata* (Bradbury and Emmons, 1974), and *Uroderma bilobatum* (Lewis, 1992), the ability of predators to predict the location of potential prey should decrease.

Species that regularly move among several alternative tree roosts during the lactation period (Barclay and Brigham, 1996) should sustain mother–pup contact and reduce the risks of predation. Females of some species (e.g., *Megaderma lyra*) move their young to night roosts while they are foraging (Marimuthu, 1988), but because of the high cost of flight associated with bats carrying extra loads (Hughes and Rayner, 1991, 1993), mothers are unlikely to transport their young on prolonged foraging flights unless they benefit by experiencing reduced risks of predation or young bats are able to learn important foraging skills (Radhamani *et al.*, 1990).

Alloparental care

Alloparental care (non-offspring care) has been observed in several species of mammals (Gubernick and Klopfer, 1981; Reidman, 1982), but reports of care-giving behavior by unrelated bats is rare. Observations of allogrooming in bats have been made mostly in captive situations. Under these conditions, females typically groom their young during and immediately following parturition (Kleiman, 1969; Kunz *et al.*, 1994a; McLean, 1995; W.R. Hood, personal observations). The most thorough documentation of allogrooming in bats was reported for *Plecotus auritus* (McLean and Speakman, 1997), where it was shown that the relative amount of time that females spent allogrooming decreased over the course of lactation. Similar observations were reported by Kleiman (1969) for *Nyctalus noctula*, although the significance of such declines in allogrooming remains unclear.

Only a few studies have reported allogrooming activities in free-ranging populations. Mothers may lick, nuzzle, scratch, rub, or hang in direct physical contact with their pups (Burnett and August, 1981; Winchell and Kunz, 1996). In *Desmodus rotundus*, allogrooming not only occurs between mother and offspring, but also this behavior is reciprocated among individuals of different age groups (Wilkinson, 1986). Allogrooming in *D. rotundus* appears to be independent of roost and ectoparasite levels, and thus may facilitate individual recognition in roosting situations and could facilitate food sharing (Wilkinson, 1986).

Alloparental care was observed at the time of parturition in captive *Pteropus rodricensis* (Kunz *et al.*, 1994a). A single female assisted and tutored an unrelated female before, during, and after parturition. The helper female groomed the mother's anovaginal region, grasped her with partially outstretched wings, fanned her, 'tutored' her in the feet-down birthing position, groomed the emerging pup, and physically nudged the pup toward its mother's nipple. Similar behaviors have been observed on other occasions in this species (W.R. Hood, personal observations). Reports of 'baby-sitting' have been made for *Myotis thysanodes* (O'Farrell and Studier, 1973) based on the presence of a few adult females in a maternity roost where large numbers of pups were present. An alternative explanation for this and similar observations is that females in late pregnancy may delay departure from a roost when parturition is imminent (Kunz, 1973), and thus give the appearance to an observer that adults were baby-sitting.

Most females selectively nurse their own offspring, although there have been reports of misdirected nursing in captive groups of *Pipistrellus pipistrellus* (Kleiman, 1969; Eales *et al.*, 1988; Hughes *et al.*, 1989; De Fanis and Jones, 1996). In free-ranging populations of *Tadarida brasiliensis* (McCracken and Gustin, 1991), pups may engage in milk stealing, and in *Nycticeius humeralis* mothers sometimes nurse unrelated offspring as the latter approach weaning age (Wilkinson, 1992a). On theoretical grounds, one could expect alloparental nursing to evolve through kin selection or reciprocity if the benefits of provisioning non-related pups outweighed the costs, especially in colonies with high levels of relatedness. However, there is no evidence for reciprocity or kin-selection in either *T. brasiliensis* (McCracken *et al.*, 1994) or *N. humeralis* (Wilkinson, 1992a). Wilkinson (1992b) suggested that females of *N. humeralis* may gain an immediate benefit by dumping excess milk to unrelated offspring, if foraging costs are lower with a reduced body mass. Alternatively, females may gain a delayed benefit if they nurse unrelated pups that return to the same colony in subsequent years and share information about feeding and roosting sites (Wilkinson, 1992b).

10.2.4 Paternal Care

Pup and mate guarding

The roosting and social habits of most tropical species remain unknown (Kunz, 1982), and evidence for a male's contribution to roost establishment, maintenance, and mate and pup guarding are known for only a few species. In most temperate zone bats, maternity roosts are inhabited largely by females and their young, where males assume little or no responsibility for parenting. However, in tropical regions, males of some species defend roosts or harems and thus may invest considerable effort during the period when females are gravid and young are present. In *Carollia perspicillata, Phyllostomus hastatus* and *Artibeus jamaicensis*, males incur costs by defending females and their pups from other males at roost sites, although such defense may occur independently of the presence of pups (Porter, 1979; McCracken and Bradbury, 1981; Morrison and Morrison, 1981; Kunz *et al.*, 1998a). Harem males of some emballonurids (*Saccopteryx bilineata* and *S. leptura*) incur costs by defending females on foraging grounds and at roost sites (Bradbury and Emmons, 1974; Bradbury and Vehrencamp, 1977a), suggesting that such defense is not directly linked to paternal care of offspring. In some monogamous species, *Lavia frons* (Vaughan and Vaughan, 1987), *Cardioderma cor* (Vaughan, 1976), and *Vampyrum spectrum* (Vehrencamp *et al*., 1977), males may protect females and their young from predators by defending the foraging territories in which young bats learn to feed.

The investment that some species make in the construction of tents, and defense of the same, could be included as paternal care, assuming that the investment that males make benefits their progeny. In one species where males have been observed in the act of tent making (*Cynopterus sphinx*, Balasingh *et al*., 1995), harem males defend tents and their female occupants at least during the pregnancy and pup rearing periods. Twice each year, males of *C. sphinx* sever and modify stems, leaves, and vines of selected plant species, forming stem tents (Kunz *et al*., 1994b; Balasingh *et al*., 1995; Bhat and Kunz, 1995). A single male may spend from 30 to 50 days constructing one tent and several additional months defending females and their pups. Similar types of paternal effort can be expected for other tent-making species (Kunz *et al*., 1994b; Kunz and McCracken, 1996; Timm, 1987; Tan *et al*., 1998).

Male lactation

Direct parental care in most mammals is the exclusive domain of females (Clutton-Brock, 1991), although anecdotal evidence of mammary development and production of milk has been reported for males of some species, including bats. The first circumstantial evidence that male bats may lactate was suggested by Dobson (1878), who observed males of *Cynopterus brachyotis* and *C. sphinx* with nipples as large as those of lactating females. Although Dobson gave no evidence that milk was expressed from the mammary glands of males, he suggested that males may assist females by nursing young when twins were born (although twins are rare in the Pteropodidae). This suggestion has not been verified from behavioral or morphological evidence for these or other species (Ryberg, 1947). However, mammary hypertrophy and secretion of milk by males (male galactorrhoea) has been observed in two plant-visiting pteropodids, *Dyacopterus spadiceus* in Malaysia (Francis *et al*., 1994) and *Pteropus capistratus* in Papau New Guinea (F. Bonaccorso, personal communication). Females and mature males of *D. spadiceus* captured in August at Kuala Lompat, Malaysia each had functional mammary glands. While 350 μl of milk was expressed from the nipple of a single adult female, only 4–6 μl of a similar fluid was expressed from males. The nipples of males were smaller and less keratinized than those of lactating females, suggesting little or no suckling had occurred (Francis *et al*., 1994). Whether lactation in male bats is an aberrant condition or has functional significance remains to be clarified.

The environmental conditions and physiological status required to promote lactation are unknown in male bats. Lactation could be stimulated by alterations in hormone production (including elevated oestrogen and progesterone), liver malfunction (which may elevate circulating oestrogen due to the inability of the liver to convert testosterone to dihydrotestosterone, an oestrogen precursor), and conversion of androgen into oestrogen locally within the mammary tissue as occurs during neonatal hypothalamic masculinization (Francis *et al.*, 1994). Consumption of plants which contain naturally occurring phytoestrogens, or consumption of plants contaminated with breakdown products of pesticides (many of which have oestrogenic properties) are likely alternative hypotheses to account for spontaneous lactation in males. It is also possible that male lactation in bats is an evolved condition. Because most mammals are polygynous, one would not expect lactation to have evolved in species with a polygynous mating system. However, monogamous relationships, where males and females share in the care of offspring, could provide the appropriate social conditions necessary for the evolution of lactation in males.

10.3 SIZE AND DEVELOPMENTAL STATE AT BIRTH

Bats exhibit several reproductive specializations which contrast with those of similarly-sized terrestrial mammals, including relatively long periods of gestation, small litters, and large neonatal body masses (Kurta and Kunz, 1987; Hayssen and Kunz, 1996). These specializations are generally considered adaptations to an aerial mode of locomotion which, among mammals, is exclusive to bats. Gestation in bats is relatively long, with the duration between copulation and parturition in many species extended by at least one of several forms of delay: delayed implantation, delayed development, and reduced foetal growth (Racey, 1973; Racey, 1982; Racey and Entwistle, this volume). Bats typically have singleton litters, but their litter mass is comparable to those of similar-size terrestrial mammals (Hayssen *et al.*, 1993; Hayssen and Kunz, 1996; Kurta and Kunz, 1987).

Litter mass among bats is highly correlated with maternal body mass, and this relationship is allometric, with smaller bats having relatively large offspring and larger bats having relatively small offspring (Hayssen and Kunz, 1996). Mean litter to maternal mass is 28.3% for microchiropterans and 17.7% for megachiropterans. Microchiropterans exhibit a greater range of litter masses relative to maternal mass, suggesting that litter size is not strictly constrained by maternal body size, as appears to be the case for megachiropterans where litter mass is more highly correlated with maternal body mass (Hayssen and Kunz, 1996).

Geometry of wing shape relative to body mass sets an upper limit on the mass that a bat can effectively carry in flight, whether the additional mass is imposed by a stomach full of food and/or a developing foetus. Bats that have lower intrinsic wing loadings should be better able to carry greater mass relative to those with higher wing loadings (Norberg and Fenton, 1988; Norberg and Rayner, 1987). A full-term foetus can impose increases in wing loading up to 44% over non-gravid conditions (Funakoshi and Uchida, 1981; Hoying and Kunz, 1998), and thus would be expected to increase the power required to sustain flight (Hayssen and Kunz, 1996).

Wing loading among the Microchiroptera is highly variable, which may reflect their diverse dietary habits and foraging strategies (Hayssen and Kunz, 1996). The highest wing loading can be found among the molossids and phyllostomids. Members of these families have wing loadings that are similar to those predicted by dimensional analysis, and thus may function near the physical limits of dimensional scaling (Hayssen and Kunz, 1996). Vespertilionids and rhinolophids have the lowest wing loading values relative to body mass, allowing these groups to carry the same proportions of their body mass as molossids and phyllostomids at lower energetic cost (Hayssen and Kunz, 1996). Thus, low intrinsic wing loadings among the vespertilionids may be a key factor in promoting the evolution of litter sizes greater than one.

Large size of bats at birth is generally associated with an advanced stage of development; yet this is true for only a handful of characteristics. Large size of neonates may support an advanced state of neuromuscular development at birth (Kurta and Kunz, 1987), as is evident by well-developed hind limbs, allowing pups to cling tenaciously to their mothers or the roost substrate. Despite their relatively large neonatal size, newborn bats are functionally altricial, based largely on the sparsity of their pelage, inability to thermoregulate (Fujita, 1986), and nutritional dependence on their mothers (Kurta and Kunz, 1987). Lactational requirements of females may be reduced if the gestational period is prolonged and young are born at an advanced size (Kurta and Kunz, 1987). This pattern appears to hold true only for members of the family Vespertilionidae, which have large young and a relatively short lactation period. By contrast, rhinolophids produce large neonates and nurse their young for extended periods (Hayssen and Kunz, 1996).

10.4 POSTNATAL GROWTH

Postnatal growth is an important life-history trait in mammals (Case, 1978; Ricklefs, 1979) because it reflects a major component of parental effort (Oftedal, 1984; Kirkwood, 1985; Costa *et al.*, 1986; Gittleman and Oftedal, 1987; Oftedal and Gittleman, 1988; McLaren, 1993; Kunz and Stern, 1995). Published analyses of postnatal growth and life-history variation in mammals have grossly underrepresented bats (e.g., Wootton, 1987; Harvey and Read, 1988; Read and Harvey, 1989; Promislow and Harvey, 1990) or excluded them entirely (e.g., Millar, 1981; Western and Ssemakula, 1982; Martin, 1984; Martin and McLarnon, 1985). Despite the relatively recent allometric analyses of postnatal growth in bats (Kunz and Stern, 1995), little attention has been given to factors influencing individual variation, and few studies have considered proximate and evolutionary forces that may shape patterns of postnatal growth in the Chiroptera.

In Case's (1978) allometric analysis of postnatal growth in terrestrial vertebrates, he included only 17 species representing two families of bats. His analysis was limited because only the early period of linear growth was examined and the effects of body size were not removed from his analysis. Postnatal growth data on bats available at the time of Case's analysis (see Orr, 1970) were strongly biased toward small insectivorous species (<30 g as adults), and most of the taxa represented were members of a single family (Vespertilionidae). Case (1978) found from his analysis that bats had intermediate growth rates, although he offered no explanation for this conclusion. Tuttle and Stevenson (1982) summarized data on postnatal growth for 24 taxa, but included species on which both longitudinal and cross-sectional methods of data collection were used (see below). The allometric analysis of Kunz and Stern (1995) included 33 taxa, representing seven families, and found that proximate factors known to influence postnatal growth rates include food supply, climate, habitat, maternal factors, and social environment. Here, we summarize interspecific and intraspecific patterns of postnatal growth in the Chiroptera by presenting empirical data, results derived from the logistic growth model, and an evaluation of both maternal and environmental factors judged to be important selective forces which may influence these patterns.

10.4.1 Growth Models and Analytical Considerations

Data on length of forearm, body mass at birth, age at weaning, and rates of postnatal growth derived from the logistic growth model are summarized in Table 10.5. Growth parameters for length of forearm and body mass were derived from the Marquardt-Levenberg algorithm (Marquardt, 1963). We used the logistic model because our previous findings (Kunz and Stern, 1995; Kunz and Robson, 1995; Stern and Kunz, 1998) showed that this model provides a better fit to empirical data than either the von Bertalanffy or Gompertz models (but see Hughes *et al.*, 1995). Kunz and Stern (1995) analysed growth data on body mass because this variable is more sensitive to environmental variation than length of forearm (see Hoying and Kunz, 1998).

Empirical methods for quantifying postnatal growth in bats are equally appropriate for free-ranging and captive populations (Kunz, 1987). Ideally, studies on postnatal growth should be based on the recapture and measurements of known-age, marked individuals. In field situations, successful recaptures of pups will vary depending on size of the colony, fidelity of mothers and their pups to the roost site, and relative access that investigators have to mothers and pups (Kunz, 1987). In captive situations, care must be taken to ensure that feeding schedules provide adequate nutrition, appropriate roosting environments, and adequate space to allow bats to exercise (McLean, 1995).

