Rick A. Adams · Scott C. Pedersen
Editors

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ISBN 978-1-4614-7396-1 ISBN 978-1-4614-7397-8 (eBook)
DOI 10.1007/978-1-4614-7397-8
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013943698

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Printed on acid-free paper

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This book is dedicated to our fallen
colleagues Elizabeth Kalko, Dave Redell,
and Björn Siemers and to Tom Kunz
with wishes for a full recovery

Preface

Recent advances in the study of bats have changed the way we understand this elusive group of mammals. This volume consists of 25 chapters by 57 authors from around the globe covering the most recent findings on the evolution, ecology, and conservation of bats. The chapters in this book are not intended to be exhaustive literature reviews but instead extended manuscripts that bring new and fresh perspectives. Many chapters provide new insights and understanding in bat evolution, ecology, and conservation. All chapters were peer reviewed and revised by the authors. Many of the chapters are multiauthored to provide comprehensive and authoritative coverage of the topics.

The book is organized into three sections: Evolutionary Patterns (Chaps. 1–7), Ecology and Behavior (Chaps. 8–16), and Conservation and Education (Chaps. 17–25). Each section is not only diverse topically but also integrative with other chapters in that section. Some chapters provide new and unpublished data for consideration, whereas others provide historical, present, and future perspectives. This volume provides novel approaches to old questions as well as new ways of understanding the complex world of bats. New perspectives are provided on many aspects of bat biology, evolution, morphology, development, natural history, cognition and behavior, emotions, formation flight, migration, dispersal to islands, emerging viruses, white-nose syndrome, speciation, bats and the human dimension, educational and conservation challenges, global monitoring, economic value, and the state of global bat populations.

This book will be of interest to students, professional biologists, wildlife managers, conservationists, educators, environmental consultants, and anyone else interested in the broad and rich array of topics brought up to date in this volume.

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Part I
Evolutionary Patterns

Chapter 1
How to Grow a Bat Wing

Lisa Noelle Cooper and Karen E. Sears

Abstract The earliest bats underwent an extraordinary limb-to-wing transition during their evolutionary history and successfully colonized the aerial habitat. Unfortunately, the bat fossil record lacks transitional fossils documenting this event, thereby challenging scientists to reconstruct these changes in their body plan based on the molecular and morphological events occurring throughout embryonic development. This chapter reviews how recent evolutionary developmental biologists have begun to elucidate how bats got their wings based on molecular studies in embryonic and fetal bats. This chapter first summarizes our current understanding of the processes regulating basic mammalian limb development in terrestrial taxa, and then discusses how bat limb development is unique in its formation of a novel limb pattern, wing membrane, and elongated digits. Lastly, this chapter outlines novel areas ripe for future study in bat evolution and development. Taken together, these data offer insights into the molecular and gross morphological events that drive innovation and molecular diversification in mammals.

1.1 Introduction

Although most mammals inhabit terrestrial habitats, one lineage, the bats (Order Chiroptera) underwent an extraordinary limb-to-wing transition during their evolutionary history and successfully invaded the skies (Gunnell and Simmons 2005b; Thewissen and Babcock 1992). This invasion enabled the diversification of bats, such that today bats comprise 25 % of living mammalian species (Arita and

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_1,

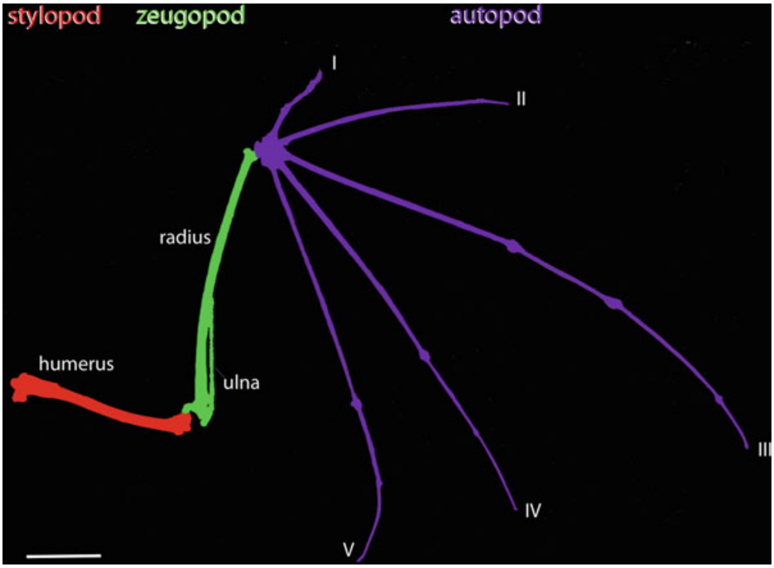

Fig. 1.1 Bones of the forelimb of bats. Scale bar is 1 cm in length

Fenton 1997; Teeling 2000; Teeling et al. 2005). As a result, a key innovations leading to bat's success was the evolution of a body plan and wing capable of powered flight (Adams 2008; Arita and Fenton 1997; Hedenstrom et al. 2007; Norberg and Rayner 1987; Pivkin et al. 2005).

Unfortunately, little is known of the morphological events that took place during the bat's evolutionary transition from terrestrial limb to wing. Bat ancestors (based on molecular and morphological data) were probably quadrupedal rodent-like mammals with pawed limbs (Gunnell and Simmons 2005a). Modern bats and the earliest fossil bats exhibit forelimbs with long finger bones (not extra finger bones) that are covered by a thin, soft tissue wing membrane (Fig. 1.1). No fossils have yet been found that document the forelimb changes between these two morphological endpoints, a pawed limb and a wing (Gunnell and Simmons 2005a; Jepsen 1966). As a result, the fossil record currently cannot provide much insight into how the bones of the bat forelimb lengthened or the wing membranes formed over evolutionary time. To overcome this paucity of data (Eiting and Gunnell 2009), scientists are charged with reconstructing the limb-to-wing transition based on molecular and morphological events occurring in bats as they develop from embryos to adults (Adams 1992, 2008; Chen et al. 2005a; Cretekos et al. 2001, 2005a, 2007a, 2008a, b; Farnum et al. 2008; Hockman et al. 2008; Kunz and Anthony 1982; Nolte et al. 2009; Sears et al. 2006; Weatherbee et al. 2006; Wyant and Adams 2007).

A relatively new branch of biology, evolutionary developmental biology (or "evo-devo"), in part, seeks to shed light on the mechanisms underlying the diversification and evolution of novel body structures by integrating data from many sources, including the fossil record, embryology, and genetics (Carroll 2008). Traditionally, the goal of most developmental research in mammals has been to understand the genetic basis of mouse development, and thereby advance the field of biomedicine. However, some evo-devo researchers have more recently begun to apply methods developed in mice to non-model mammals [e.g., marsupials (Sears 2004, 2005), cetaceans (Thewissen et al. 2006), and bats (Chen et al. 2005a; Cretekos et al. 2001, 2007a, 2008a; Hockman et al. 2008; Sears et al. 2006; Weatherbee et al. 2006)]. This novel application of techniques to atypical taxa has answered fundamental questions about the diverse evolutionary and molecular mechanisms patterning the mammalian body plan (Behringer et al. 2005, 2009). For example, recent research has illuminated how whales lost their legs (Thewissen et al. 2006), bats lengthened their wing bones (Cretekos et al. 2008a; Sears et al. 2006), and embryonic marsupials developed the limb morphology necessary to crawl from the birth canal to their mother's teat (Sears 2004, 2005). This powerful integration of fossil and modern developmental data offers insight into the mechanisms driving the evolution of novel variations in the mammalian body plan on both macro- and microevolutionary scales.

Bats, because of their unusual body plan, have recently emerged as model "non-model" organisms for evo-devo study. Within the last decade, researchers have engaged in extensive fieldwork to collect embryonic and fetal tissues from bats in the wild, and used these tissues to explore molecular patterning in the laboratory (Cretekos et al. 2005b). Most evo-devo research has focused on Seba's short-tailed bat (*Carollia perspicillata*) collected from the tropical island of Trinidad. This small, leaf-nosed bat is an agile flier and females can give birth to two pups a year. Females mostly synchronize their pregnancies, so embryos of a variety of closely spaced ontogenetic stages can be collected in a single field season. In collaboration with the University of West Indies, a team of researchers from the United States (University of Illinois, Northeast Ohio Medical University, Idaho State University, University of Texas M. D. Anderson Cancer Center, State University of New York Downstate Medical Center) go into the field to collect embryonic and fetal *Carollia* twice a year for evo-devo research. During the Trinidad field collection, roosts are typically found in culverts, abandoned water tanks and houses (Fig. 1.2).

In addition, a handful of researchers have studied wing development in other bat species [e.g., *Miniopterus* (Hockman et al. 2008), *Molossus* (Nolte et al. 2009), *Myotis* and *Rhinolophus* (Ray and Capecchi 2008)]. These studies suggest a general conservation of the genes controlling limb development across mammals. That is, the same genes control bat and mouse limb development. However, the regulation of these shared genes differs in bats and terrestrial mammals (e.g., mouse) when generating their divergent limb phenotypes.

This chapter reviews the current state of research on limb development in bats and outlines ongoing and future avenues of study. This chapter will initially offer a review of the fundamental molecular mechanisms known to drive limb morphogenesis in mammals. Next, published articles focusing on molecular events driving bat wing membrane development and limb patterning will be reviewed. The role of molecular signaling in bat forelimb connective tissues will then be discussed as it

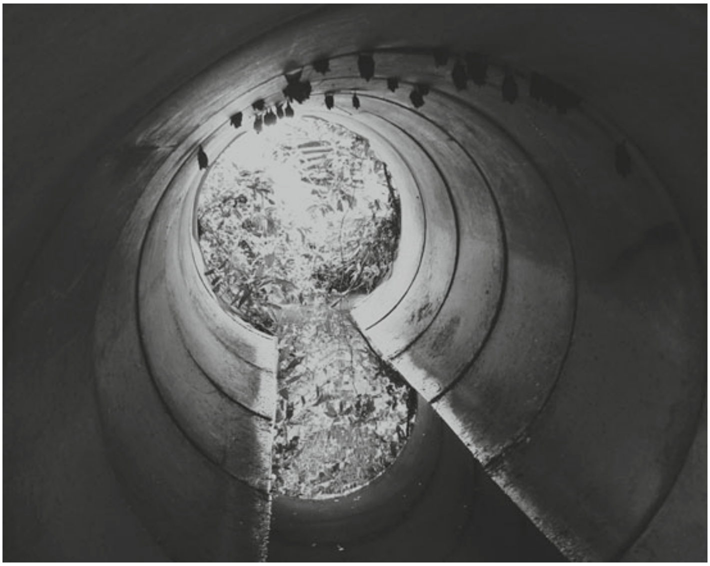

Fig. 1.2 Bats hanging from the ceiling of a culvert. Photo credit to Merla Hübler

partially explains how bat wing bones have lengthened over evolutionary time. Continuing with the connective tissue theme, a literature review of our current understanding of bat skeletogenesis will be combined with some preliminary data detailing recent efforts in exploring the evolution of bat skeletal structure. This review ends by listing potential future areas of research into the unique body plan of bats. Taken together, these reviewed and proposed focus areas of bat research hold promise to unravel the molecular and gross morphological events that drive the process of innovation and molecular diversification in mammals, and animals in general.

1.2 Fundamentals of Limb Development

For the past 60 years, an intense interest in vertebrate limb development has generated tremendous insights into the morphological events and molecular mechanisms shaping vertebrate organogenesis (Saunders 1998a, b; Wellik et al. 2011). Biomedical interest in limb organogenesis typically aims to identify the causes of and treatments for human diseases. However, a new generation of evo-devo researchers are investigating variation in limb organogenesis to identify molecular drivers of innovations and diversification in limbs (Abbasi 2011; Reno et al. 2008; Davis et al. 2007; Fröbisch and Shubin 2011; Hodgkinson et al. 2009; Larsson et al. 2010; Shapiro 2002; Shapiro et al. 2003, 2004; Tamura et al. 2011; Thewissen et al. 2006).

To accomplish this, an understanding of the basic and conserved mechanisms shaping vertebrate limb morphogenesis, which was discovered during study of traditional model organisms (e.g., chick, mice, etc.), is required (Gilbert 2006).

Vertebrate limb outgrowth and patterning occurs along three primary axes (Cooper and Tabin 2009; Duboc and Logan 2011; Harfe 2011; Hopyan et al. 2011; Welten et al. 2011). Outgrowth occurs along a line running longitudinally down the limb, from shoulder-to-fingertip, called the proximo-distal (PD) axis. Proper development along the PD axis results in a limb that is segmented into the humerus (stylopod), radius and ulna (zeugopod), and the wrists and digits of the hands and feet (autopod). Patterning of the limb also occurs in a line from the thumb to the little finger along the antero-posterior (AP) axis. AP patterning, among other things, partially drives variation in digit number and size. Finally, developing limbs are also patterned on the axis from the back of the hand to the palm, or the dorso-ventral (DV) axis. DV patterning, in part, shapes asymmetries in joints and muscles of fingers so that flying, grasping, and crawling are possible. Activity of these three axes begins early in limb development with the expression of molecular signals. Although development of each axis is regulated by a characteristic set of molecular pathways, these pathways interact among axes to generate skeletal and soft tissues. Failure of one axis to properly develop can cause a complete failure in limb outgrowth and patterning (Crossley et al. 1996).

A thickened epithelium called the apical ectodermal ridge (AER) guides outgrowth of the limb along the PD axis (Cooper et al. 2011b). The AER is a mass of cells (Fig. 1.3a) that extends over the distal-most AP axis of a developing handplate (Fernandez-Teran and Ros 2008; Saunders 1998a, b). Signals originating in the AER act to at least permit, and potentially instruct, outgrowth of the limb and patterning of distal limb structures. Among many other genes, the AER expresses fibroblast growth factors (*Fgfs*) 4, 8, 9, and 17 to stimulate cellular proliferation and inhibit apoptosis of the underlying mesoderm (Martin 1998; Sun et al. 2002).

The homeobox (*Hox*) genes, as well as a dynamic relationship between retinoic acid (*RA*) and *Fgf*s, partially control segmentation and patterning along the limb's PD axis (Boulet and Capecchi 2004; Cooper et al. 2011a; Montavon et al. 2008; Rosello-Diez et al. 2011; Zakany et al. 2004). The most studied tetrapod models, mouse and chick, have 39 *Hox* genes divided into four clusters (A–D). Most important for limb development are 5′ group A and D genes. The *Hox11* paralogue is essential for zeugopod development (i.e., *HoxA11* and *HoxD11* in the forelimb), while the *Hox13* paralogue is specific for autopod development (i.e., *HoxA13-HoxD13*) (Cooper and Tabin 2009). If these *Hox13* homologues are absent during development, the autopod fails to develop. Evidence also suggests that major segments of the limb (e.g., stylopod, zeugopod, and autopod) are partially patterned by a temporal dynamic between proximally expressed retinoic acid (*RA*) and distally expressed *Fgf*s and *Wnt* (Cooper et al. 2011a; Rosello-Diez et al. 2011). Distally expressed *Fgf*s and *Wnt*s act to keep cells in an undifferentiated state, while the more proximally expressed *RA* acts to specify proximal segment cell fates.