Linear models, using least square regression for quantifying rates of change in length of forearm, body mass, and cartilaginous epiphyseal gaps can be used effectively to describe rates of change during different phases of the postnatal period (Kunz and Anthony, 1982; Burnett and Kunz, 1982; Cosson *et al.*, 1993; Kunz and Robson, 1995; Stern and Kunz, 1998). Equations derived from these analyses also can be valuable for assigning ages to bats during the postnatal period (Anthony, 1988) and stages of lactation to mothers when mother–pup pairs can be captured (Kunz *et al.*, 1995a).

Postnatal growth rates derived from measurements of pups captured on different dates (cross-sectional or grab samples) usually yield highly biased results as compared to mark-recapture data (longitudinal samples). As the growth period progresses, the capture of smaller and younger pups is often easier and, as a consequence, the empirical growth curves based on these data have lower slopes than those based on recaptured individuals. Baptista *et al.* (in press) quantified growth rates in free-ranging little brown bats (*Myotis lucifugus*) to compare longitudinal (mark-recapture) with cross-sectional samples. They found that cross-sectional samples significantly underestimated growth rates for length of forearm,

Table 10.5 Logistic growth in body mass and length of forearm for bats and their ecological and physiological correlates. Growth data and equations are derived as described by Kunz and Stern (1995). Only those studies where the exact age of pups is known are included. TR = tropical, TE = temperate.

						Body mass[c]					Length of Forearm[c]					
Taxa	Wild or Captive[a]	Diet[b]	Climate	Litter Size	Weaning (d)	Mass at Birth (g)	Adult Mass (g)	Sample Size[d]	Asymptotic Mass (g)	Growth Constant *K* (1/time)	Forearm at Birth (mm)	Adult Forearm (mm)	Sample Size[d]	Asymptotic Forearm (mm)	Growth Constant *K* (1/time)	Reference
Megachiroptera																
Pteropodidae																
Cynopterus brachyotis	C	F	TR	1.0	–	–	36.4	12 (81)	32.16	0.04	–	65.1[e]	12 (81)	60.4	0.06	W.R. Hood & T.H. Kunz, unpublished
Cynopterus sphinx	C	F	TR	1.0	–	–	–	(8)	42.10	0.04	–	–	–	–	–	Krishna & Dominic, 1983
Epomophorus wahlbergi	C	F	TR	1.0	81.1	16.1	129.6	–	–	–	30.9	–	(13)^{M} (16)^{F}	82.4^{M} 78.0^{F}	0.03^{M} 0.04^{F}	Sowler, 1983
Hypsignathus monstrosus	C	F	TR	1.0	–	40.0	420.0^{M} 234.0^{F}	(8)^{M} (17)^{F}	435.0^{M} 191.9^{F}	0.01^{M} 0.52^{F}	–	134.0^{M} 120.0^{F}	(15)^{M} (8)^{F}	126.1^{M} 74.8^{F}	0.03^{M} 0.01^{F}	Bradbury, 1977b
Pteropus hypomelanus	C	F	TR	1.0	–	74.0	570.7	(417)	515.2	0.01	62.0	157.4	(419)	153.3	0.02	W.R. Hood & T.H. Kunz, unpublished
Pteropus poliocephalus	C	F	TR/TE	1.0	–	76.0	639.5	1(18)	583.9	0.02	–	149.1	1 (17)	153.2	0.03	W.R. Hood & T.H. Kunz, unpublished
Pteropus pumilus	C	F	TR	1.0	–	40.6	174.8	(95)	171.8	0.02	44.9	152.2	(102)	102.7	0.03	W.R. Hood & T.H. Kunz, unpublished
Pteropus rodricensis	C	F	TR	1.0	–	39.2	264.7	(166)	248.5	0.01	51.9	124.4	(180)	121.5	0.02	W.R. Hood & T.H. Kunz, unpublished
Pteropus scapulatus	C	F	TR	1.0	–	–	362.0	6 (81)	379.1	0.02	–	161.0	6 (69)	146.1	0.02	G.M. O'Brien, pers. comm.
Pteropus vampyrus	C	F	TR	1.0	–	133.3	1132.6	(273)	755.5	0.02	79.5	224.7	(269)	190.4	0.03	W.R. Hood & T.H. Kunz, unpublished
Microchiroptera																
Emballonuridae																
Peropteryx kappleri	W	I	TR	1.0	–	1.5	12.8[e]	2(18)	15.4	0.08	20.0	48.0[e]	2 (18)	50.5	0.07	Giral *et al.*, 1990
Taphozous longimanus	W	I	TR	1.0	17.5[e]	–	29.0[e]	(9)	20.6	0.07	–	61.6[e]	–	–	–	Krishna & Dominic, 1983
Molossidae																
Molossus molossus	C	I	TR	1.0	65.0	3.6[e]	15.1	14 (140)	12.3	0.08	17.0	38.1	15 (319)	40.7	0.05	Häussler *et al.*, 1981
Tadarida brasiliensis	W	I	TE	1.0	–	3.2	12.0[e]	49 (161)	10.6	0.17	18.5	43.0[e]	49 (161)	44.6	0.09	T.H. Kunz & S.K. Robson, 1995
Noctilionidae																
Noctilio albiventiris	C	I	TR	1.0	80.5	–	37.7[e]	20 (290)	25.4	0.06	–	65.0[e]	20 (293)	57.9	0.07	Brown *et al.*, 1983
Phyllostomidae																
Artibeus jamaicensis	C	F	TR	1.0	66.3	13.9	45.0[e]	22 (97*)	52.8	0.04	33.5	60.5	22 96)	60.5	0.08	Taft & Handley, 1991
Carollia perspicillata	C	F	TR	1.0	23.0	5.0	17.6	17 (21*)	17.2	0.10	24.2	43.6[e]	17 (21*)	42.1	0.01	Kleiman & Davis, 1979
Desmodus rotundus	C	B	TR	1.0	285.0	7.0[e]	29.0[e]	4 (179)	29.9	0.01	–	59.0[e]	–	–	–	Schmidt & Manske, 1973
Phyllostomus discolor	C	F	TR	1.0	–	7.0[e]	39.7[e]	6 (108)	42.5	0.05	–	62.4[e]	6 (108)	65.5	0.01	Rother & Schmidt, 1985
Phyllostomus hastatus	W	F, I	TR	1.0	–	16.4	84.0[e]	21 (197)^{M} 18 (168)^{F}	77.9^{M} 68.3^{F}	0.06^{M} 0.06^{F}	34.4	81.9	20 (172)^{M} 18 (200)^{F}	82.1^{M} 83.52^{F}	0.07^{M} 0.07^{F}	Stern & Kunz, 1998

Rhinolophidae																
Rhinolophus ferrumequinum	C	I	TE	1.0	60.0[e]	5.8[e]	22.9[e]	–	15.6	0.13	–	58.7[e]	–	–	–	Ransome *et al.*, unpublished
Vespertilionidae																
Antrozous pallidus	C	I	TE	1.8	–	3.1[e]	18.8[e]	(377)	22.3	0.96	–	54.5[e]	(417)	54.6	0.10	Brown, 1976
Eptesicus fuscus	W	I	TE	2.0	36.0[e]	3.5[e]	20.2[e]	(256)	11.5	0.14	–	45.2[e]	(254)	44.8	0.13	W.R. Hood, unpublished data
Eptesicus serotinus	C	I	TE	1.5	24.0	5.8	23.5[e]	3 (28*)	28.9	0.04	–	53.0[e]	4 (22*)	49.3	0.14	Kleiman, 1969
Myotis daubentonii	W	I	TE	1.3	–	2.3	7.0	5–7 (10*)	7.5	0.10	14.9[e]	37.6	5–7 (10*)	37.2	0.15	Krátky, 1981
Myotis macrodactylus	W	I	TE	1.0	–	1.5[e]	7.1[e]	–	–	–	–	37.6[e]	–	37.6	0.12	Maeda, 1976
Myotis lucifugus	W	I	TE	1.0	25.0[e]	2.2	7.3[e]	(75)	6.8	0.22	15.8	36.4[e]	(65)	41.9	0.27	Kunz & Anthony, 1982
Myotis myotis	W	I	TE	1.0	31.5	6.2	24.9	53 (45)	26.6	0.09	23.7	63.7	53 (61*)	59.5	0.15	de Paz, 1986
Myotis thysanodes	W	I	TE	1.0	21.0	–	8.0[e]	–	–	–	–	–	–	47.6	0.13	O'Farrell & Studier, 1973
Myotis velifer	W	I	TE	1.0	43.0[e]	3.2[e]	8.9[e]	(280)	8.9	0.17	–	39.6[e]	(280)	44.8	0.12	T.H. Kunz & S.K. Robson, unpublished
Nyctalus lasiopterus	C	I	TE	1.7	–	5.3[e]	58.5[e]	(205)	26.2	0.12	22.1	62.0	5 (201)	60.6	0.14	Maeda, 1972
Nyctalus noctula	C	I	TE	1.2	60.0	5.7	20.4	10 (30)	27.6	0.12	–	51.0[e]	10 (27*)	49.3	0.11	Kleiman, 1969
Nycticeius humeralis	C	I	TE	2.1	30.0	2.0	10.2[e]	(63)	6.4	0.06	–	36.7[e]	28(162)	33.1	0.09	Jones, 1967
Plecotus auritus	C	I	TE	1.4	42.3	–	7.1[e]	–	5.9	0.25	–	38.0[e]	–	36.9	0.19	De Fanis & Jones, 1995a
Pipistrellus mimus	W	I	TR	2.0	52.0	0.5[e]	2.0[e]	20 (92)	3.4	0.11	–	27.0[e]	20 (109)	27.0	0.12	Isaac & Marimuthu, 1996
Pipistrellus pipistrellus	W	I	TE	2.0	51.0	1.1	5.1	14–36 (13*)	3.7	0.04	–	31.4[e]	14–36 (13*)	31.2	0.12	Rakhmatulina, 1972
Pipistrellus savii (= *Hypsugo savii*)	W	I	TE	1.8	–	–	4.0[e]	(113)	5.5	0.19	–	–	(118)	41.3	0.16	Tiunov, 1992
Pipistrellus subflavus	W	I	TE	2.0	–	1.6	6.4	50 (383)	5.2	0.13	13.2	34.9	50 (401)	34.8	0.15	Hoying & Kunz, 1998
Scotophilus heathi	W	I	TR	1.9	21.0	5.0	33.5	(5*)	28.6	0.10	–	61.5[e]	–	–	–	Krishna & Dominic, 1983
Vespertilio murinus	C	I	TE	2.0	28–36	–	–	–	–	–	14.8	45.1	8	43.4	0.17	Kozhurina, 1998
Vespertilio superans	W	I	TE	2.0	35.0[e]	2.0[e]	18.4[e]	(64)	15.3	0.11	–	–	(71)	53.7	0.10	Tiunov, 1989

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[a] Indicates whether bats were wild caught (W) or in captivity (C) prior to milking.

[b] Indicates typical diet of species. Diets include insects (I), fruit (F), nectar (N), and blood (B). Note: Captive bats may have also received nutrient supplements, however, this is generally not stated in published reports.

[c] Mass and forearm measurements differentiated as male (M) and female (F) for sexually dimorphic species.

[d] Number of individuals (number of data points on which growth curve is based) is given in parentheses; *published data represent means for each given age.

[e] Virginia Hayssen, personal communication.

body mass, and length of epiphyseal gaps. Growth rates derived from cross-sectional samples underestimated length of forearm and body mass by 40% and 30%, respectively. In a study where cross-sectional sampling was used, corrections based on growth rates of known-age individuals were needed to compensate for potential biases (see Tuttle, 1975). Because no independent comparisons are available to validate this and other growth curves where cross-sectional samples were used (Dwyer, 1963; Pagels and Jones, 1974; Short, 1961; Thomas and Marshall, 1984), no further considerations are given to these studies in the present chapter.

Postnatal growth rates of some species reared in captivity may differ from those derived from free-ranging populations. For example, captive *Antrozous pallidus* and *Pipistrellus pipistrellus* grew faster than individuals of the same species in free-ranging populations (Kunz, 1987), and similar differences were reported for *Epomophorus wahlbergi* (Sowler, 1983). Although differences in roost environment and social conditions invariably exist between captive and free-ranging colonies, contrasting planes of nutrition may account for most of the reported differences in postnatal growth patterns. In several previous studies where bats have been reared in captivity, individuals were fed questionable diets and were seldom, if ever, given an opportunity to fly. Free-ranging bats also may experience depressed growth rates owing to fluctuating and sometimes unpredictable environmental conditions that reduce the availability of food to mothers and/or depress metabolic rates of pups and mothers (Hoying and Kunz, 1998).

10.4.2 Interspecific Variation

Among the species of bats compared allometrically by Kunz and Stern (1995), a significant negative correlation was found between postnatal growth rates and body mass (Figure 10.2). No significant phylogenetic effect (Megachiroptera v. Microchiroptera) on postnatal growth was found after removing the effect of body mass. The absence of a significant phylogenetic effect was not surprising, given the small sample of megachiropterans (n = 5) available for analysis. After removing the effects of body mass, latitude (temperate v. tropical) was the only extrinsic variable that significantly affected postnatal growth rate, suggesting that temperate zone bats have higher growth rates than their tropical counterparts (Figure 10.3A). A significant negative allometric relationship was found separately for insectivorous bats, as species from temperate regions showed higher growth rates than those from tropical regions (Figure 10.3B). When tropical species were examined alone, no significant relationship between asymptotic body mass and postnatal growth was observed, perhaps because of small sample size.

Postnatal growth rates in frugivorous species were more associated with asymptotic mass than insectivorous species (Figure 10.4). This latter observation supports the hypothesis that postnatal growth rates in tropical frugivorous species may be influenced by a relatively constant diet, whereas insectivorous bats may more likely experience greater variation in food quality and quantity and, thus, exhibit more variation in growth rates. No effects of whole animal basal metabolic rate or mass-specific metabolic rate on postnatal growth rates of bats were found (Kunz and Stern, 1995).

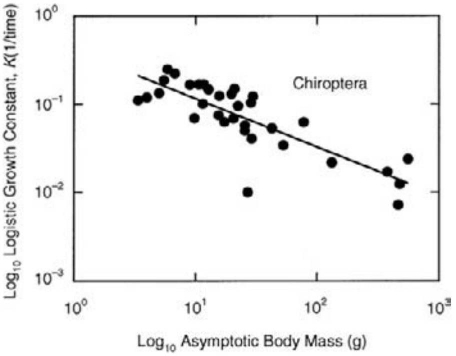

Figure 10.2 Allometric relationship between postnatal growth rates and asymptotic body mass in the Chiroptera (after Kunz and Stern, 1995).