Patterning along the AP axis, from thumb to little finger, is largely controlled by the zone of polarizing activity (ZPA) (Harfe 2011). The ZPA is located in the

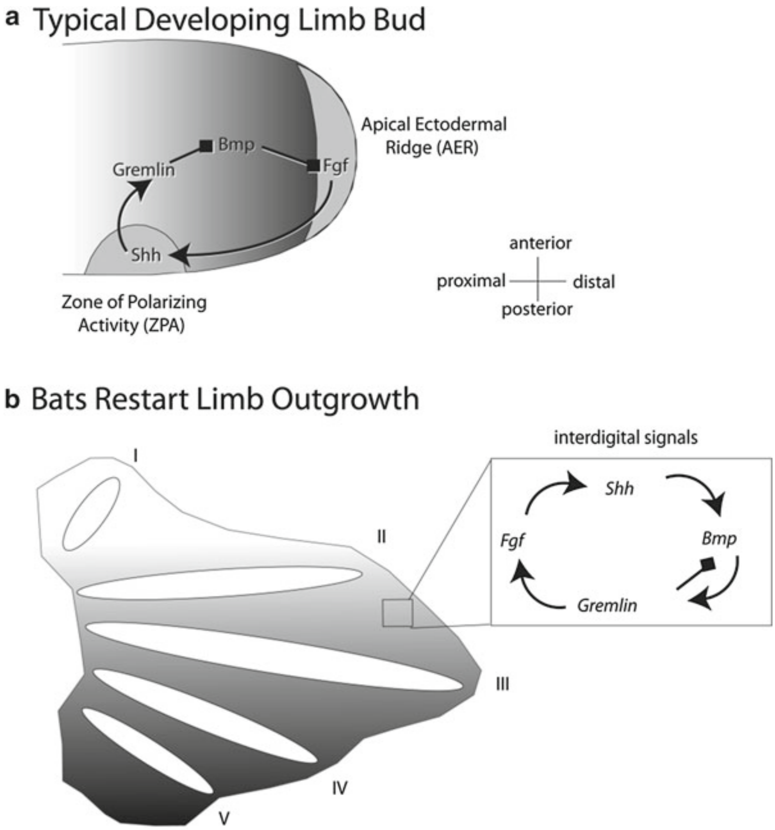

Fig. 1.3 Schematics of the developing limb of a generalized vertebrate (**a**) and a fetal bat (**b**)

posterior portion of the tetrapod limb bud, but lacks defining morphological characteristics. This signaling center characteristically expresses Sonic Hedgehog (*Shh*). This gene, named after a video game character, is essential for proper AP patterning of the limb, and for the formation of all posterior limb structures including the ulna and fibula, and all digits except for the thumb (Harfe 2011; Harfe et al. 2004; Zhu and Mackem 2011).

DV patterning of the limb is controlled by asymmetrical expression of genes along the longitudinal axis of the limb. *Wnt-7a* is expressed in the ectoderm on the dorsal surface of the limb bud (Dealy et al. 1993; Davis et al. 1991), while the transcription factor Engrailed1 (*EN1*) is expressed in the ventral ectoderm (Davis et al. 1991). *EN1* directs the formation of ventral limb structures, including paw pads.

Although usually discussed independently, the development of these axes is interconnected. For instance, a positive feedback loop between the AER and ZPA is required for proper limb development (Laufer et al. 1994). This AER-ZPA feedback loop (Fig. 1.3a) is partially regulated by bone morphogenic protein (*Bmp*) and *Gremlin*. *Bmps* downregulate *Fgf* expression in the AER and thereby repress limb outgrowth (Niswander and Martin 1993). *Gremlin* is induced by *Shh* expression in the ZPA and acts to inhibit the action of *Bmps*. *Gremlin* therefore maintains activity of the AER during limb outgrowth. Expression of *Shh* in the ZPA is positively regulated by *Fgfs* expressed in the AER. *Shh* expression also connects activity of the AP and DV axes. Pythons and dolphins each fail to express at least one of the genes essential to this AER-ZPA feedback loop, contributing to truncation of their limb development (Cohn and Tickle 1999; Thewissen et al. 2006). This cessation of this gene activity during limb development partially explains why adult snakes are limbless, and adult dolphins lack hind limbs.

1.3 Interdigital Tissues and Wing Patterning

During embryogenesis, mammalian limbs, including those of bats and mice, initially form as buds protruding from the body (Hopyan et al. 2011). This limb bud then elongates and forms a handplate, and finally takes on an adult-like form during the fetal period. In mice, this sequence is accompanied by a period of programmed cell death, or apopotosis, in the tissues connecting the digits. The presence of interdigital apoptosis removes the soft tissues connecting the developing digits, creating separated digits (Chen and Zhao 1998). The same process occurs in humans to shape our fingers. In bat forelimbs, however, interdigital apoptosis is inhibited and the interdigital tissues are retained to form the wing membrane (Weatherbee et al. 2006).

Essential to the pathway that activates interdigital apoptosis is the expression of bone morphogenic protein (*Bmp*) (Chen and Zhao 1998; Dahn and Fallon 2000; Ganan et al. 1996; Guimond et al. 2010; Laufer et al. 1997; Merino et al. 1999; Pajni-Underwood et al. 2007; Yokouchi et al. 1996; Zou and Niswander 1996). In the developing limbs of both mice and bats, *Bmp* is expressed in the interdigital tissues, indicating the apoptotic pathway is activated. However, bats utilize two mechanisms to keep this pathway from resulting in interdigital cell death. First, bats express the *Bmp* inhibitor *Gremlin* within the interdigital tissues (Weatherbee et al. 2006). Second, bats extend *Fgf-8* expression from the AER to the interdigital tissues, where it probably acts as an anti-apoptosis survival factor. *Gremlin* probably inhibits the apoptotic effects of *Bmp*, and the interdigital expression of *Fgfs* probably drives cellular proliferation of the membrane tissues, resulting in the membrane's growth between the digits (Weatherbee et al. 2006).

The developing interdigital tissues of bats also display novel spatial and temporal expression patterns of genes active in typical limb development (e.g., *Shh*, *Ptc1*, *Fgf8*) (Hockman et al. 2008). Bats display a novel expression domain for *Shh* and its downstream target *Patched 1* (*Ptc1*). During the incipient stages of bat limb development, the ZPA is comparatively expanded and *Shh* exhibits a larger expression domain than that of mice (Hockman et al. 2008). It could be that this domain expansion is a response to the increased *Fgf* expression in the enlarged AER of bats.

Furthermore, during fetal limb development, *Shh* expression extends from the ZPA and is redeployed in the interdigital tissues.

Because limb patterning and outgrowth occurs on three axes (e.g., proximodistal, antero-posterior, and dorso-ventral) and signals among these axes are interdependent, the effects of novel expression domains in the bat interdigital spaces may help shape the novel morphologies of the bat limb. Besides inhibiting apoptosis of the wing membrane, these novel expression domains likely act to generate the bat wing by reactivating the *Shh–Fgf* positive feedback loop, after it usually stops in mice, thus extending the duration of limb development (Hockman et al. 2008). Furthermore, several genes (i.e., *Fgf8*, *Shh*, *Gremlin*, and *Bmp2*) are expressed in an antero-posterior gradient in the developing limb of the bat. Besides being expressed in the bat limb AER, *Fgf8* is expressed in a gradient from digit V to digit I, and is thought to activate *Shh* expression in these tissues (Fig. 1.3b). *Shh* then activates *Bmp2* in a similar expression pattern. *Bmp2* then activates *Gremlin* expression (graded from anterior to posterior). Expansion of these expression domains into the interdigital tissues therefore plays an essential role in patterning the autopod of bats. The gradient of *Shh*, *Fgf8,* and *Bmp2* expression probably contributes to the shortened digit I and elongated digits III–V (Hockman et al. 2008).

1.4 Connective Tissue Development: From Cartilage to Bones

1.4.1 Mechanisms Generating Long Wing Bones

Bat forelimbs possess radically modified autopodal skeletal elements that support the wing membrane (Norberg 1972; Swartz et al. 1992). For example, the bones of digits III–V of all modern and known fossil bats are elongated compared to terrestrial taxa. At the formation of their initial cartilaginous condensations, the developing digits of bats and mice are equivalent in length (Sears et al. 2006). However, during fetal development the relative length of bat wing metacarpals and phalanges dramatically increases (Cretekos et al. 2005a; Sears et al. 2006). Over the past decade, evo-devo researchers have identified some of the molecular processes driving this digital elongation by comparing molecular signals in the developing digits of mice and bats.