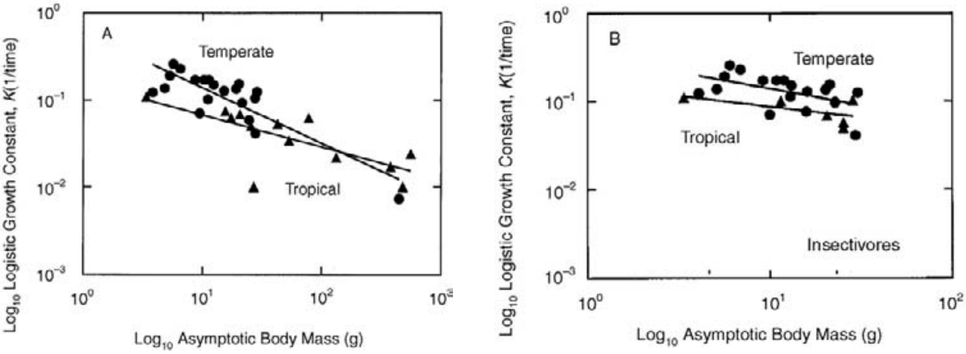

Figure 10.3 A. Allometric relationships between postnatal growth rates and asymptotic body mass in temperate (●) versus tropical zone (▲) bats (F = 9.63, P = 0.004). B. Allometric relationship between postnatal growth rates and asymptotic body mass for temperate (●) and tropical (▲) insectivorous bats (F = 4.72, P = 0.042) (after Kunz and Stern, 1995).

Postnatal growth rates of bats from tropical regions were among the lowest among the Chiroptera (Kunz and Stern, 1995), and the vampire bat, *Desmodus rotundus*, is a conspicuous further outlier. This species has the lowest growth rate among all bats that were examined by Kunz and Stern (1995). Whether this exceptionally slow growth rate reflects dietary constraints imposed by an exclusive blood meal, social conditions, or other factors invites further study.

The analysis by Kunz and Stern (1995) suggests a strong selection pressure for rapid growth rates in temperate climates. One hypothesis to account for these higher growth rates of bats in temperate, as opposed to tropical latitudes, may be explained by selection pressures in response to a shorter growing season. Faster growth rates in temperate latitudes would allow individuals to achieve maximum

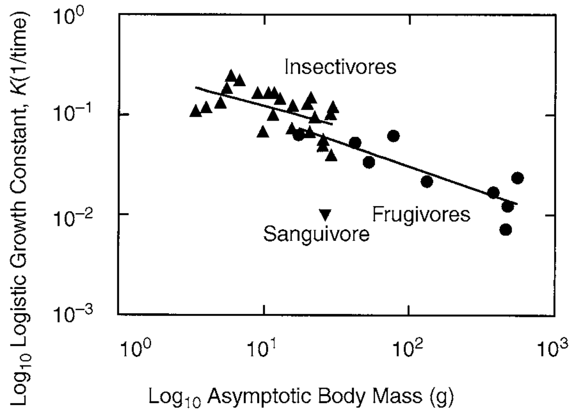

Figure 10.4 Allometric relationship between postnatal growth rates and asymptotic body mass in insectivorous (▲), frugivorous (●), sanguivorous (▼) bats. After removing the effect of asymptotic body mass, there was no significant effect of diet (insects v. fruit) on postnatal growth ($F = 1.23$, $P = 0.289$) (after Kunz and Stern, 1995).

somatic growth and to deposit important fat reserves before the onset of winter hibernation or migration (Kunz and Stern, 1995; Hoying and Kunz, 1998). This interpretation is consistent with Boyce's (1979) hypothesis that accelerated growth should be found among mammals living in highly seasonal environments, a characteristic of temperate regions. In this context, it would be interesting to compare postnatal growth rates of tropical species known to occur over a range of different altitudes.

10.4.3 Intraspecific Variation

When data on intraspecific variation in postnatal growth rates are examined, both quantitative and qualitative differences can be seen. Here we evaluate environmental and biotic factors known or suspected to affect intraspecific variation in postnatal growth rates in bats, including food supply and climate, latitude, diet, gender, litter size, colony size, season, and growth conditions (captive v. free-ranging). Because many of these factors are interdependent, we also consider possible interactions in an effort to interpret observed patterns of postnatal growth.

Local climate and food supply

Case (1978) postulated that feeding requirements of young mammals and availability of food to their parents were sufficient to account for much of the variability observed in postnatal growth rates. Growth rates of young animals should be higher if parents are subjected to higher planes of nutrition, either through natural abundance of food or through nutritional enrichment in captivity.

In free-ranging bats, meteorological conditions directly affect both food abundance and quality as well as the behavioral and physiological condition of mothers and pups. The activity of flying insects may be reduced or altered by wind, moonlight, low temperature, and precipitation (Anthony and Kunz, 1977; Anthony *et al.*, 1981; Richards, 1989; Rydell, 1989; Jones *et al.*, 1995; Hoying and Kunz, 1998).

Field observations suggest that food available to lactating females is an important factor explaining growth rates in bats. In their study of postnatal growth in *Pipistrellus subflavus* in eastern Massachusetts, Hoying and Kunz (1998) found significant inter-year variation in postnatal growth rates (Figure 10.5). They attributed this difference, in part, to the contrasting abundance of insects during the two years of study. Postnatal growth in body mass was slower in 1982 than in 1981. Births were delayed by nearly one week in 1982 and pups that were born during this inclement period experienced depressed growth. Not only were fewer flying insects available to lactating mothers, but unseasonably cool temperatures caused daily torpor in lactating females and their pups, thus contributing to the depressed postnatal growth rates. When ambient temperatures and levels of precipitation returned to normal conditions in subsequent days, the pups experienced compensatory growth (Hoying and Kunz, 1998).

Latitude and regional climate

Theoretically, populations of the same species at higher latitudes should grow faster than populations at lower latitudes, thus ensuring that individuals at higher latitudes complete somatic growth and fat deposition before the onset of migration and hibernation (Fujita, 1986). Fujita found that postnatal growth rates (body mass and length of forearm) from a population of *Myotis lucifugus* in Alberta, Canada (54°56′N) were lower than those in New Hampshire (42°25′N). Although the general trend of higher growth rates in temperate v. tropical bats may hold in an

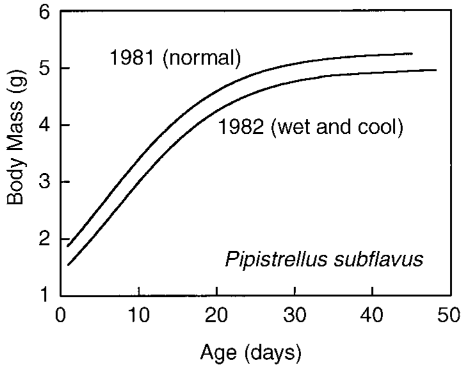

Figure 10.5 Effects of insect availability on postnatal growth rates in *Pipistrellus subflavus* in two different years (1981 and 1982). Bats increased in body mass at a significantly greater rate in 1981 than in 1982 ($F = 32.3, P < 0.001$). The year 1981 was characterized by normal seasonal temperature and precipitation, whereas 1982 experienced above normal precipitation and low ambient temperature (after Hoying and Kunz, 1998).

allometric analysis of different species (Kunz and Stern, 1995), Fujita's observation suggests that growth rate in the most northern part of a species' range may actually be lower, as influenced by energetic considerations (reduced availability of food and increased costs of thermoregulation). Cooler summer temperatures at northernmost latitudes may also directly affect availability of insects and the female's ability to convert energy and nutrients into milk.

If maintenance costs are higher for mothers and pups at colder, more northerly temperate latitudes, then less energy would be available for reproduction. For species with a geographic distribution that extends from intermediate into cool temperate latitudes, postnatal growth rates may indeed be lower at the highest latitudes. Thus, for species with geographic distributions that span from low to high latitudes (e.g., *Eptesicus fuscus*), we would predict a hyperbolic curve for growth rates, with the lowest rates occurring at the extremes and the highest rates at intermediate latitudes. Reduced postnatal growth rates, low fat deposition, and lower levels of fecundity at the northernmost latitudes of a species' range in the northern hemisphere (Kunz *et al.*, 1998b) may characterize a suite of life-history traits that limits its breeding distribution.

Season

In tropical environments, female bats that produce two or more litters per year may experience marked differences in the availability of food and water at different times of year, which in turn may influence nutrient and energy transfer from mothers to pups. McWilliam (1987) showed that early postnatal growth rates in *Coleura afra* were higher during the short rainy season in Africa than during the long rainy season which followed an extended dry period. He attributed this difference to the poor condition of females following a pregnancy when food resources were low. In India, qualitative differences in postnatal growth rates were observed between spring and summer litters of *Cynopterus sphinx* (Krishna and Dominic, 1983), further suggesting that growth rates may vary seasonally, especially in polyoestrous species.

Gender

Gender-specific differences in postnatal growth rates should be most pronounced in sexually dimorphic species. Several species of insectivorous bats are weakly sexually dimorphic, with females being slightly larger than males (Myers, 1978; Williams and Findley, 1979), but overall differences in postnatal growth rates appear to be correspondingly slight or nonexistent (Kunz, 1973; Kunz and Anthony, 1982; Burnett and Kunz, 1982; but see Kunz, 1974). Notwithstanding, some tropical frugivorus species (e.g., *Artibeus jamaicensis*, *Pteropus hypomelanus*, *Hypsignathus monstrosus*, and *Epomophorus wahlbergi*) and one omnivorous species (*Phyllostomus hastatus*) show significant sexual dimorphism in adults; this may be a consequence of gender-specific differences in postnatal growth rates (Kunz and Stern, 1995). In *P. hastatus*, where reproductive variance is much greater for males, and where body size may affect reproductive success (Stern and Kunz, 1998), mothers should invest more in their sons than in their daughters (see Trivers and Willard, 1973). Such differential allocation of maternal

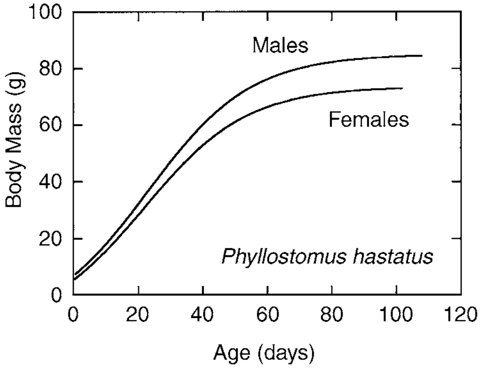

Figure 10.6 Effects of gender on postnatal growth in body mass in *Phyllostomus hastatus*. Males grew at a significantly greater rate ($t = 4.39$, $P < 0.001$) than did females from the same population in the same year (from Stern and Kunz, 1998).

resources to male pups often results in a high degree of sexual dimorphism in polygynous species (Figure 10.6 and Bradbury, 1977b), suggesting that postnatal growth rates are greater or growth continues longer in males than in females.

Litter size

Most bats produce singletons, although a few species produce litters of two or more (Tuttle and Stevenson, 1982; Kurta and Kunz, 1987; Hayssen and Kunz, 1996). Species in which twinning occurs can provide valuable data for comparing the effects of litter size on postnatal growth rates. Among North American bats which have litter sizes greater than one, postnatal growth rates have been quantified for *Antrozous pallidus*, *Pipistrellus subflavus*, and *Eptesicus fuscus*. Populations of *E. fuscus* in the western USA typically produce litter sizes of one, whereas those in the east typically produce twins (Burnett, 1983; Holroyd, 1993).

Comparisons of postnatal growth rates between populations from different geographic regions may be confounded by differences in climate, food availability, or both (see below). To avoid such geographic or other confounding effects on postnatal growth, ideally singletons and twins should be sampled from the same population. Bassett (1984) compared growth rates for length of forearm in singletons and twins of *A. pallidus* collected from the same population in California. In his captive study, pups from both groups grew at the same rate, although singleton pups were larger at birth than those from litters with twins. However, because body mass is more sensitive to environmental effects during the postnatal period than is length of forearm (Hoying and Kunz, 1998), conclusions based on a comparison of length of forearm between singletons and twins may be less revealing than those based on body mass. Holroyd (1993) compared growth rates of twins versus singletons from a free-ranging population of *E. fuscus* in Alberta, Canada, and found that both length of forearm and body mass of twin pups grew significantly more slowly than the length of forearm and body mass of singleton pups.

Roost temperature and colony size

Conditions in the roosting environment of bats (Kunz, 1982) potentially have important direct and indirect effects on intraspecific variation in postnatal growth rates (Tuttle and Stevenson, 1982). Tuttle (1975) compared pre-weaning growth rates in *Myotis grisescens* from several caves, each with contrasting roost temperatures, and found that postnatal growth rates of pups were highest in the warmest roosts. Tuttle postulated that females and pups from cooler caves allocated more energy to maintenance, and thus less energy was available for production (milk output by females and accretionary growth of pups). He also suggested that roost temperature was directly proportional to colony size, because large numbers of bats generated more heat and could more effectively increase the roost temperature of the cave (and thus reduce maintenance costs). Post-weaning growth in *M. grisescens* may also be influenced by colony size (Tuttle, 1976), as individuals from large colonies are expected to fly greater distances to feed than bats from small colonies. Tuttle argued that pups from large colonies allocated a greater amount of energy to nightly foraging flights, as pups from these colonies experienced slower post-weaning growth rates. These results suggest that interactions between roost location (relative to suitable foraging habitats, colony size and microclimate) should be considered when evaluating postnatal growth rates in bats.

10.4.4 Ontogeny of Flight, Echolocation and Feeding Behavior

Two important benchmarks for successful postnatal growth and development in bats are the attainment of flight and independence from parental care. Early postnatal development of the neuromuscular system of bats and other animals establishes the crucial circuitry for specialized tasks such as flight, navigation, and feeding skills (Powers *et al.*, 1991; Stern *et al.*, 1997). For Microchiroptera, development of echolocation is essential for feeding success, survival, and reproduction. Early development of vocal and auditory systems in bats appears to be important for the development of echolocation and social calls used to capture prey and to locate and identify mates and offspring (McCracken and Gustin, 1991; Moss *et al.*, 1997).

The extent to which either or both parents assume roles in the development of communication and echolocation skills in bats has not been thoroughly investigated. For most species, only a maternal influence is expected, because adult sexes of many species are segregated during the maternity period. Young bats of some species may accompany their mothers on foraging flights. They include *Noctilio albiventris* (Brown *et al.*, 1983), *Eptesicus fuscus* (Brigham and Brigham, 1989), and *Desmodus rotundus* (Wilkinson, 1985, 1987). Other species, such as *Myotis myotis* (Audet, 1990), *M. lucifugus* (Buchler, 1980; Kunz and Anthony, 1996; Adams, 1996), *Pipistrellus pipistrellus* (Racey and Swift, 1985), and *Rhinolophus ferrumequinum* (Jones *et al.*, 1995) appear to develop foraging skills independent of their mothers. Thus, what role mothers may assume in the development of echolocation and early foraging success of their pups will require long-term studies where both mother and pups are followed simultaneously.

In monogamous species and in polygynous species that form harems, and in species where the putative father is present during the developmental period of pups, paternal influence on the development of vocalizations (including echolocation) and early flight and foraging can be expected. In the monogamous megadermatid, *Lavia frons*, males and females typically roost near one another and hunt from perches as sit-and-wait predators (Vaughan and Vaughan, 1987). Within a week of making their first foraging flights, pups practice wing flapping as they cling to their mothers. Pups may accompany either parent to a shared feeding territory, and may also use their parent's foraging perches. Similar observations have been reported for *Cardioderma cor*, where mother–pup contact is maintained by calls produced by the mothers at foraging sites (Vaughan, 1976).

10.5 CONCLUSIONS, PREDICTIONS AND FUTURE DIRECTIONS

Future studies on parental care and postnatal growth in bats should focus attention on frugivorous, nectarivorous, sanguivorous, and carnivorous species. Most published studies on mother–pup recognition have focused on temperate insectivorous species, thus research on tropical species with other dietary habits is warranted. Although the role of acoustic communication in mother–pup reunions has received considerable attention, the role of spatial memory, olfactory, and visual cues have largely been ignored. These modes of communication are likely to play an equally important role in the identification of kin. The role of tactile stimuli, especially associated with allogrooming in pup recognition, has not been investigated.

Analysis of milk composition has been reported for relatively few species of bats. Future studies should focus on milk composition of nectarivorous, frugivorous and carnivorous species, and include analysis at different stages of lactation along with estimates of milk yield. Comparisons of milk composition and milk energy output from different species and different life histories will ultimately make it possible to evaluate the relationship between milk yield and growth rates in bats. Among species represented by different feeding guilds, we would expect maternal effort and postnatal growth rates to be greatest in insectivorous and carnivorous species, intermediate for frugivorous species, and least for nectarivorous species. Why the postnatal growth rate of a sanguivorous species is so low relative to other species invites further study.

Future studies on postnatal growth rates should be designed to integrate data on local climate, food availability, maternal diet, milk composition, milk energy yield, and non-energetic parental effort. Studies should be designed to compare parental effort and postnatal growth in species from different geographic areas, different colony sizes, and seasons. We would expect postnatal growth rates in populations that occur in highly seasonal, temperate environments to exhibit higher growth rates than those from subtropical and tropical environments, except that growth in extreme latitudes may be reduced. In tropical environments, where food resources are often highly variable in time and space (including flowers, fruits, and insects), we would expect to find seasonal differences in growth rates in those species which produce two or more litters per year.

Relationships between pup growth, colony size and the thermal environment of roosts, and the development of thermoregulatory capacity of young, should be thoroughly investigated. We would expect higher growth rates in individuals that roost in the warmest environments and maintain elevated body temperatures during the postnatal period. Parental effort and growth rates in bats should be sensitive to environmental conditions, especially if the habitat is degraded (which may increase foraging time and reduce energy transfer to pups), sizes of maternity colonies are reduced (with a decrease in thermal advantages that may accrue from warm roosts), and there are changes in the abundance and availability of food resources (seasonal and inter-year variation in insect, fruit, and nectar production). Finally, studies on parental care in bats should be extended to investigate how parental effort is adjusted to variation in benefits to offspring and costs to the parents as reflected in the fitness of individuals.

ACKNOWLEDGMENTS

We are grateful to numerous individuals who have assisted in the field and laboratory. These individuals include K. Atkinson, T. Baptista, D. Barber, F. Bonaccorso, S. Davidson, A. Field, D. Le Blanc, O.T. Oftedal, and J. Seyjagat, and R.M.R. Barclay, G. O'Brien, and E. Kozhurina made unpublished data available on postnatal growth. We also thank E. Studier, C. Voigt, and A. Stern for their critical reviews of an early version of this manuscript. Preparation of this paper was made possible by grants from the National Science Foundation (BSR 87-00585) and the Lubee Foundation, Inc. to T.H.K. This chapter is publication no. 60 of the Lubee Foundation.

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11

Environmental Regulation of Reproduction

Paul D. Heideman

11.1 INTRODUCTION

Reproductive and life-history strategies vary tremendously among bats, even within species (Chapter 9). Understanding the nature of this variation and the evolution of these strategies requires an understanding of the mechanisms responsible. Bat populations are in decline worldwide. Effective conservation strategies require some knowledge of reproduction; in some cases knowledge of cues and mechanisms may be crucial (e.g. if reproduction is impossible without a particular environmental cue – say, a plant secondary compound – and the source of that cue is missing in a particular habitat). Finally, bats are perhaps the most

Reproductive Biology of Bats
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stubbornly seasonal mammals known, following a wide range of seasonal cycles in essentially all habitats in which they occur. This suggests that bats are excellent subjects for the study of environmental regulation of seasonal reproduction. Insights gained from seasonal tropical bats, for example, might even offer valuable insights into the sources of the more subtle seasonal reproductive patterns shown by humans (Bronson, 1995), another tropical mammal.

Paul Racey (1982) produced a comprehensive and synthetic review of the reproductive ecology of bats that influenced my thinking greatly. Two aspects of Racey's review were particularly striking. First, bats followed an amazing variety of reproductive strategies, and there was abundant documentation for them. Second, we knew very little about the environmental regulation or evolution of those reproductive strategies. In particular, there were a great many studies in which environmental events were correlated with reproduction, but there was almost no information on whether particular environmental events actually caused reproductive changes in bats. In the past 15 years, our knowledge has grown, as can be recognized in this volume by dividing coverage of the topic into life-history strategies (Chapter 9) and environmental regulation (this chapter). Nevertheless, the conclusions drawn here are from a small number of studies on a small number of species.

This chapter reviews what is known about environmental factors that affect reproduction in bats in the context of both ultimate causes and underlying neuroendocrine mechanisms. The focus will be on the results of experimental laboratory studies, but some field studies that offer insights into mechanisms are also discussed. Four general questions will be addressed. First, what are the ultimate factors that favor or disfavor the environmental regulation of reproduction in bats? Second, how do signals from the environment become translated into physiological signals that alter reproductive events? Third, what additional insight is offered by field studies? Finally, what else do we need to know, and why do we need to know it?

11.2 ULTIMATE FACTORS

Having and raising offspring is the most difficult part of life. Reproduction is energetically and nutritionally expensive (Kurta *et al.*, 1989; Studier *et al.*, 1972; Wilde *et al.*, 1995). Additional time spent foraging and in reproductive behaviors presumably increases the vulnerability of bats to predators. Young bats are extremely vulnerable to both biotic and abiotic threats (Tuttle and Stevenson, 1982). In an absolutely constant environment, no one time is better for reproduction than any other, and we would expect to find no environmental effects on reproduction. However, because no terrestrial habitats are constant, there will be times and places during which reproduction will be more successful. Thus, environmental variability both directly affects reproduction and creates potential selective advantages to the use of environmental cues to regulate reproduction.

The major effect of the environment on reproduction in mammals is to change the timing of reproductive events. The timing of the reproductive cycle sets the timing of the most demanding and important events in the life of any mammal – birth, dispersal, mating, pregnancy and lactation. Differences of only a few weeks in the timing of any of these events may turn success into failure. Accurate timing is possible only if the environmental cues available are good predictors of later events and are detectable by the neuroendocrine systems of the animal. Furthermore, for a neuroendocrine link between a particular cue and reproduction to be favored by selection, an environmental cue used to regulate reproduction must have a high enough signal to noise ratio that it offers a sufficiently unambiguous signal to the animal. There is no guarantee that the environmental signals available are good enough predictors to optimize reproductive timing consistently. Nor is it likely that animals always have the sensory and neuroendocrine circuitry to make use of the best possible environmental cues. In fact, in some (or many) habitats, it may be impossible to optimize reproductive timing precisely. None of these possibilities can be addressed in bats without knowing the environmental signals and physiological mechanisms of reproductive regulation.

When is the best time for bats to reproduce? Natural selection should favor reproductive timing that maximizes lifetime reproductive success. Because the direct investment of male bats in their offspring is negligible, and because quality sperm are rarely a limiting resource, the optimal timing of reproduction for females generally controls the timing of reproduction. Females are under two potentially conflicting selective pressures in timing their reproduction. Late pregnancy, lactation and weaning are the most demanding stages of reproduction in female bats (Kurta *et al.*, 1989; Studier *et al.*, 1972; Wilde *et al.*, 1995), and the probability of survival for a female will be maximized by matching these stages with optimal environmental conditions. For a young bat, however, the most demanding period of life may be either while it is still dependent on its mother, or in the period immediately post-weaning, when many young of many species must disperse and/or gain skills and fat to survive a harsh season of the year (Bradbury and Vehrencamp, 1977). Because the survival and success of offspring is the ultimate currency of natural selection, maximum lifetime fitness may be achieved by timing reproduction to maximize the likelihood of survival of offspring instead of the mother, or to compromise between these two strategies.

Animals can follow strategies of reproductive timing that range from strict opportunism to complete reliance on an environmental cue (reviewed by Bronson and Heideman, 1994). Animals that reproduce opportunistically simply attempt to reproduce whenever conditions are good. Alternatively, animals may follow a strategy of reproducing when a predictive environmental cue indicates a likely future period of good conditions for reproduction. Because most predictable environmental variation is seasonal, in both the temperate zones and the tropics, predictive cueing generally results in seasonal reproduction. Opportunism often results in seasonal reproduction as well, simply because favorable conditions often occur seasonally (Heideman and Bronson, 1992). However, opportunism may be rare as a seasonal strategy in bats because the relatively long gestation periods of bats (2–11.5 months) make it unlikely that a female could produce a follicle, ovulate, mate, and then reach the most demanding stages of reproduction (lactation and weaning) before good conditions have passed. Instead, most seasonally reproducing bats probably react to cues that predict oncoming periods when climatic, dietary and other conditions permit successful reproduction.

Seasonal reproduction may or may not be highly synchronized among individuals. In highly seasonal reproduction, clusters of births fall at exactly the same point in each year. In highly synchronous reproduction, births are clustered in a narrow period within the year, which may or may not fall at the same time in each year. Thus, synchrony is the tendency for births to be clustered, and seasonality is the tendency for clusters of births to fall at approximately the same point in each of two or more years. Heideman (1995) discusses methods to identify synchrony and seasonality in bats. It is possible for births to be seasonal but only weakly synchronized, in which case births are spread over many months of the year. Synchrony of reproduction may be favored by factors that are unrelated to seasonality (see Tuttle and Stevenson, 1982, p. 110), and it is possible for births to be very highly synchronous but completely non-seasonal in mammals (Higgens, 1993).

The specific factors that are the ultimate causes of seasonal breeding include four major components of the environment: abiotic, dietary, social interaction and interspecific interaction (e.g. seasonal changes in parasites or predation). Any of these factors that make reproduction more successful at some times than at others can favor seasonal regulation of reproduction. As defined in the classic studies by Baker and colleagues (Baker, 1938), these are the 'ultimate' factors that affect the evolution of the annual reproductive effort of animals. The most important of these ultimate factors for many species appear to be related to energetic and nutritional requirements. Because of their effects on plant and animal production and/or energetic demands, cold winters and seasonal rainfall appear to be major ultimate causes of seasonal reproduction. However, there is evidence that other factors also play a role in seasonal reproduction (Heideman, 1988).

Most species of bats exhibit at least a tendency toward seasonality in their reproductive effort, and many breed only during a single, sharply restricted, part of the year (Racey, 1982; Wilson, 1979). Many species that have been described as reproductively aseasonal, because females that are either pregnant or lactating are present in every month of the year, actually have two (or, rarely, more) narrow temporal windows during which births occur in each year. Even species in which births may occur in every month often have seasonal peaks in reproduction (Racey, 1982).

No seasonal cycle can persist in the absence of environmental regulation, and this implies that most species of bats are under some form of environmental regulation of reproduction. Internal, physiological seasonal clocks cannot precisely match an external rhythm (Gwinner, 1986); thus, even species with a precise internal circannual clock must reset that clock periodically, or else the timing of events under clock control will drift gradually over the years. In species that are known to reproduce seasonally, reproduction must be regulated by environmental signals, either directly or via resetting of an internal circannual clock that in turn regulates reproduction. In bats, clear circannual rhythms of reproduction have been shown in *Antrozous pallidus* and *Anoura geoffroyi* (Beasley, 1985–86; Heideman and Bronson, 1994).

11.3 MECHANISMS OF ENVIRONMENTAL REGULATION OF REPRODUCTION IN BATS

In many species of mammals, environmental factors are known to affect the timing of reproductive maturation, sexual receptivity and mating, ovulation and fertilization, implantation, rates of post-implantation growth (and thereby of parturition), embryo loss, birth, gonadal regression, and gonadal recrudescence (= gonadal regrowth and development) in a variety of species (Bronson, 1989; Bronson and Heideman, 1994; for bats see Racey, 1982; Tuttle and Stevenson, 1982). In bats, it is known experimentally (see references later in this chapter) that environmental factors can affect the timing of ovulation and fertilization (temperature), gonadal recrudescence (photoperiod), rates of post-implantation growth (temperature, food, and possibly stress), and embryo loss (stress). In addition, there is indirect evidence for bats that environmental factors can affect the timing of all of the other reproductive events above.

The environment can act on bat reproduction in two fundamentally different ways (Figure 11.1). First, extreme physical conditions or starvation can directly alter developmental rates, kill embryos, or inhibit reproductive physiology and behavior (reviewed by Tuttle and Stevenson, 1982). Second, the environment can affect reproduction via specific neuroendocrine pathways that have evolved to play a role in the optimal timing of different stages of reproduction. As an example of the latter, reproduction may be stimulated or inhibited by a particular photoperiod (e.g. Beasley and Zucker, 1984). In this case, specific environmental cues are

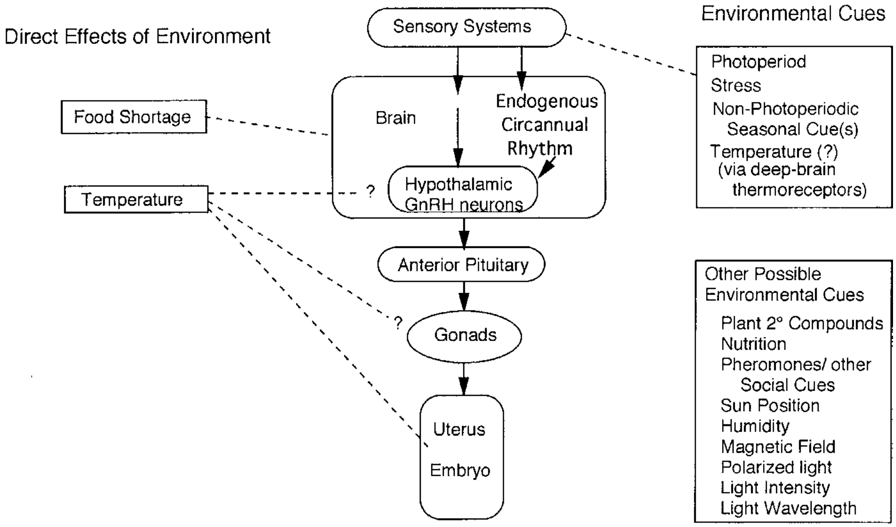

Figure 11.1 Major pathway of reproductive regulation in mammals (solid lines; note that feedback pathways are not included). Known environmental factors affecting reproduction in bats are connected with their probable site of action by dashed lines. Other cues that could, potentially, regulate reproduction in bats are listed in the box at lower right.

perceived by a sensory system – photoreceptors in the eye – are then converted into a neural or hormonal signal, and then either stimulate or inhibit some portion of the neuroendocrine system that regulates reproduction (commonly termed the ‘reproductive axis’) (Bronson and Heideman, 1994). It is important to separate these two modes of environmental regulation conceptually, because the latter are adaptations that presumably evolved to optimize reproduction, while direct effects of the environment can result in non-optimal reproductive timing. Unfortunately, it is not always clear whether a particular environmental factor is acting via a specific regulatory pathway, as a cue, or whether the effect is imposed on the reproductive system in a non-specific manner. In addition, it is possible that some species of bats sometimes actively seek particular physical environments because of their direct effects on reproduction.