The cartilaginous precursors of bony skeletal elements are composed of chondrocytes progressing through multiple developmental stages (de Crombrugghe and Akiyama 2009; Farnum et al. 2008; Kronenberg 2003; Kronenberg et al. 2009). Bone morphogenic proteins (*Bmps*) play a critical role in chondrocyte proliferation, initial hypertrophic differentiation, and inhibiting terminal hypertrophic differentiation (Kronenberg 2003; Kronenberg et al. 2009; Minina et al. 2001; Pizette and Niswander 2000). During skeletogenesis, *Bmps* are expressed in hypertrophic chondrocytes as well as the osteogenic perichondrium, a connective tissue sheath encasing a developing metacarpal. Previous studies have found that the levels of *Bmp* expression were 30 % higher in bat than mouse forelimb digits. Increased signaling was also documented in a target of the *Bmps* – *phospho*-Smad 1/5/8 (Sears et al. 2006). The functional effects of these signaling differences in bat and mouse cartilages were tested during limb culture assays. In these experiments, developing bat and mouse metacarpals were excised and exposed in vitro to the protein of *either BMP* or its antagonist Noggin. Cultured limbs significantly lengthened in the presence of the BMP protein, and shortened in the presence of Noggin. Therefore, alterations in BMP levels are sufficient to lengthen the metacarpals, and presumably other autopodal elements, of the bat wing (Sears et al. 2006). These data offer compelling insights into the novel morphologies that indicate that a small modification in the level of gene expression can result in dramatic morphological changes.

The genes *HoxD13 and Prx1* and *Prx2* have been shown to have different expression patterns in bats compared to mice, but the sequences of these genes are almost identical (Cretekos et al. 2008b; Ray and Capecchi 2008). As a result, researchers looked for differences in the expression and sequence patterns of upstream enhancers, or regulatory elements, of these genes. Differences were found in the *Prx1* enhancer, and researchers genetically replaced the limb-specific *Prx1* enhancer in mice with its bat homolog. In the resulting mutant mice, limb expression of *Prx1* was upregulated. Furthermore, the limbs of the mutant mice displayed a modest but significant lengthening of bones relative to those of wild-type mice. Therefore, at least evolutionary modifications in the bat *Prx1* enhancer could have played a role in driving forelimb elongation in bats. Researchers have also investigated the possible role of evolutionary changes to a *HoxD13* limb enhancer, Global Control Region (GCR), in bat development (Ray and Capecchi 2008). Compared to humans and mice, the bat GCR displays additional activity domains in the limbs and outer ears, and a lineage-specific alteration of 26 nucleotide sequences.

1.5 Mechanisms Regulating Bat Bone Architecture (Ongoing Research)

Forelimb bones of bats are unique compared to terrestrial mammals because they contain less mineral, and have thinner cortices (Papadimitriou et al. 1996; Swartz et al. 1992; Swartz 1997; Swartz and Middleton 2008). Unfortunately, the cellular and molecular events shaping mineral deposition during endochondral ossification of the bat skeleton are unknown.

Compared to terrestrial mammals, bats alter the microanatomical characteristics of their forelimb bones, probably to accommodate the high bone bending strains that result from powered flight (Kirkpatrick 1994; Papadimitriou et al. 1996; Swartz 1997; Swartz et al. 1992; Swartz and Middleton 2008). Radiographs (Swartz 1997) and density assays (Dumont 2010; Papadimitriou et al. 1996; Swartz and Middleton 2008) suggest that bat forelimb elements display a PD gradient in mineral content

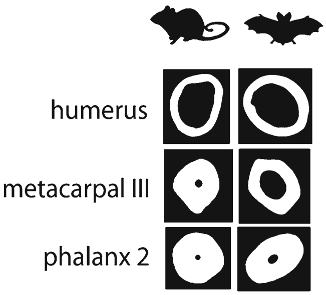

Fig. 1.4 Results of high resolution micro-CT scans of some forelimb bones of adult bats and mice. Not to scale. Bones sized to show relative dimensions of medullary cavities

and cross-sectional geometry. As a result, the bat humerus and radius display the greatest mineral concentration (Dumont 2010; Swartz and Middleton 2008) and largest medullary cavity among sampled mammals, while the phalanges have the lowest mineral concentration of mammals and are typically amedullary. High resolution scans and precise nanoindentation tests are needed to validate these findings (Swartz and Middleton 2008). Furthermore, the molecular events shaping development of the unique aspects of bat forelimb architecture remain unknown. This is unfortunate, as the unique skeletal microstructure of bats provides an outstanding opportunity for skeletal biologists to use bats as a "natural mutant" to investigate the alternative modes of skeletogenesis naturally employed by mammals.

To begin to remedy this situation, a set of preliminary studies were performed that compared limbs of the short-tailed bat (*Carollia*) and mice (*Mus*, *Peromyscus*). Developing bones of an ontogenetic series of bats and mice were longitudinally sectioned and stained to quantify diaphyseal dimensions. Preliminary results indicate that, compared to rodents, bats begin long bone ossification earlier in ontogeny and increase the rate of diaphyseal elongation just prior to birth. This finding is consistent with reports that most bat endochondral ossification occurs postnatally (Adams 1992, 2008; Farnum et al. 2008). Additionally, high-resolution micro computed tomography (μCT) scans were used to quantify bone cross-sectional geometries. Scans indicated that adult bat bones display larger midshaft medullary cavities and thinner cortices than those of mice (Fig. 1.4). Humeral, femoral and tibial cross-sectional dimensions were roughly equivalent in these taxa, however distal bones (radius and elements of the autopod) of the bat displayed 8–40 % larger medullary cavities compared to sampled rodents. Furthermore, nanoindentation tests revealed that the midshaft cortex of metacarpal III of the bat was 40 % as stiff and 36 % as hard than that of mice. Whole bone bending tests (i.e., humerus and radius) allowed for the generation of load–displacement curves. Both bat and mouse humeri, and the mouse radius displayed steeply-sloped curves indicative of stiff bone, while the bat radius was much more compliant, suggesting a decreased mineral content and/or increased elasticity. These preliminary findings are generally consistent with previous studies of bat anatomy, and support the assertion that bats have altered their bone architecture relative to rodents.

These data indicate that the bones of bats display a decreased mineral composition and are more compliant compared to the bones of mice. The combination of a low-bone-mass phenotype with a compliant behavior during loading events represents an intriguing opportunity for skeletal biologists interested in biomedical applications for human diseases. The human bone diseases of osteopenia and osteoporosis create low-bone-mass pathologies that frequently result in bone fracture. Such fractures are a major source of morbidity and mortality in the elderly. Rather than continuing to utilize the mouse as a model taxon for identification of treatments for bone diseases, it could be that bats represent a "natural mutant" that holds the key to rescuing bone elasticity in low-bone-mass pathologies. To explore the mechanisms shaping the unique material properties of bat bones, a variety of molecular techniques will be used to identify key gene expression differences in the developing bones of bats and mice.

To compare the genetic mechanisms shaping limb dimensions in the typical limbs of mice versus the novel elongated and lightweight bone of bats, the transcriptomes of these bones will be compared. Resulting data will identify the relative expression levels of those genes actively patterning bone cross-sectional geometries (e.g., cortical versus medullary bone dimensions), and mineral deposition (e.g., osteoblast and osteoclast activity). Candidate genes identified in the transcript analysis will then have spatial and temporal expression patterns visualized via in situ hybridization and immunohistochemistry. Functional tests (e.g., limb culture) will then be used to experimentally manipulate long bone development. Taken together, these proposed methods aim to identify the molecular function of those genes that are expressed differentially in the developing bones of bats and mice.

1.6 Future Research

1.6.1 Early Limb Patterning: When do Genes Stop Making a Pawed Limb and Start Making a Wing?

Despite its highly specialized adult form, bat forelimbs superficially resemble those of other mammals during their earliest outgrowth and patterning. Not until the handplate is fully formed is the bat forelimb easily distinguishable (Cretekos et al. 2005b). As discussed above, the extreme digit elongation of adult bat wings arises even later, predominately during the fetal period (Sears et al. 2006; Sears 2008). In contrast, the minimal gene expression data available suggest that the molecular divergence of bat wings from the limbs of other mammals occurs much earlier in development. For example, spatial expression of *Fgf8*, a gene essential to limb outgrowth, is expanded in bat forelimbs relative to those of mouse at the limb bud stage (Cretekos et al. 2007b). Consistent with this, preliminary data suggests that the AER extends further along the AP border in the bat than mouse forelimb. No studies have elucidated the genetic modifications that establish the enlarged AER and ZPA signaling centers in the bat wing, compared to mice.

To begin to remedy this situation, researchers are currently taking a comparative approach to investigate how evolutionary changes during initial forelimb outgrowth and patterning (from initial outgrowth through the formation of the limb bud) contribute to the generation of the divergent forelimbs of bat and other mammals (e.g., mouse, opossums, cats, dogs, horses, etc.). As the evolutionary flexibility of earliest limb development has profound implications for how mammalian limbs diversify, this ongoing research should prove integral to our understanding of mammalian evolution and innovation.

1.7 Sensing Flow and Capturing Prey: Mechanoreceptors in the Wings, Muscles, and Tendons of Bats

During aerial maneuvers, the wing membrane of bats stretches and billows with flow of air over the membrane (Watts et al. 2001). Bats actively regulate these membrane behaviors during impressive aerial maneuvers like turns, hovering, and recovering from stalls (Pivkin et al. 2005; Muijres et al. 2008; Hedenstrom et al. 2007). Research into the anatomy of the wing has revealed that the architecture of the wing includes an intricate network of collagen and elastic fibers in the dermis (Holbrook and Odland 1978), microscopic muscles controlling membrane deformation (Swartz et al. 1996), and an extraordinary array of cutaneous receptors (Sterbing-D'Angelo et al. 2011). These cutaneous Merkel cell receptors are found on both the dorsal and ventral surfaces of the wing, and are associated with microscopic hairs that protrude out of dome-like structures. This sensory complex acts to send information regarding air flow to the bat somatosensory system.

Some bat wings lack pelage, or body, hair, and instead display two types of hairs: long, thick hairs near the ventral forearm (where aerodynamic pressure is pronounced), and short sensory hairs arranged in a grid pattern along the wing, and along the trailing edge of the wing. These sensory hairs, when associated with the domes, are typically located in areas along the wing membrane that are exposed to turbulence, or vortices, and flow reversal, as occurs during aerodynamic flow separation. These hairs therefore act as stall sensors, and experimental evidence shows they are sensitive even at low airflow speeds.

In terrestrial taxa, such sensory hairs are typically found on the head in the form of vibrissae. The presence of these receptors on the wings of bats offers evo-devo researchers an outstanding opportunity to explore epithelial structure diversification at the molecular level. No molecular analyses have addressed the mechanisms regulating sensory hair development in the bat wing. It is currently unknown how hair types are specified along the wing surfaces, and whether the loss of pelage hair on the wing was a critical step in the gaining of such an extensive network of cutaneous sensory receptors. It is also unknown how regular spacing of these hairs is regulated during development. As with murine hair follicle formation, it could be that sensory hair cell distribution along a wing is controlled by a reaction–diffusion mechanism associated with *Wnt* and its inhibitor *Dkk.* In rodents, these two genes in part determine epithelial appendage density (Sick et al. 2006).

1.8 Conclusions

Although evo-devo research is shedding light on the numerous changes that characterize the limb to wing transition in bats, it is unlikely that we will be able to combine these manipulations to create a flying rodent in the laboratory. This level of molecular manipulation is currently the stuff of dreams, but experiments do offer profound insights into the mechanisms that may have shaped the evolution of the body plan in the ancestors of modern bats. Slight temporal and spatial modifications to the expression of genes essential for proper limb development (e.g., *Bmp*, *Fgf*, *Shh*, *Gremlin*) probably played critical roles in the evolutionary generation of the bat wing's membrane and elongated bones.

The picture beginning to emerge from comparative studies of mammalian limb development, including that of bats, is that the evolution of the earliest stages of limb development may be constrained to some degree, such that extreme modifications (e.g., loss of complete limb bud or digit anlagen) tend to be selected against. Consistent with this, the gross morphology of the limb during its earliest development is similar in mammals with highly divergent adult limb morphologies (e.g., bats, cetaceans, etc.). However, this constraint is by no means absolute, and mammalian limbs seem to have experienced a suite of moderate evolutionary modifications during their earliest development (Richardson 1999). Minor alterations in earliest limb development have been documented in bats (e.g., *Fgf8* expression and AER formation), and in other non-traditional mammals that have been studied, such as opossums and pigs (Sears et al., in press).

One example of a minor modification in early limb development that is emerging is that the morphology of the AER varies among mammals (for a review of AER morphologies among vertebrates see Cooper et al. 2011a, b). For example, bats have an expanded forelimb AER width and length along the AP axis that likely contributes to the formation of their larger adult forelimbs. In contrast, opossum (e.g., *Monodelphis*) forelimb AERs are reduced. In opossum, the AER is formed of discontinuous masses of cells along the limb's tip, rather than the characteristic ridge of tissue. This morphology has yet to be documented in other vertebrates, and its effects on signaling during limb development are the subject of ongoing study. Results of this and other ongoing studies may call into question fundamental assumptions about the overall conservation of the morphological and signaling pathways that are necessary to generate a limb (Stopper and Wagner 2005).

Taken together, evo-devo researchers are exploring gene regulation in the developing limbs of mammals with novel phenotypes to understand how molecular and genetic variation has shaped organogenesis over geological time scales. This fundamental shift in scientific approach borrows heavily from the techniques utilized in a laboratory focused on biomedical research, but asks questions that answer mechanistic questions on different time scales. As a result, the field of evo-devo is able to provide links between micro- and macro- evolution, and thereby illuminating not only how morphological evolution has proceeded, but why and the mechanics of how it has proceeded.

Acknowledgements The authors thank Drs. Rick Adams and Scott Pedersen for the invitation to contribute to this edited volume. Drs. Chris Cretekos, Richard Behringer, John J. Rasweiler IV, and Mr. Simeone Williams are thanked for conversations and continued field efforts in collecting *Carollia* specimens. Laura Catherwood and Alex Rockwell are thanked for assistance in sectioning and staining bat wings. Lastly, the authors thank the Department of Life Sciences at the University of West Indies, as well as the Ministry of Agriculture, Land, and Marine Resources of Trinidad and Tobago, Trinidad for supporting our field work. L.N. Cooper thanks the American Association of Anatomists for supporting this research via an AAA Postdoctoral Fellowship. K. Sears thanks the University of Illinois for funding field collection of *Carollia*.

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Chapter 2
Time's Arrow in the Evolutionary Development of Bat Flight

Rick A. Adams and Jason B. Shaw

Abstract Conceptualizing the evolution of flight in mammals is confounded by a lack of empirical evidence. In this chapter, we quantify functional ontogeny to model the evolution of flight in bats to fill in transitional gaps between a hypothetical nonvolant ancestor and volant descendents. Our data thus far indicate that bats evolved flapping flight mechanics directly with no gliding intermediate forms and that bats most likely evolved from a terrestrial, rather than arboreal, ancestor. We predict that future analysis of locomotor ontogeny in contemporary bats will be instrumental in bridging the significant gaps and discontinuities between fossil, molecular, and mechanical evidence thus far used to interpret flight evolution in mammals.