The environmental signals that mammals use as cues to regulate their reproduction apparently act primarily in the brain, by affecting the secretory output of the neurons that produce the master hormone of reproduction, GnRH (see Chapter 1, general reviews by Bronson and Heideman, 1994; Morgan *et al.*, 1994). These environmental factors are known to include food, stress, temperature, photoperiod and social factors (including visual, pheromonal, auditory and tactile cues). Many other types of environmental input are also potential cues (Figure 11.1). In most cases, the precise mechanisms through which these environmental inputs affect GnRH neurons (or other parts of the reproductive axis) are still uncertain (reviewed by Bronson and Heideman, 1994; Turek and Van Cauter, 1994). GnRH neurons and the neuroendocrine events that they control in bats are described in detail in Chapters 1–3. In the sections below, non-specific environmental effects and seasonal cues will be discussed.

11.3.1 Photoperiod

The best-known seasonal cue regulating reproduction in mammals is day length, or photoperiod (Bronson, 1989). Photoperiod is commonly used to regulate reproduction by mammals in the temperate zone, and it is an important predictive cue for some mammals in the subtropics and higher latitudes in the tropics as well. Because the annual change in photoperiod declines to zero at the equator (Figure 11.2), mammals on the equator cannot use photoperiod as an environmental cue. Mammals within 10° of latitude to the equator appear not to use photoperiod as a seasonal cue (Heideman and Bronson, 1994), and those at higher tropical or subtropical latitudes may respond to photoperiod weakly or not at all (McGuckin and Blackshaw, 1992; O’Brien *et al.*, 1993).

In mammals in general, day length exerts effects on the mammalian reproductive system via a complex pathway (Figure 11.3) (reviewed by Morgan *et al.*, 1994; Tamarkin *et al.*, 1985; Turek and Van Cauter, 1994). This pathway has not been studied in detail in bats, but it appears to be fairly similar across the different orders of mammals. Light is detected by retinal photoreceptors whose output is directed through fibers in the retinohypothalamic tract (RHT) to the suprachiasmatic nuclei (SCN). The SCN is the central circadian oscillator controlling daily rhythms of mammals (reviewed by Turek, 1994), and it can maintain a free-running circadian rhythm even in the absence of a daily light cycle.

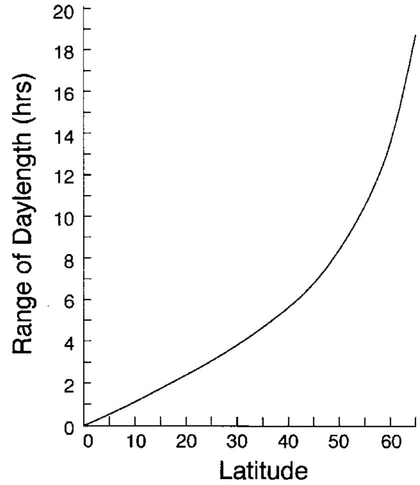

Figure 11.2 The total annual change in daylength as a function of latitude.

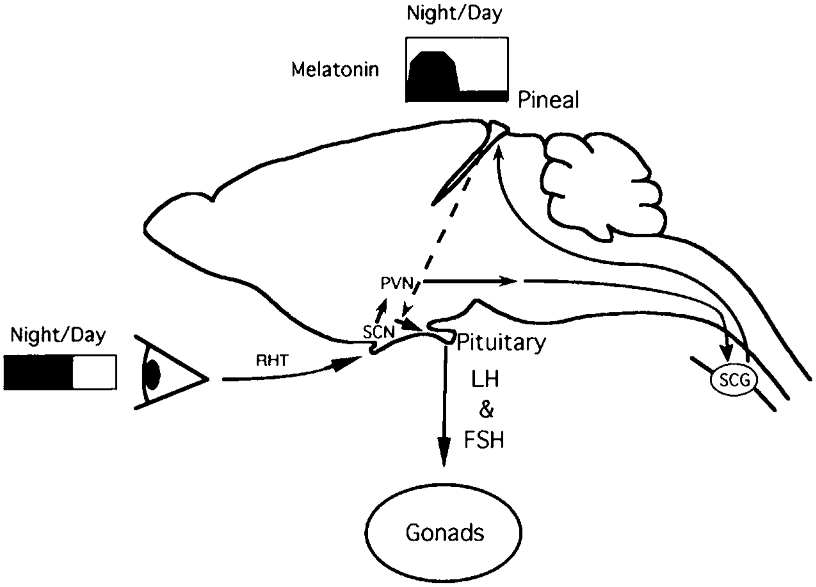

Figure 11.3 An outline of the pathway through which photoperiodic information regulates reproduction in mammals. The precise connection between melatonin released by the pineal gland and the GnRH neurons that regulate reproduction (dashed line) is unknown. (Abbreviations: follicle stimulating hormone, FSH; luteinizing hormone, LH; paraventricular nuclei, PVN; retinohypothalamic tract, RHT; superior cervical ganglia, SCG; suprachiasmatic nuclei, SCN.)

The SCN provides input to the paraventricular nuclei (PVN) of the hypothalamus, and from there neuronal connections extend to the superior cervical ganglia, which in turn act on the pineal gland via sympathetic adrenergic neurons. The pineal gland secretes large amounts of its hormone, melatonin, at night. The presence of melatonin provides a physiological signal for night, and the duration of the nightly rise in circulating melatonin provides a general physiological signal for night length (and thereby for day length) (Bartness *et al.*, 1993). A circadian rhythm of melatonin secretion can be maintained by the SCN even in the absence of a daily light cycle. In reproductively photoresponsive mammals, the melatonin signal appears to be both necessary and sufficient for a reproductive response (Bartness *et al.*, 1993; Turek and Van Cauter, 1994). Melatonin acts in some way, almost certainly indirectly (Maywood *et al.*, 1996; Morgan *et al.*, 1994; Turek and Van Cauter, 1994), to modify the release of GnRH and thereby regulate reproduction (see Chapters 1–3 for reviews of GnRH neurons and reproductive regulation). Reproductive effects of photoperiod appear to be integrated largely in the brain, by their action on the secretory activity of GnRH neurons, but there is evidence for at least minor actions at the level of the anterior pituitary as well (Everett, 1994; Kordon *et al.*, 1994; Page, 1994).

In gross morphology, the pineal gland in bats is similar to that of other mammals (reviewed by Bhatnagar *et al.*, 1986). The gland typically arises in the vicinity of the third ventricle and choroid plexus of the brain, and either forms a roughly spherical body in that region or takes a more or less cylindrical form, with or without thicker and thinner regions, extending dorsally between the cerebral hemispheres to near, or just under, the skull (Bhatnagar *et al.*, 1986). A few species of bats have been reported to lack a pineal (Bhatnagar *et al.*, 1986), but it is possible that in at least some of these species the pineal is simply extremely small and difficult to find, as is the case for *Anoura geoffroyi*, in which the pineal is minute and lies concealed within the wall of the great cerebral vein for most of its length (Heideman *et al.*, 1996). Interestingly, the largest pineal gland in any mammal, relative to body size, occurs in the megachiropteran bat *Dobsonia praedatrix* (Bhatnagar *et al.*, 1990). The relationship between the pineal gland and both vascular and neural tissue is highly variable in bats (Bhatnagar *et al.*, 1990). The significance of these differences in size and morphology remains unknown. In an examination of seasonal changes in the ultrastructure of the pineal gland of *Pipistrellus pipistrellus*, Pévet and Racey (1981) found no significant seasonal differences, which may reflect either insensitivity to photoperiod or simply a lack of change discernible at the ultrastructural level. It is worth noting that a common surgical procedure, pinealectomy, used in the study of photoperiodic effects may be difficult or even impossible in a number of species of bats because of the complexities of pineal architecture, the nearness of large blood vessels or cisternae to the pineal, and the substantial portions of the pineal that lie too deep within the brain to remove easily and safely.

A variety of sources of data suggest that ancestral vertebrates had functional extraretinal photoreceptors, possibly including receptors in the pineal gland itself (Turek, 1994). In many non-mammalian vertebrates, the pineal gland contains functional photoreceptors which collect sufficient light to distinguish day from night. However, in all mammals tested to date, the pineal gland must receive input from the retina in order to function normally, and this seems likely to be true of bats (Bittman, 1993; Morgan *et al.*, 1994; Turek, 1994). The complex pathway from retina via the SCN to the pineal allows filtering and processing of the photic signal, and permits mammals to maintain daily rhythms of melatonin even on days when photoperiodic information is incomplete or missing. Photoreceptor elements have been described in mammalian pineal glands including those of bats (Bhatnagar, 1988, 1992, 1994; Pévet *et al.*, 1977), although these are poorly developed and probably non-functional. It is conceivable, however, that some bats may be able to detect light directly in the pineal gland.

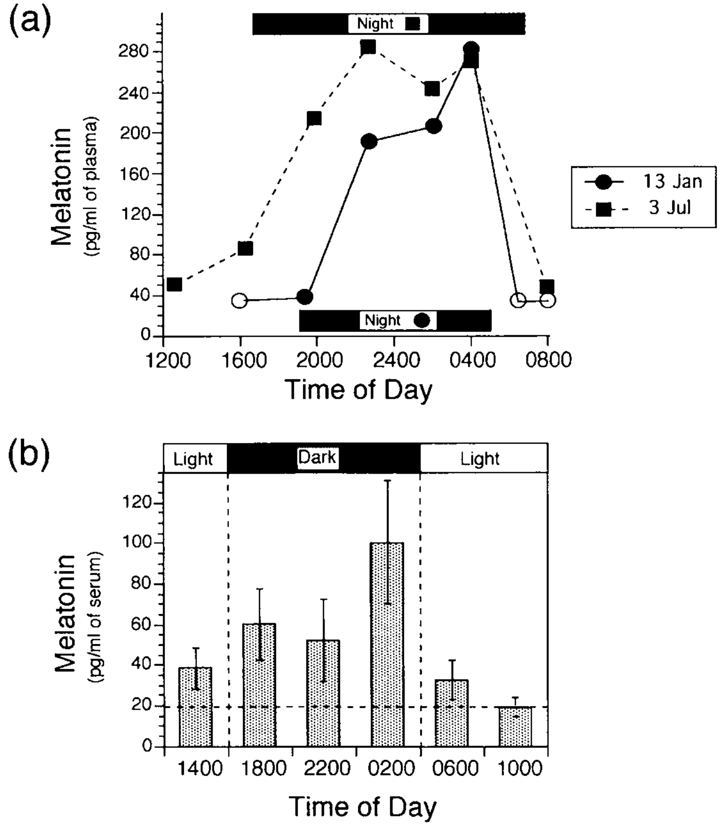

Figure 11.4 Melatonin profiles from two species of bats. (a) Plasma melatonin profiles from pools of plasma collected from adult male *Pteropus poliocephalus* (Megachiroptera) obtained in January and July, showing the difference in the duration of the melatonin rise in longer and shorter day lengths. These bats were exposed to natural light; the dark bars at the bottom and top of the figure show the dark periods in January and July, respectively. The three open symbols represent samples with melatonin levels below the limit of detection of the assay. (Data taken from McGuckin and Blackshaw, 1992, and converted to picograms/ml). (b) Serum melatonin profile for *Anoura geoffroyi* (Phyllostomidae) in a controlled 12-hour photoperiod, showing the mean ± standard error of serum melatonin concentration at each time point. The bar at the top of the profile shows the dark period, and the dashed line at the bottom of the figure shows the limit of detection of the assay. (Figure from Heideman *et al.*, 1996.)

Only two studies have assessed circulating melatonin levels in bats (Figure 11.4). McGuckin and Blackshaw (1992) measured serum melatonin levels of the megachiropteran *Pteropus poliocephalus*. They reported serum levels under 90 pg/ml during the day and 100–300 pg/ml during the night. Heideman *et al.* (1996) reported night-time serum melatonin levels in a microchiropteran, *Anoura geoffroyi*, of about 50–100 pg/ml, whereas levels during the light period were below the limit of detection (20 pg/ml). Serum samples from another microchiropteran bat, *Eptesicus fuscus*, gave serum melatonin values below 20 pg/ml in a pool of day-collected serum, and 46 pg/ml in a pool of night-collected serum (see Heideman *et al.*, 1996). All of these values fall within the range previously reported for mammals.

It is clear from the studies above that bats can produce a nocturnal rise in serum melatonin levels similar to that of other mammals. In addition, work by McGuckin and Blackshaw (1992) showing seasonal differences in the nightly melatonin profile in *P. poliocephalus* indicates that the melatonin signal of bats can vary in duration in response to seasonal changes in ambient photoperiod.

Melatonin receptor sites in the brain and pituitary gland of many mammals have been identified by the use of 2[^{125}I]iodomelatonin (I-MEL), which has biological effects similar to melatonin (Bittman, 1993; Glass, 1988; Morgan *et al.*, 1994). The little brown bat (*Myotis lucifugus*) has been found to have I-MEL binding mostly in the preoptic area, SCN, mediobasal hypothalamus, and in the area of the pars tuberalis (unpublished data described by Bittman, 1993). A recently-cloned family of high-affinity melatonin receptors is likely to be the receptors identified by I-MEL studies (Reppert *et al.*, 1994, 1995a,b). The melatonin receptor exerts its effects via inhibition of cAMP, apparently through inhibition of adenylate cyclase (Morgan *et al.*, 1994; Reppert *et al.*, 1994).

A major question in this neuroendocrine system is in the details of how melatonin exerts its effects on reproduction. Melatonin receptor binding is found in the brains of both reproductively photoresponsive and nonphotoresponsive species of mammals, and both categories of species often bind melatonin in similar brain regions (Bittman, 1993). Potential target sites are the pars tuberalis on the stalk of the pituitary gland, the median eminence of the hypothalamus, and sites in the mediobasal preoptic regions of the hypothalamus (Morgan *et al.*, 1994). Weaver *et al.* (1991) and others (Bittman, 1993; Morgan *et al.*, 1994) noted that the only site consistently high in melatonin receptors in all seasonally breeding mammals is the pars tuberalis, and suggested that melatonin might act there to regulate reproduction. Recent work suggests that the dorsomedial nucleus of the basal hypothalamus is another likely target site (Maywood *et al.*, 1996; Morgan *et al.*, 1994). There is good evidence that the action of melatonin on mammalian GnRH neurons probably involves interneurons (Maywood *et al.*, 1996; Morgan *et al.* 1994), but none of this work has been done on bats.

11.3.2 Photoperiodic Regulation of Seasonal Reproduction in Bats

Racey (1978) tested for effects of photoperiod on testicular size and development in *Pipistrellus pipistrellus* late in hibernation. There were no statistically significant differences in reproductive tissue weights or spermatogenesis between bats in long or short photoperiods, but his sample sizes were low. Racey (1982) concluded that the evidence for photoperiodic effects was equivocal in these experiments.

Beasley and Zucker (1984) studied the pallid bat (*Antrozous pallidus*) to produce the first clear experimental evidence of reproductive effects of photoperiod in a bat. Male pallid bats in California follow the typical temperate zone reproductive cycle for a male vespertillionid bat. Spermatogenesis occurs from July through September, testis size peaks in August and September, and accessory glands enlarge and the epididymides contain sperm beginning in September and continuing into the winter (Figure 11.5). Beasley and Zucker (1984) captured wild bats in April and May and placed them in short (10 h) or long (14 h) photoperiods for three, four, five or six months, at which times reproductive condition was evaluated. Treatment with short photoperiod accelerated reproductive events. Bats in short photoperiods entered the 'autumnal condition' in August, one month earlier than bats in long photoperiod (Figure 11.5).

Beasley *et al.* (1984b) provided evidence that melatonin mediates the effect of photoperiod on reproduction in pallid bats, as it does in other mammalian orders (reviewed by Bronson, 1989; Bronson and Heideman, 1994). Male pallid bats captured in July were implanted subcutaneously with either an empty silastic capsule or a silastic capsule that delivered a constant dose of melatonin. Bats in each group were then housed in either short (10 h) or long (14 h) photoperiods.

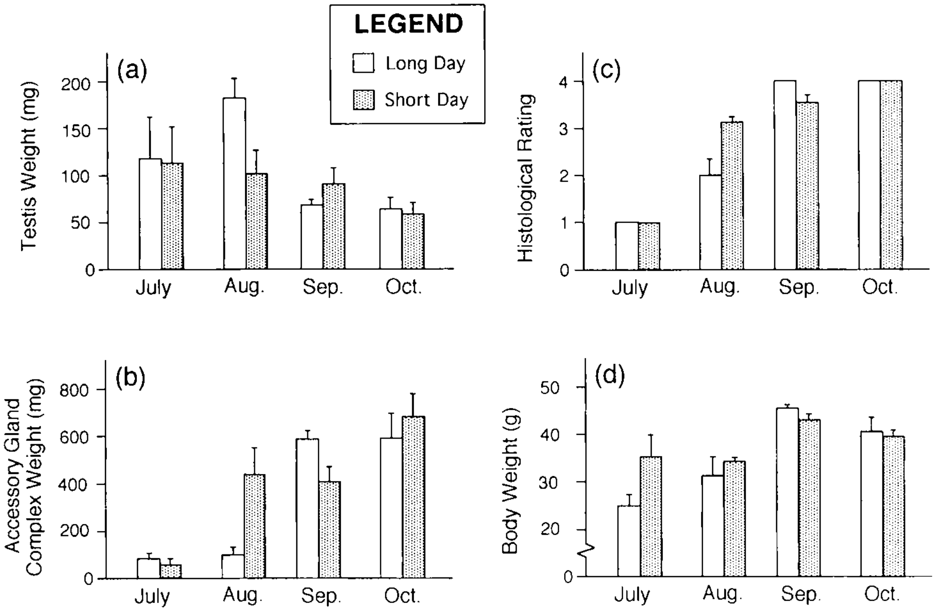

Figure 11.5 Mean (+ standard error) of reproductive organ weights, histological rating of testes and epididymides, and body weight of *Antrozous pallidus* under long or short photoperiods from July to October. (a) Testis weight, (b) accessory gland complex weight, (c) histological rating of testes and epididymides, and (d) body weight. (Redrawn from Fig. 3 in Beasley and Zucker, 1984.)

Melatonin treatment had the same effects as short days (Figure 11.6), accelerating the attainment of the 'autumnal' reproductive condition by one month. Beasley and colleagues (Beasley, 1985–86; Beasley *et al.*, 1984a) described circannual rhythms of body weight and reproduction in male pallid bats (*A. pallidus*), and reported that melatonin apparently acts by shifting the phase of these rhythms. Singh and Krishna (1995) reported that melatonin may have direct inhibitory effects on the secretion of androstenedione by the testes of *Scotophilus heathi* (Microchiroptera) *in vitro*.

O'Brien *et al.* (1991) and McGuckin and Blackshaw (1992) reported reproductive responses to photoperiod in a megachiropteran bat (*Pteropus poliocephalus*) (Figure 11.7). However, the differences were so small relative to seasonal reproductive changes of wild males that O'Brien (1993) concluded that photoperiod is unlikely to be a major proximate environmental cue regulating reproduction in this species. *Pteropus poliocephalus* produces a short-duration nocturnal rise in melatonin in short nights and a long-duration nocturnal rise in melatonin in long nights (McGuckin and Blackshaw, 1992), suggesting that the weak reproductive effects of photoperiod may be mediated by melatonin; however,

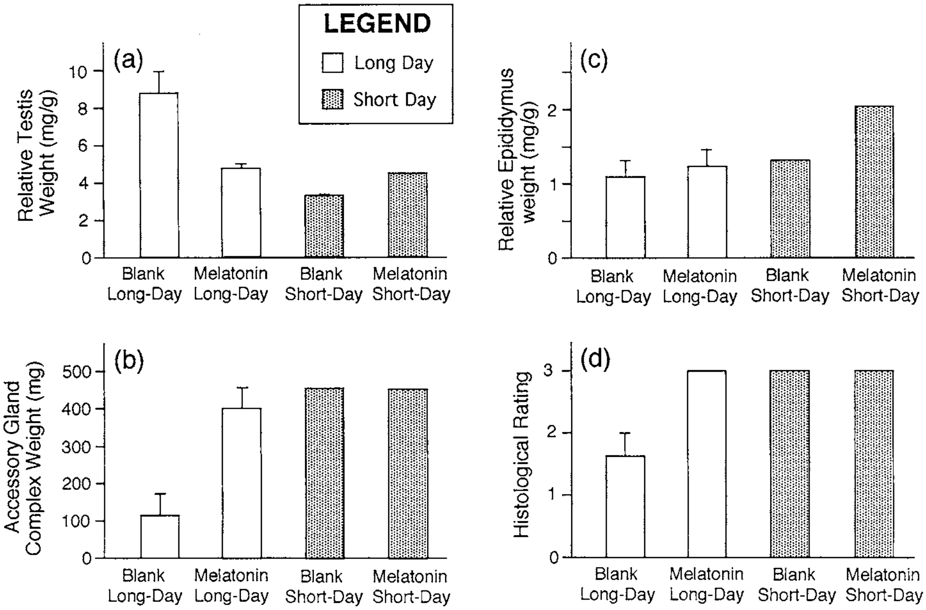

Figure 11.6 Differences in reproductive organ weights (testis weight relative to body mass, reproductive accessory gland weight, and epididymus weight relative to body mass) and histological rating of testes and epididymides of male *Antrozous pallidus* given either a silastic capsule filled with melatonin or a blank control capsule, and held in either long (14 h) or short (10 h) photoperiods. Standard errors are not shown for animals housed in short days because there were too few for calculation of standard errors. (a) Testis weight relative to body weight, (b) accessory gland complex weight, (c) epididymus weight relative to body weight, and (d) histological rating of testes and epididymides. (Redrawn from Fig. 2 in Beasley *et al.*, 1984b.)

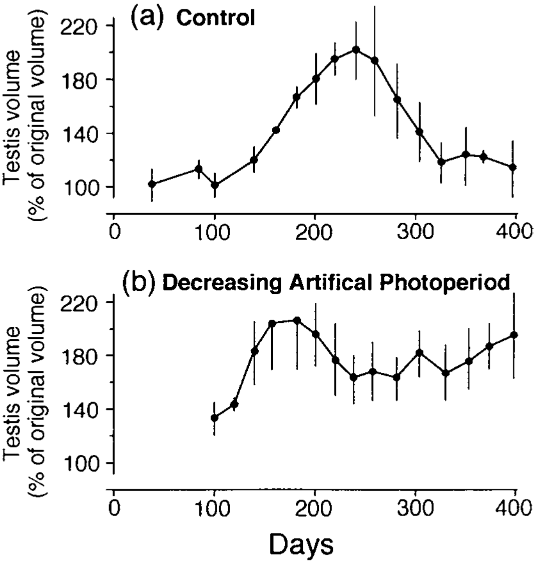

Figure 11.7 Testicular volume (mean ± SD; n = 3 animals per group), as a percentage of initial testicular volume, of *Pteropus poliocephalus* in Australia receiving either natural photoperiod or a gradually decreasing photoperiod. Volume was estimated from the formula for a prolate spheroid using measurements of testis length and width. (a) Natural photoperiod change, beginning during the winter on 16 July (Day 0), (b) 100 days of long days (L16:D8), beginning on 16 July (Day 0), followed on days 100–172 by 72 days of decreasing photoperiod, and then from day 172 until the end of the study given a constant photoperiod of L8:D16. (Redrawn from Fig. 3 in McGuckin and Blackshaw, 1992.)

the effects of melatonin have not been tested directly. Finally, Halder and Ghosh (1993) reported briefly that melatonin injections inhibited gonadal recrudescence in both males and females of another megachiropteran bat, *Cynopterus sphinx*, in India.

11.3.3 Non-photoperiodic Regulation of Seasonal Reproduction in Bats

There are three species of seasonally-breeding bats for which there is experimental evidence that photoperiod is *not* an important environmental cue. *Pteropus rodricensis* from 23 °S latitude breed seasonally on Rodrigues Island (Cheke and Dahl, 1981). Animals in a captive colony maintained on the island of Jersey on the photoperiod typical of 23 °S, but six months out of phase with the natural photoperiod, began breeding asynchronously and did not entrain to the new photoperiod cycle (Carroll, 1988; West, 1985).

Female *Anoura geoffroyi* (Phyllostomidae) on the South American island of Trinidad (10 °N latitude) produce one young per year, and males have a four-month peak in testis size from May through August, which also matches the period of peak spermatogenesis (Heideman *et al.*, 1992). Groups of 10–13 males from this population were subjected experimentally to six months of (a) constant photoperiod, (b) a gradually increasing photoperiod matching the natural cycle of civil-twilight photoperiod at 10 °N latitude, or (c) an accelerated photoperiod cycle which compressed the natural cycle to six months. Testes were measured externally at two-week intervals. There were no significant differences in testis size among treatments, and in all three treatments the peak in testis size matched the timing of peak testis size in the wild population (Figure 11.8). A second study (Heideman and Bronson, 1994) described an internal rhythm of reproduction in these bats. Males housed in constant photoperiod for just under two years underwent cycles of testicular regression and growth that averaged about 7.5 months in length (Figure 11.9). A series of four different photoperiod treatments designed to to test exhaustively for responses to photoperiod failed to alter this pattern (Figure 11.10). These bats produce a nocturnal melatonin secretion pattern (Heideman *et al.*, 1996) that is similar in amplitude to that of many photoresponsive mammals, suggesting that the reproductive system of *A. geoffroyi* might be insensitive to seasonal changes in the melatonin signal.

Pteropus poliocephalus (Megachiroptera) occurs from tropical to low temperate latitudes in Australia, where it reproduces seasonally. Males attain a peak in testis size from July to November, with most mating occurring in November and December (O'Brien *et al.*, 1993). O'Brien *et al.* (1993) reported 18 months of data on groups of two to five male *Pteropus poliocephalus* in three different photoperiod treatments (Figure 11.11). One group received a natural pattern of

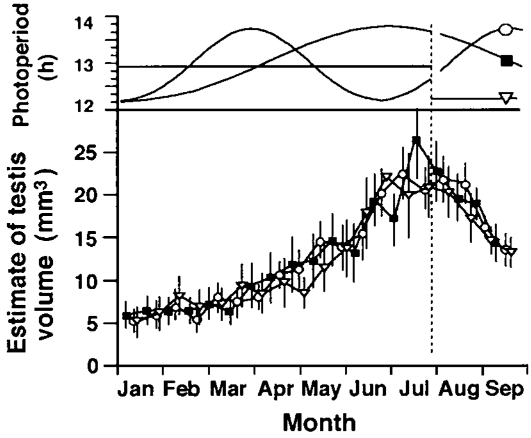

Figure 11.8 Testicular volume (mean ± SE; calculated using the formula for a prolate spheroid from measurements of the width and length of one testis) of three groups of *Anoura geoffroyi* under different photoperiod treatments. One group (solid squares) experienced a 12-month cycle of natural change; a second (open circles) experienced the same cycle accelerated to six months; and a third (open triangles) experienced a constant day length (see day length treatments at top). The dashed line identifies a point at which the photoperiod cycle was changed at the end of this experiment, after the maximum testis size in each group had been attained. The points for testis volume are offset slightly along the x-axis to improve readability. (From Heideman *et al.*, 1992.)

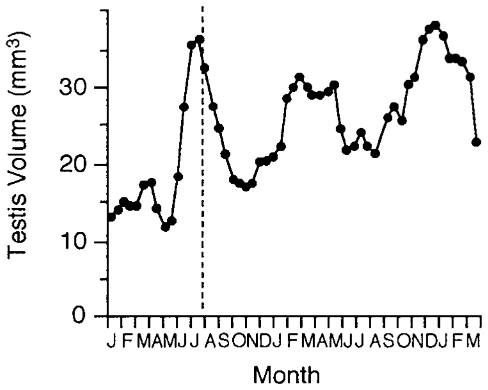

Figure 11.9 An endogenous circannual rhythm of testicular size (volume calculated from width and length measurements of one testis taken every two weeks) of a representative individual *Anoura geoffroyi* from a group held in constant daylength. The treatment began at the point of the dashed line. In the previous 7 months, the light period was L12 h 54 min, and for the 20-month constant photoperiod treatment the light period was 12 h 19 min. The curve has been smoothed by plotting the running means of three adjacent measurements. (Drawn from data in Fig. 4 in Heideman and Bronson, 1994.)

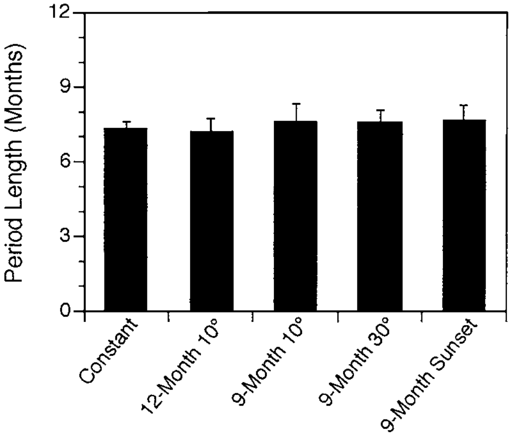

Figure 11.10 Mean ± SE of period from peak to peak of testis size of *Anoura geoffroyi*, from 10° N latitude on the island of Trinidad, in five photoperiod treatments. The treatments were: constant photoperiod of 12 h 19 min, a 12-month gradually changing cycle mimicking the civil twilight photoperiod at 10° N latitude, a similar cycle accelerated to 9 months, a 9-month gradually changing cycle mimicking the civil twilight photoperiod at 30° N latitude, and a 9-month cycle mimicking, but accelerating, the change in the time sunset at 10° N latitude and holding day length constant. In no group did the bats entrain to their photoperiod cycle. (From Heideman and Bronson, 1994.)

photoperiod ranging from 11 h 15 min to 14 h 45 min (lengthened in order to include the approximate period of twilight in the light phase). The other groups received alternating three month periods of short (11 h) and long (16 h) photoperiod, one beginning the experiment in short photoperiods and the other in long photoperiods. All three groups showed similar patterns of change in testicular volume, as calculated from measurements of testis length and width (Figure 11.11).