2.1 Introduction

The evolution of flight in mammals is one of the most compelling events in vertebrate history. Although some fossil evidence and molecular analyses provide insight into how, and possibly where, the evolutionary transition(s) to flight took place, there persist two unresolved central and interrelated questions (1) *Was the ancestor of bats arboreal, semiarboreal, or terrestrial?* (2) *Did flight evolve in bats via an intermediate gliding form or did flapping flight evolve directly?*

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_2,

Darwin (1859) proposed that bats evolved from a quadrupedal arboreal ancestor and went through a transitional gliding state that eventually gave rise to true flight. In efforts to visualize an evolutionary pathway from nonvolant ancestor to volant descendant, the tree shrew (Scandentia, *Tupaia glis*) and the colugo (Dermoptera, *Galeopterus variegatus*) frequently became asserted as representative transitional states (Hill and Smith 1984). For example, Allen (1939) wrote about anatomical similarities between tree shrews and the Middle Eocene *Palaeochiropteryx* and wrote that colugos are "almost bat-like in some respects," and Romer (1959) stated "It seems obvious that the bats, essentially insectivores in their beginnings, have been derived from an arboreal insectivorous group…" and also considered colugos illustrative of an "intermediate stage in the evolution of flight." However, tree shrews are not likely demonstrative of an arboreal ancestor for bats because their probable origin (63 mya) coincides with a time when protobats would have conceivably evolved already (Gunnell and Simmons 2005; Janečka et al. 2007) and colugos are no longer thought representative of a hypothetical intermediate but instead as an evolutionary end point for highly derived gliding (Janečka et al. 2007; Norberg 1985, 1987; Simmons et al. 2008). Many researchers, nonetheless, still continue to view bat evolution as beginning with an arboreal insectivore ancestor that transitioned into bats via a gliding intermediate form (see Giannini 2012 for review). The appealing nature and resilience of the arboreal-gliding hypothesis seems to reside in two well-accepted ways in which key evolutionary innovations may arise (1) transitional states leading to highly derived outcomes are commonly incremental and additive and arise over generations, thereby minimizing the degree of deleterious discordance between form and function, and (2) the origin of patagia was for an established and related mode of locomotion and has easily provided the foundation for co-option into a flapping wing for powered flight (although see Speakman 1999, 2001 for an alternative hypothesis).

Some researchers have argued conversely that bats evolved flapping flight directly with no gliding intermediate (a more punctuated scenario). Moody (1962) asserted that "gliding does not provide the means of entering the flying insectivorous niche" and Jepson (1970) wrote "…no eutheres or the 'flying' marsupials are known to forage or eat while volplaning, the whole function of the glide seems to be transportation." Perhaps most damning of the arboreal-gliding-intermediate hypothesis is that empirical tests on gliding squirrels revealed that transitions from efficient gliding to flapping flight would be severely confounded and unlikely to succeed (Bishop 2008). There is also no fossil evidence showing that gliders of any vertebrate group transitioned to powered flight (Grande 1994; McMillan et al. 2006; Wilf 2000). However, some researchers have shown mathematically plausible aerodynamic models for the transition from gliding to flight, and thus the potentiality remains (Dudley et al. 2007; Norberg 1985).

If a gliding protobat did indeed exist, some have suggested that it may have been omnivorous or herbivorous thereby avoiding the improbable nature of aerial hawking of insects while gliding (Ferrerezi and Giménez 1996; Jepson 1970; Padian 1987; Speakman 2001). Although some Paleocene-age jaw fragments with frugivorous teeth were originally thought to be from bats (Mathew 1917), this has been challenged (Storch et al. 2002), and teeth of the earliest identifiable bat fossils are tribosphenic, matching those of Early Tertiary insect-eating mammals (Jepson 1966; Rose 2006; Simmons et al. 2008).

Dudley et al. (2007) contends that the many independent lineages leading to the plethora of contemporary vertebrate gliders are strong, albeit indirect, support for the gliding-intermediate hypothesis. However, an equally valid argument would suggest that the existence of mammalian gliders from at least nine independent origins (Jackson 2000; Meng et al. 2006; Scheibe and Robins 1998; Stortch et al. 1996) would have likely given rise to more than a single origin of flight if gliding was an inherent intermediate state.

The goal of this chapter is to integrate baseline data on the ontogeny of flight with likely evolutionary scenarios. We focus on four underlying principles (1) *presence of the past*, that contemporary species retain developmental locomotor pathways laid down during earlier phyletic evolution; (2) *minimize highly risky (maladaptive) behaviors*, selection favors behaviors that reduce the probability of mortality during ontogenetic and evolutionary transitions; (3) *locomotor stability*, selection favors those individuals with the best locomotor skills during ontogeny and evolution; and (4) *Occam's Razor*, when there are two competing hypotheses that make exactly the same predictions, the simpler one is the better.

Descriptive ontogeny has been well documented for several bat species (see Kunz and Parsons 2009 for review). Instead, in this chapter, we pursue a *functional* evo-devo perspective (Breuker et al. 2006) in hopes of providing a more integrative, empirically based blueprint for flight evolution. We use high-speed video to observe previously unnoted details in the ontogeny of flight and tie these data in with molecular and fossil estimates for flight evolution in bats.

2.2 Stability of Gliding Versus Flapping Flight

Flapping flight is commonly considered to be less aerodynamically stable than is gliding (Brown 1953; Maynard Smith 1952; Weis-Fogh 1973), but others argue using mathematical models that neither is inherently less stable than the other (Taylor and Thomas 2002; Norberg 1990). Whatever the case, the transition from nonvolant to volant form, both ontogenetically and evolutionarily, would require minimizing maladaptive discordance between form and function (Norberg 1990). Inherent control of roll, pitch, and yaw angles during gliding or powered flight (Fig. 2.1) must be maintained and is governed by an individual's ability to passively induce opposing forces to deviations in the angles of rotation and to serially dampen out oscillations that would perturb the intended flight trajectory (Maynard Smith 1952).

Flight kinematics in bats is complex because each wing has typically 15 joints that are active throughout a wingbeat cycle. In addition, highly flexible metacarpal and phalangeal joints are capable of hyperextension, and the distal phalanges of digits 2–5 are usually cartilaginous producing highly flexible wing tips (Swartz and

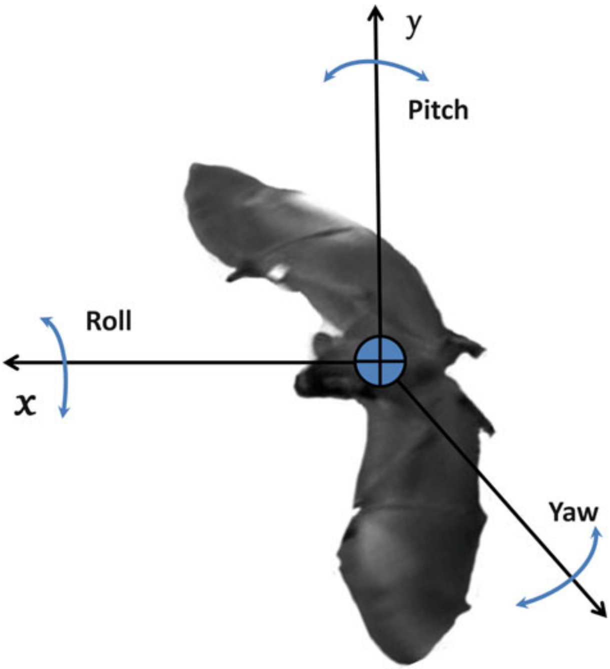


Fig. 2.1 During flight or gliding, the animal is free to rotate about, or translate along, each of three orthogonal axes originating at the center of gravity marked by the *blue circle*

Middleton 2008) that bend and twist in unprecedented ways (Norberg 1990). Clearly the evolution and ontogeny of bat flight is challenging anatomically, physically, and behaviorally.

2.3 Growth and Development of Bat Wings

Shaw (2011) quantified ontogeny of flight morphology in two phyllostomids (*Artibeus jamaicensis* and *Carollia perspicillata*). After standardizing for differences in body size, newborns of the larger bodied *A. jamaicensis* were significantly less developed than were newborns of *C. perspicillata* in the following characters: birth mass; area of wing surface, armwing, and handwing; as well as length of forearm, total wing, armwing, and handwing.

Wing loading and aspect ratio developed similarly in the two species in that as aspect ratio increased, wing loading decreased throughout the first half of development with just the opposite trends during the second half of ontogeny. Curiously, the relationship between these variables is less symmetrical in *A. jamaicensis* than in *C. perspicillata* (Fig. 2.2), and the relationship between the growth and development of the armwing (providing major lift and power) and the handwing (providing

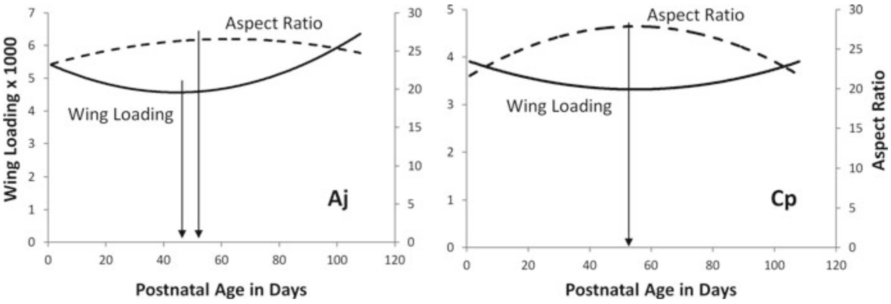


Fig. 2.2 Relationship between changes during ontogeny of aspect ratio (*dotted line*) and wing loading (*solid line*) for *Artibeus jamaicensis* (Aj) and *Carollia perspicillata* (Cp)

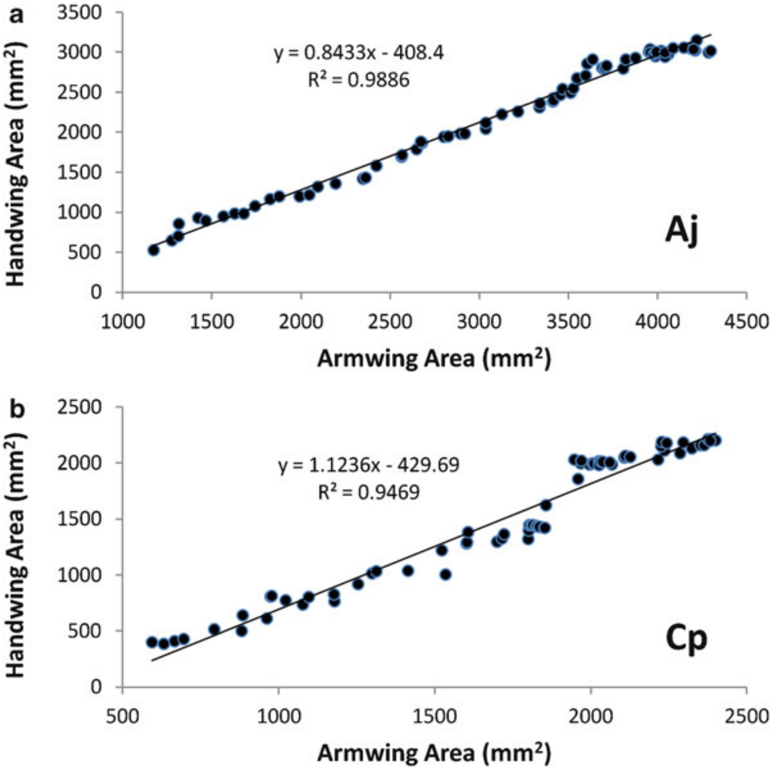


Fig. 2.3 Regression plot of armwing area against handwing areas for (**a**) *A. jamaicensis* and (**b**) *C. perspicillata*

control and maneuverability) is more highly correlated and linear in the larger bodied *A. jamaicensis* (Fig. 2.3).

The shape of the wing tips (wing area ratio/wing tip length ratio – wing tip area ratio) affects maneuverability and thus agility in bats (Norberg et al. 2000).

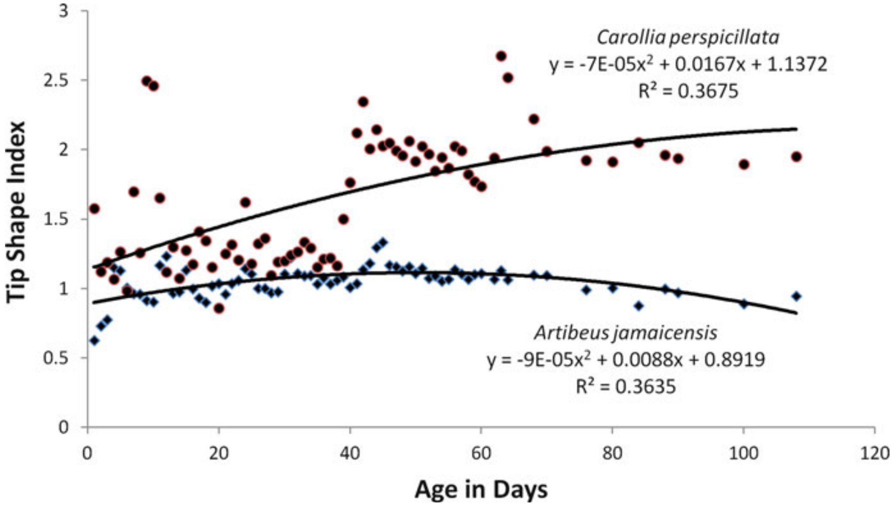


Fig. 2.4 Regression plot of tip shape index for *A. jamaicensis* (*diamonds*) and *C. perspicillata* (*circles*)

Juvenile *A. jamaicensis* showed much tighter variation in wing tip growth and development ($\bar{x} = 1.05$, sd = 0.136) than did *C. perspicillata* ($\bar{x} = 1.64$, sd = 0.44) (Fig. 2.4). Furthermore, proportional changes between the handwing and armwing areas showed tighter correlation in *A. jamaicensis* (Fig. 2.3) which may be a consequence of higher gravitational force (Force = *MA*) acting as a greater selective influence due to consistently higher body mass during ontogeny (Fig. 2.5). Moreover, the asymmetrical relationship between wing loading and aspect ratio in *A. jamaicensis* (see Fig. 2.2) may also be body size related.

2.4 Ontogeny of Flight in Bats

Powers et al. (1991) quantified flight abilities of known-age little brown bats (*Myotis lucifugus*) using a trapdoor and drop box and categorizing four flight stages: *flop*, vertical descent with no flapping and no horizontal displacement; *flutter*, wing motion with no horizontal displacement; *flap*, wing motion resulting in horizontal displacement (20–200 cm), but no sustained flight; and *flight*, sustained with adult-like maneuverability. They observe first wing flapping at day 10, short horizontal flights at day 17, and sustained flight at day 24.

However, with the use of high-speed video taken on newborn of our two species of fruit bats, we revealed that individuals are capable of flapping their wings immediately after birth. Thus, we redefined Powers et al. (1991) *flop* stage as falling to the pad with <3 wing flaps over the 1 m descent and the flutter stage as >3 wing flaps with no horizontal displacement. We found that both species followed the same pattern during the early stages of flight development (flop and flutter) and there were no significant differences in the age at which fluttering began.

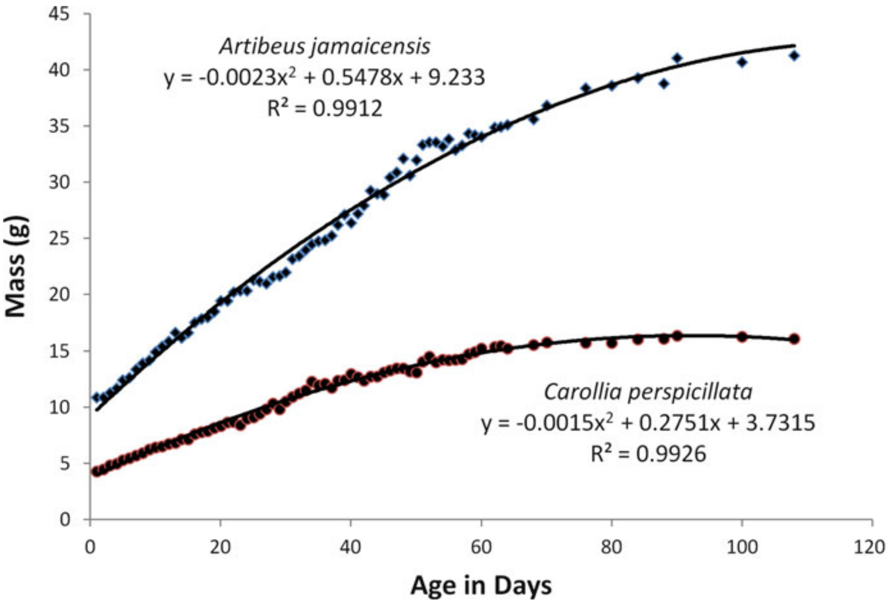


Fig. 2.5 Polynomial growth rate curves for *A. jamaicensis* and *C. perspicillata*