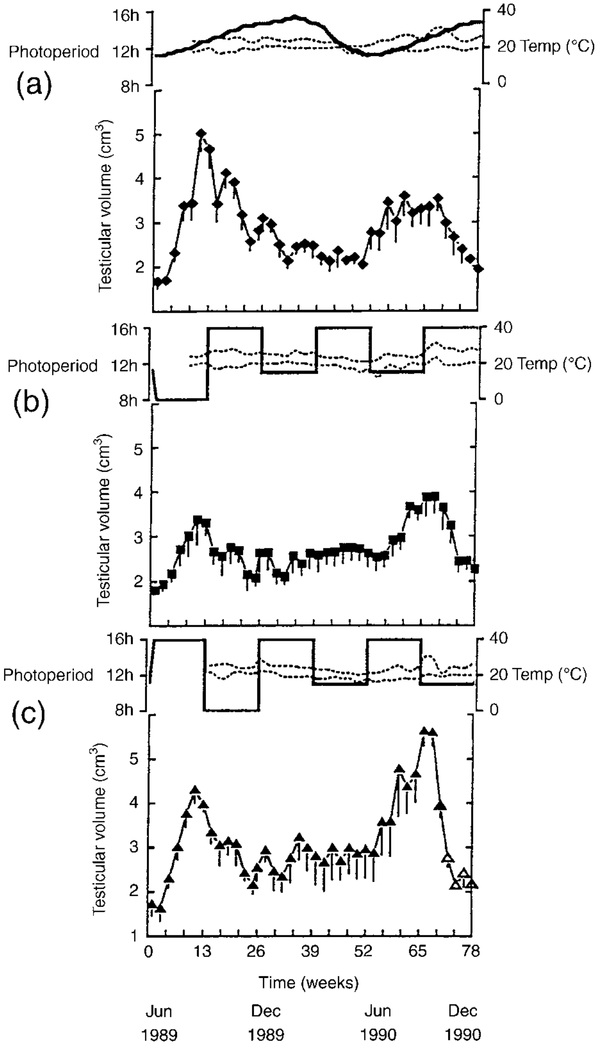

Figure 11.11 Cycles of testis volumes (mean ± SE, calculated using the formula for a prolate spheroid from length and width testis measurements) of *Pteropus scapulatus* maintained under (a) simulated natural photoperiod, (b) alternating 13-week periods of short (LD 11 : 13) and long (LD 16 : 8) photoperiod, beginning with short photoperiod, and (c) alternating 13-week periods of short (LD 11 : 13) and long (LD 16 : 8) photoperiod, beginning with long photoperiod. (From O'Brien, 1993.)

This species has a relatively small pineal gland and a low-amplitude nightly rise in melatonin (McGuckin, 1988), which suggests that the failure to respond reproductively to photoperiod could be due either to a weak melatonin signal, given their small pineal and relatively small nightly rise in melatonin (Martin *et al.*, 1995), or insensitivity of the reproductive axis to melatonin.

11.3.4 Temperature and Reproduction

The reproductive axis of insectivorous bats can be remarkably sensitive to temperature. Because many species of microchiropterans can decrease their body temperature during hibernation and/or daily torpor, changes in body temperature are a mechanism by which the environment can act upon reproduction. Racey (1982) provided an excellent and comprehensive review of the literature on this topic, which is summarized in this section along with some more recent results. There is good evidence for two kinds of effects of temperature on reproduction: ovulation or mating in response to increased temperature and slowed embryonic development in response to low temperatures. In both temperate and tropical species, field and laboratory evidence indicates that decreases in body temperature due to torpor can decrease the rate of development of embryos, and thereby affect the timing of parturition. Pregnant female *Pipistrellus pipistrellus* that are given food *ad libitum* are less likely to enter daily torpor, while those deprived of food enter torpor (Racey, 1973; Racey and Swift, 1981). Thus, the ability to enter daily torpor may permit pregnant bats to reduce energy demands during periods of cold weather or food shortage, but at a cost of decreasing embryonic growth rates and delaying parturition (Racey, 1982).

In many species of hibernating temperate zone bats, ovulation appears to be triggered by warm temperatures, but this trigger is gated by other factors. In these species, the ovarian follicles develop in the autumn, females typically mate in the late fall, and ovulation occurs upon arousal from hibernation in the spring (reviewed by Oxberry, 1979), and ovulation can be induced in the spring by warming and arousal. However, during the early part of hibernation, ovulation cannot be triggered by warm temperatures, and even treatment with gonadotrophins may be ineffective in causing ovulation (Guthrie and Jeffers, 1938; Smith, 1951), although the hormone treatments are sometimes effective (see Racey, 1982). After the early period of hibernation, ovulation can be induced by administration of gonadotrophins, but not by a short period of higher temperatures alone (Guthrie and Jeffers, 1938; Racey, 1976; and see Smith, 1951; Racey, 1982). Finally, later in hibernation, from around the time of midwinter to the normal time of arousal in the spring, ovulation can be induced by placing hibernating females into warm rooms (Guthrie and Jeffers, 1938; Smith, 1951; Pearson *et al.*, 1952; Racey, 1976, 1982; Buchanan, 1987). As the time of normal arousal from hibernation and ovulation approaches, placement of bats in higher temperatures induces ovulation more rapidly and in more individuals (Racey, 1982). Increases in temperature may have similar effects in stimulating spermatogenesis in males of hibernating species of bats late in hibernation (Racey and Tam, 1974; Gustafson, 1979), while being ineffective early in hibernation (Gustafson, 1979). These studies show that temperature can regulate ovulation (and perhaps spermatogenesis) in these species, but that something normally blocks responses to temperature early in hibernation. Either some additional environmental stimulus or some kind of endogenous biological timer acts as a gate to make the reproductive axis of females increasingly sensitive to temperature as hibernation progresses. More recently, Mendonça *et al.* (1996) provided correlational evidence that arousal from hibernation triggered mating behaviors, and experimental evidence that a period of hibernation followed by an increase in temperature can induce mating behavior, even in gonadectomized males with very low levels of plasma androgens (Mendonça and Hopkins, 1997).

Many species of bats are heterothermic, and low body temperature can alter the duration of gestation in these species (Racey, 1969; Racey and Swift, 1981). Racey (1973) found that pregnant *Pipistrellus pipistrellus* induced to enter torpor by low temperatures and food deprivation for periods up to 14 days delayed parturition by an average of 14 days relative to controls. Even in the presence of abundant food, Racey (1973) found that low temperatures (5 °C) delayed parturition. Conversely, high temperatures (30° and 35 °C) accelerated parturition dates.

A number of field studies have provided correlational evidence that the effect of temperature is important in natural populations (for a more detailed review of the earlier literature see Racey, 1982). Racey and Swift (1981) found that the duration of gestation of *P. pipistrellus* in England in 1978, which had a warm spring and abundant insects, was 41 days, 10 days shorter than the gestation length in 1979, which had a two-week period of cold temperatures and few trappable insects during the early part of gestation (Figure 11.12). *Rhinolophus ferrumequinum* in England has shown a strong relationship between cold temperature and average birth date, with cold temperatures apparently acting by slowing post-implantation development (McOwat and Andrews, 1995; Ransome, 1973; Ransome and McOwat, 1994). The latter authors developed linear models to relate mean birth date and the growth rate of young bats to rainfall and temperature. While rainfall was not found to be an important factor, temperatures in April, May and June were strongly related to mean birth date, and temperatures in later months were strongly related to growth rate. Audet and Fenton (1988) presented evidence that, during a colder spring in southern Canada, *Eptesicus fuscus* (Vespertilionidae) were forced to enter torpor more frequently, thus extending gestation and delaying parturition. Lewis (1993) reported dramatic effects of a cold spring on reproductive timing in a population of *A. pallidus* in Oregon. Mean maximum monthly temperatures in Oregon were cooler by 2° to 5 °C in the spring of 1991 than in 1990, and parturition dates were later by a month or more in 1991. In contrast, Pearson *et al.* (1952) found no correlation between average temperature and average date of parturition in *Corynorhinus rafinesquii* (now *Corynorhinus townsendii* = *Plecotus townsendii*) in the Western USA, but these authors did not have records of temperatures and conditions within and near their colonies. In spite of the lack of correlation between temperature and gestation, Pearson *et al.* (1952) suggested that the range in calculated gestation lengths (56–100 days) among years and colonies is due to slowed embryonic development in some females, populations, or years due to low environmental temperatures. Krishna and Dominic (1982) reported a 19-day difference in duration of gestation in the two annual pregnancies in *Taphozous longimanus* (Emballonuridae). The longer gestation period occurs during the

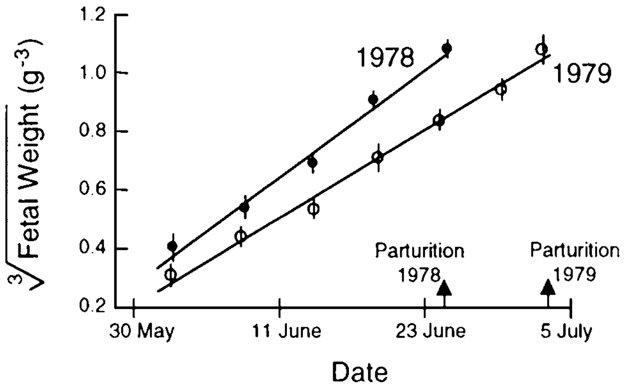

Figure 11.12 Foetal growth of *P. pipistrellus* at Aboyne, United Kingdom, in one year (1978) with a warm spring and another (1979) with a cold period during the spring. The vertical bars are s.e.m. for 6 bats/group; the regression equations in 1978 and 1979 were, $y = 0.028x + 0.37$, ($r = 0.97$), and $y = 0.024x + 0.28$, ($r = 0.99$), respectively. (Redrawn from Text-fig 1 of Racey and Swift, 1981.)

colder months of the year, and lowered body temperature might explain the increase.

The effects of temperature can interact with food availability. In the laboratory, pregnant female *Pipistrellus pipistrellus* provided with *ad libitum* food were less likely to enter torpor (Racey, 1973; Racey and Swift, 1981), and bats that entered torpor less often had shorter gestation periods (Racey, 1973). Thus, while lowered body temperature may be the proximate factor extending gestation, low food availability may force pregnant bats to enter torpor, and thereby slow the developmental rate of their embryos. Bats with access to abundant food may maintain high body temperature even at low ambient temperatures and suffer no extension of gestation. There is also evidence from field studies that low food availability can induce slowed development. Bernard (1994) reported variation in foetal growth rate among three years in *Miniopterus schreibersii* in South Africa. Average temperatures did not differ among the three years, but rainfall (and presumably insect abundance) was much lower in the year in which developmental rate was slowed. Audet and Fenton (1988) presented evidence that *Eptesicus fuscus* (Vespertilionidae) in southern Canada actively controlled their torpor bouts. During a colder spring, lactating females defended higher body temperatures than pregnant or non-reproductive females. Grinevitch *et al.* (1995) showed that male *Eptesicus fuscus* were more likely to enter torpor and had longer torpor bouts than reproductive females, and that lactating females were less likely to enter torpor than pregnant females (no non-reproductive females were included in the study). In addition, males were more likely to remain in the roost without foraging, especially on nights in which temperatures were low. All of these authors (Audet and Fenton, 1988; Grinevitch *et al.*, 1995) suggested that the additional foraging by reproductive females may be part of a strategy to obtain sufficient food to defend high body temperatures and maintain rapid rates of development.

The phenomenon of slowed embryonic growth rate due to lowered body temperature can be viewed both as a constraint of heterothermy and as a specific adaptive response. Heterothermy as an adaptation gives insectivorous bats the ability to tolerate the variability in food supply of the temperate zone (see discussion in McNab, 1982), but the retarded embryonic growth and delay of parturition that comes with heterothermy may have serious fitness costs (Racey, 1982). In some species, natural selection appears to have favored female behaviors that minimize the use of torpor during pregnancy and lactation (Audet and Fenton, 1988; Grinevitch *et al.*, 1995). Selection favoring rapid spermatogenesis may have similar effects on males in some species (Kurta and Kunz, 1988). Under particularly harsh conditions, however, females are unable to obtain enough food to maintain a high body temperature and are forced to accept the reduced rate of embryonic development accompanying their entry into torpor.

11.3.5 Other Environmental Factors and Reproduction

Heideman and Bronson (1994) reviewed non-photoperiodic environmental cues that could, potentially, be used by bats to regulate reproduction in the tropics. The possibilities include the annual cycle of the time of sunrise and sunset, food abundance, plant secondary compounds, rainfall and temperature, changes in light intensity, changes in magnetic field, and the changing position of the setting or rising sun. Some of these have the potential to be cues with high signal to noise ratios, while others would appear to be so high in noise as to offer little value as predictors. There is no evidence, however, that any bat actually uses any of them as predictive cues.

Heideman and Bronson (unpublished data) subjected groups of 10 to 12 male *Anoura geoffroyi* to experimental treatments to test for non-photoperiodic environmental cues that might regulate reproduction seasonally, with a control group in constant conditions (Figure 11.13). The experiment was designed to test whether any of the cues provided could entrain the endogenous seven-month cycle of reproduction in this species (Heideman and Bronson, 1994) to 12 months. One treatment provided a change in the position and intensity of the light source (spotlights on a moveable frame that were lit sequentially to mimic the position of the sun through the day and the seasons at 10 °N latitude). A second treatment altered the plane of polarized light at dawn and dusk to mimic the changes at dawn and dusk in nature. A third provided dry and wet conditions in an attempt to mimic dry and wet season moisture differences, and a fourth altered food quality by decreasing the protein content of the diet to an experimentally determined minimum. The control and all four experimental groups had similar patterns of reproductive timing, with peaks in testis size separated by seven months (Figure 11.13), indicating that none of the cues affected the reproductive rhythm in these bats. The only effect (Figure 11.13) was a slight depression of the first peak in testis size in males in wet conditions, which could have been a direct effect of stress or cold. Unfortunately, the results of this experiment did not rule out the possibility that any of these cues could have affected seasonal reproduction in this species if they had been presented in a more natural manner.

Recent work by Widmaier *et al.* (1997) indicates that the hormone leptin may be

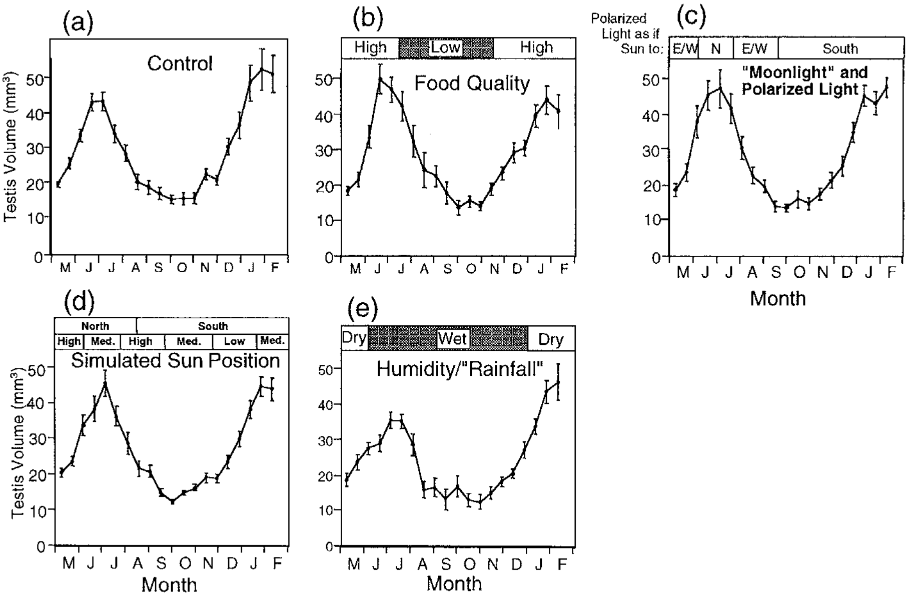

Figure 11.13 Changes in testis size (volume calculated using the formula for a prolate spheroid from width and length measurements of one testis taken every two weeks) of *Anoura geoffroyi* in five different treatments (mean ± SE; n = 10–12 per treatment). (a) A control group was held in a constant photoperiod. Four other groups were exposed to treatments designed to mimic 12-month natural cycles of change in a variety of environmental factors. (b) One group was exposed to a pattern of high and low food quality designed to mimic a potential decrease in food quality during the mid dry season. (c) One group was given low intensity light (designed to mimic moonlight) for five out of every 28 nights, and exposed to changes in the position of the plane of polarized light and 'dawn' and 'dusk'. (d) One group was exposed to changes in both 'sun' position, provided by sequentially lit floodlights on a moveable frame, and light intensity designed to mimic natural changes in sun position and light intensity. (e) One group was held in low humidity, high humidity and low humidity in a sequence designed to mimic the pattern of dry and wet seasons. The experiment was ended when all four experimental groups underwent increased testis size and peaked synchronously with the controls, four months earlier than would be expected if they had entrained to a treatment. (From Heideman and Bronson, unpublished data.)

involved in regulating pregnancy and/or lactation in *Myotis lucifugus*, suggesting that food or body condition might affect reproduction via this pathway.

11.3.6 Social Factors and Reproduction in Bats

Social factors can play an important role in fine-tuning the onset or end of a breeding season in mammals, and may be able to exert equally potent effects on reproductive success during a breeding season (Bronson, 1989). Because so many species of bats are highly social, and many species produce secretions seasonally that are likely to have some function as pheromones (e.g. Heideman *et al.*, 1990), it seems likely that pheromones affect reproduction in at least some species of bats.

Pheromones could exert effects through the vomeronasal organ, which is present in some species of bats but absent in others (Bhatnagar, 1980; Cooper and Bhatnagar, 1976) or olfactory receptors in the nasal epithelium. There appear to be no controlled experimental studies on the effects of pheromones or other social factors on reproduction in bats.

11.3.7 Stress

In mammals, a variety of environmental factors that increase corticosteroid concentrations can inhibit reproduction; these factors are lumped under the general term 'stress'. Stress effects can come from a wide range of factors, including harassment by predators, lack of food, adverse climatic conditions, competition, or adverse social interactions. The effects may be almost immediate (e.g. aborting of late embryos following handling) or more subtle (slight to strong inhibition of the reproductive axis). An intriguing recent report suggests that periods of delayed embryonic development may be induced in *Carollia perspicillata* by periods of stress caused by storms, lack of food, or loss of a roost (Badwaik and Rasweiler, 1995; Rasweiler and Badwaik, 1997).

There is evidence for termination of pregnancy following stress in some species of bats, although much of this is anecdotal. Many authors have reported abortion or reabsorption of embryos in some females following handling or upon introduction to captivity (e.g. Pearson *et al.*, 1952; Novick, 1960; Rasweiler and Bonilla, 1972; Rasweiler and Ishiyama, 1973; Towers, 1987). Dukelow *et al.* (1990) attempted to test the effects of stress caused by handling, bleeding, and/or laparoscopy on pregnancy in captive-bred *Pteropus scapulatus*. However, abortion rates were high even in the undisturbed control groups, and there was no clear evidence of increased pregnancy loss due to handling or other procedures. Rates of embryo loss ranged from 80–100% in all of the treatment groups, and some bats aborted even before handling or other treatments. Near-term females of some species of pteropodids seem particularly likely to abort embryos following or during handling (Heideman, unpublished data). Recent work has shown that several species of megachiropterans have an exceptionally strong stress response. Captive megachiropterans had basal glucocorticoid levels that were at the extreme upper end of the range for mammals, while microchiropterans were within the typical mammalian range (Widmaier and Kunz, 1993; Widmaier *et al.*, 1994). Handling and/or restraint of megachiropterans induced levels of glucocorticoids that were even higher. Widmaier *et al.* (1994) reported that levels of the pituitary hormone, adrenocorticotropic hormone, that stimulates the synthesis of adrenal glucocorticoids, were not unusually high in these megachiropterans. The functional significance of the high levels of glucocorticoids is not known, but the unusually strong stress response could be a causal factor in the loss of embryos of pteropdodids in response to capture.

While loss of a near-term embryo may be simply a pathological result of acute stress, it is conceivable that aborting embryos under stressful conditions could be adaptive in bats. Carrying a large near-term embryo can substantially reduce both speed and maneuverability in the mother. Thus, the ability to lose an embryo rapidly may occasionally allow females to escape from predators. If the average increase in lifetime reproductive success from such escapes is higher than the lifetime reproductive costs from the occasional aborted embryo that could have been raised successfully, then adaptations to permit rapid abortion in response to stress could be favored by natural selection. Alternatively, the tendency to lose embryos due to stress may confer no increase in fitness.

There is, as yet, no direct evidence for stress as a regulator of the entire reproductive axis in bats. However, many species of bats do not readily breed in captivity (Rasweiler, 1975; Wilson, 1988), which may be a response to stress. It seems likely that high stress has inhibitory effects on reproduction in bats, as it does in other mammals.

11.3.8 A Speculative View of Environmental Regulation of Reproduction in a 'Typical' Bat

The information available permits speculation on how the environment regulates reproduction in bats (for a similar discussion focused on *Pteropus*, see O'Brien, 1993). The typical species of bat probably has an endogenous circannual rhythm of reproduction. The endogenous rhythm is entrained by photoperiod in some species, especially in the temperate zone, and by non-photoperiodic cues in many other species, especially in the tropics. Most species probably use a single environmental cue as the major entraining agent for their rhythm, but also use modifiers to phase shift either their circannual rhythm, or the reproductive response to their circannual rhythm. Many temperate zone female bats use increased ambient temperature and their arousal from hibernation as an environmental signal to trigger reproductive events at the end of winter. This response to arousal is probably gated in the typical temperate zone bat by some other factor, most likely the phase of the endogenous rhythm. Low temperature and lack of food can affect reproduction directly by causing females to enter torpor, and thereby slowing embryonic development in the typical temperate zone bat, and probably in many tropical species as well. Temperature and food may be used by some species to fine-tune reproductive timing to optimize reproduction, but, in the typical bat species, low temperature and lack of food may force changes in reproductive timing that are not optimal. Finally, acute or chronic stress can induce abortion in the typical bat.

Environmental factors that act on the timing of regression and recrudescence of gonads in the typical bat probably act by affecting the activity of GnRH neurons and other neurons in the hypothalamus. Environmental factors that control the timing of some other reproductive events, possibly including ovulation, implantation, the initiation or termination of a period of delayed development, and parturition, may be as likely to act either downstream from hypothalamic GnRH neurons, at the level of pituitary gonadotrophins, the gonads, or the uterus, and/or on other hypothalamic neurons.

11.4 INSIGHTS FROM FIELD STUDIES

How far can we extend the results of laboratory studies using the results of field studies? Are there are other possible environmental effects hinted at by field studies? Several kinds of field studies offer insights into the environmental regulation of reproduction in bats.

First, many studies have demonstrated seasonality in bats (Chapter 10). Tropical as well as temperate zone bats reproduce seasonally, even within one degree of latitude of the equator (Mutere, 1967, 1973), and these bats must regulate reproduction using a non-photoperiodic seasonal environmental factor. In each species for which seasonal reproduction is clearly demonstrated, there must exist one or more environmental factors which regulate seasonality. Such regulation may either be direct (e.g. periodic semi-starvation inducing reproductive inhibition or failure), or may involve the use of a predictor or cue to regulate reproduction adaptively.

Second, studies that document variation among different years can provide information on constraints on the precision of timing mechanisms or adaptive variation in seasonal timing. Studies showing that cold spring temperatures (or low food abundance) reduce embryonic growth rates and delay parturition (see references above) illustrate the plasticity of reproductive timing in some species. In contrast, evidence from other species in which reproductive timing varies only slightly among years (e.g. Heideman, 1988, 1995) suggests that a particular population or species may be relatively unresponsive to year-to-year variation.

Third, a large number of field studies have correlated reproductive timing with various environmental factors (see Chapter 10). Because correlation cannot demonstrate causation, these studies offer only indirect evidence on the causes of seasonal reproduction. Any two events which are seasonal must be correlated, irrespective of any causal relationship or lack thereof. The fact that an increasing number of particularly detailed studies on bats has shown that peak reproduction is often precisely correlated with peak food abundance or peak food quality is evidence for diet as an important ultimate cause of seasonal reproduction in bats. However, the proximate environmental causes are largely unknown.

11.4.1 A Caveat

Unfortunately, it is problematic to use field studies to attempt to identify specific environmental cues that regulate reproduction. In principle, endogenous reproductive rhythms can be entrained by environmental cues occurring at any time of year. In addition, environmental cues at one time of year could trigger so-called 'hour-glass' timers that initiate or terminate reproduction weeks or months later. Thus, there is no reason, *a priori*, to expect that regulatory environmental cues must occur at any particular phase of the reproductive cycle in a given species. Some studies have attempted to correlate reproductive timing in different populations with specific environmental signals (e.g. latitudinal studies searching for a specific critical daylength that triggers gonadal development). Unfortunately, this approach is also likely to be unreliable in identifying environmental cues that regulate reproduction. There is now good evidence that different populations of a given species of mammal may time reproduction using environmental cues in different ways (reviewed by Bronson and Heideman, 1994). Most wild and domestic breeds of sheep, for example, use photoperiod to regulate their reproduction (Karsch *et al.*, 1984; O'Callaghan *et al.*, 1992). However, breeds from lower latitudes that reproduce earlier in the year respond differently to a particular photoperiod cycle than do breeds from higher latitudes that reproduce later in the year (Lincoln *et al.*, 1990). O'Callahan *et al.* (1992) showed that the difference in timing of reproduction between two strains of sheep was due to genetic differences in the timing of reproductive events governed by an endogenous circannual rhythm. Recent experimental evidence with rodents suggests that reproductive timing in response to photoperiod can be altered very rapidly by natural selection (Desjardins *et al.*, 1986; Spears and Clarke, 1988; Lynch *et al.*, 1989; Heideman and Bronson, 1991). This suggests that the regulation of reproduction by environmental cues may be evolutionarily plastic.

Some kinds of field studies will continue to provide very useful information on the environmental regulation of reproduction. For environmental factors that act directly on the reproductive system, such as low body temperatures during pregnancy, field studies can provide particularly valuable data, albeit indirect, on environmental regulation. In addition, studies which show that reproductive events have the same phase relationship to an environmental signal in different populations may indicate that a species is locked to the use of a particular pattern either by high gene flow between populations or through a relatively inflexible neuroendocrine pathway. Still, it is important to note that in most cases insights from field studies will need to be supplemented with laboratory experiments to identify and understand the environmental factors that regulate reproduction in bats.

11.5 WHAT SHOULD WE DO NEXT?

There are conspicuous gaps in our understanding of the evolution and physiology of reproductive regulation in bats. There are abundant field data describing cycles, a few of which report levels of variation across years or locations. The field data suggest strongly that a range of environmental factors affect reproduction, and that environmental cues stimulate physiological events that optimize reproductive timing. There are brief reports of seasonal rhythms of reproduction in captive colonies of breeding bats (e.g. Häussler *et al.*, 1981). Laboratory work to explore the mechanisms of this variation has lagged far behind the field data, and only a handful of studies have addressed these questions under controlled conditions. Much of our understanding of the impact of environmental signals on bats will build on knowledge obtained first in conventional laboratory species rather than on bats. On bats, these kinds of laboratory studies are logistically challenging because they require manipulations of environmental factors under precisely controlled conditions, in addition to the maintenance of healthy and unstressed bats for months or years, and often large flight cages in which to house them. Bats can be more expensive to maintain than laboratory rodents, and, because they are often collected in the wild from populations that may recover slowly from depletion, the use of bats can involve conservation concerns. Nevertheless, carefully designed laboratory studies on bats will be necessary to describe the effect of environmental factors on their reproduction.

What kinds of studies are particularly appropriate now? First, we need to identify the environmental factors that affect reproduction in more species of bats.

A large body of literature from field studies argues strongly that food abundance and seasonal cold or rainfall are ultimate causes that favor seasonal reproduction in bats (and other mammals). However, no mechanisms are known through which either rainfall or food can directly regulate seasonal reproduction. Do these factors ever regulate reproduction directly in bats, or do bats use other predictive cues such as photoperiod instead? At present, we know that temperature can have profound effects on reproduction in a growing number of species of bats, but temperature may simply act directly, rather than as a predictor, in some or all of these cases. In addition, a small body of evidence suggests that stress can suppress reproduction in bats or cause embryos to abort. It is almost certain that social cues can affect reproduction in bats, but we have no direct evidence for their importance. We know that photoperiod can have important effects on reproduction in a very few species of temperate and subtropical bats, but in some tropical species we have evidence that photoperiod does not regulate seasonal reproduction. A major unsolved question is how bats achieve precise seasonal timing of reproduction. It is clear that photoperiod cannot be this cue for many tropical species, and it is likely that the environmental signal(s) involved acts on the reproductive system of tropical bats via a neuroendocrine pathway that is currently unknown.

It is clear also that we need a better understanding of the physiological mechanisms through which environmental events act on reproduction in bats. What are the neuroendocrine pathways by which environmental events affect reproduction? Are there redundant pathways? How flexible is the response of the reproductive axis to these inputs? How are inputs from different sensory stimuli integrated? Do bats commonly rely on endogenous circannual clocks, annually reset by an environmental cue, to regulate their reproduction?

11.6 CONCLUSION

The environment has two types of effects on reproduction in bats. Low body temperatures act directly on reproduction by slowing down the metabolic rate and slowing or delaying reproductive events. Other environmental factors, such as photoperiod, act indirectly on the reproductive axis, through regulatory neuroendocrine circuits that process sensory input and modify reproductive status. These regulatory circuits are likely to include an endogenous annual rhythm (or rhythms) of reproduction. Relatively few laboratory studies have been done to determine which environmental factors affect reproduction or how they act on the reproductive system. Much more work, especially on bats in controlled conditions, will be necessary to provide a good understanding of how bats respond reproductively to the environment.

ACKNOWLEDGMENTS

I am grateful for comments on earlier drafts of the manuscript by an anonymous reviewer and the editors. Previously unpublished observations were obtained in research supported by NIH grants HD 24177 and F32 HD 07294. Writing of the manuscript was supported in part by a William and Mary Summer Grant.

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Species Index

Subject Index