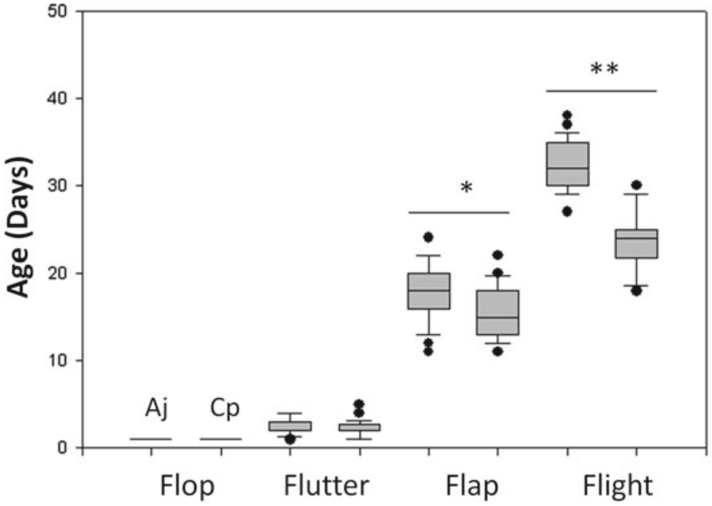


Fig. 2.6 Mean (±SD) age at first observation of each flight development category. *Left boxes* Aj = *Artibeus jamaicensis* and *right boxes* Cp = *Carollia perspicillata*. Wilcoxon Rank Sum test *P=0.01, **P=0.001

However, there were significant differences between species in mean age at which later flight stages came on-line (flapping and flight), with *C. perspicillata* showing earlier aptitude (Fig. 2.6) and beginning to fly on less developed wings than did *A. jamaicensis* (Table 2.1). There was no significant difference in the number of days

Table 2.1 Mean proportions of adult body mass, forearm length, wingspan, and wing area of each species with initial flight capacity

Variable	*Artibeus jamaicensis* (%)	*Carollia perspicillata* (%)
Body mass	59	56
Forearm	94	82
Wingspan	90	82
Wing area	77	67

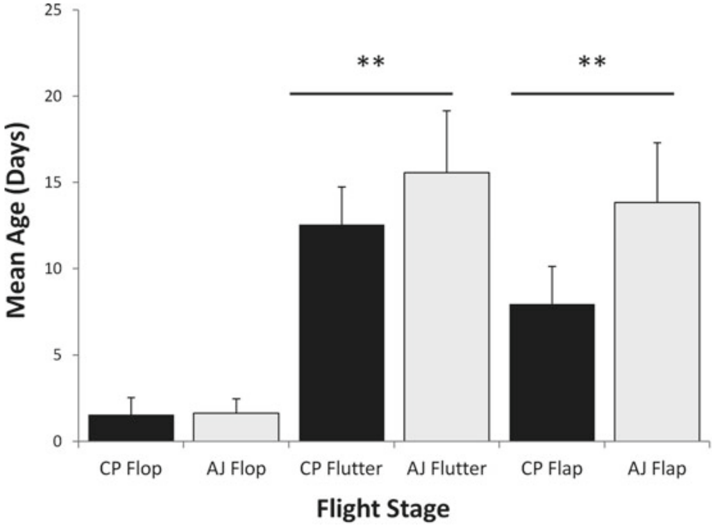


Fig. 2.7 Mean (±SD) days spent within each developmental flight category. *Black bars* represent *A. jamaicensis* and *gray bars* represent *C. perspicillata.* $^{**}P \leq 0.001$

each species spent in the flop stage, but *A. jamaicensis* juveniles spent significantly more time in flutter and flap stages (Fig. 2.7).

Shaw (2011) quantified agility of 20 *A. jamaicensis* and 15 *C. perspicillata* juveniles by flying them through a flight maze constructed of four rows of offset 1 m long, 5 mm diameter dowel rods hung on string. Spacing between dowels was set at the full wingspan of each individual and then adjusted to 75 % and 50 % of an individual's wingspan for successive trials. Agility was quantified by frequency of dowel rods contacted during each trial, and mean juvenile agility was compared with mean agility of five adults of each species.

Thirty five-day-old juvenile *A. jamaicensis* were able to maneuver through the maze at the full wingspan setting with agility equal to adults. The performance of juveniles when dowels were set at 75 % wingspan spacing was equal to adults at 45 days and at 50 % wingspan spacing at 65 days of age. Juvenile *C. perspicillata* were able to successfully maneuver the course at full wingspan dowel spacing with adult agility at 30 days postnatal development, at 75 % wingspan at 40 days, and at 50 % wingspan at 50 days. Differences between the two species in age-specific agility relative to adults were not apparent at the full wingspan setting; however, at 75 % of

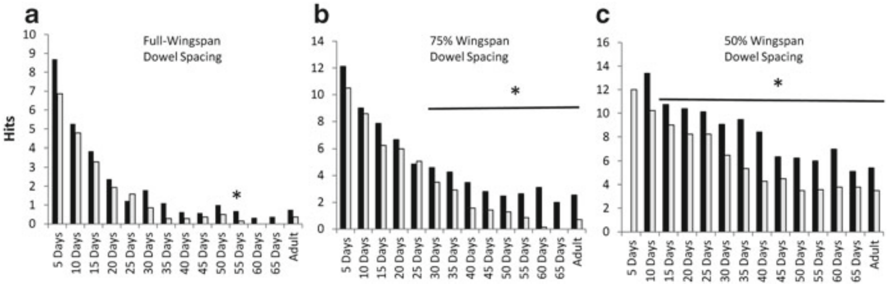


Fig. 2.8 Number of dowel rod contacts at different ages of postflight agility for *C. perspicillata* (*black bars*) and *A. jamaicensis* (*open bars*). (**a**) Dowel rods spaced at full wingspan. (**b**) Dowel rods spaced to 75 % of wingspan. (**c**) Dowel rods spaced at 50 % of wingspan

wingspan, there was a significant difference between the two species from day 30 onward and at 50 % of wingspan from day 10 onward (Fig. 2.8).

Although agility at any age is controlled, in part, by wing loading and aspect ratio, muscle development also contributes greatly to flight control and stability. Locomotor muscles in mammals are composed of up to three different fiber types, belonging to motor units that have distinct functional properties controlling various performance parameters. Several classification paradigms are based on the properties of myosin heavy chains broken down into type I, type IIa, and type IIb motor units (Brooke and Kaiser 1970; Guth and Samaha 1969, 1970). In bats, the pectoralis muscles are used for sustained forward motion, specifically performing the downstroke of the wings (Hermanson and Altenbach 1981, 1985; Vaughan 1970), whereas the acromiodeltoideus muscles are one of a set of paired abductor accessory flight muscles that are active throughout the upstroke (Hermanson and Altenbach 1985).

Pectoralis and acromiodeltoideus muscles in adult little brown bats (*M. lucifugus*) were composed entirely of fast-twitch fibers by the time of weaning (Powers et al. 1991). Growth and development of the pectoralis in juvenile *C. perspicillata* was similar to *M. lucifugus*, whereas in *A. jamaicensis*, what begins as 90 % fast-twitch fibers switches to 30 % slow twitch in the flap stage and 60 % slow twitch in adults. Shaw (2011) hypothesized that the increase in slow-twitch fibers during ontogeny in *A. jamaicensis* may afford greater efficiency for sustained commuting flight in open habitats by adults.

Flight muscle development and agility of two species showed a consistent pattern wherein the ability to successfully maneuver the maze was dependent upon the percentage of (Fig. 2.9) and diametrical growth (Fig. 2.10) of pectoralis fast-twitch muscle fibers (PFT) rather than those of the acromiodeltoideus (DFT). Curiously, diametric growth of both the pectoralis and acromiodeltoideus fast-twitch fibers appears to be equally invested in increasing agility for *A. jamaicensis* (Fig. 2.10), whereas in *C. perspicillata*, agility appears most related to pectoralis development (Hermanson and Altenbach 1981, 1985; Vaughan 1959, 1970).

As juveniles began to fly, they had grown to similar ratios of mean adult body mass for their respective species. However, the relationship between body mass and wing dimensions was quite different between the two species on the first day of

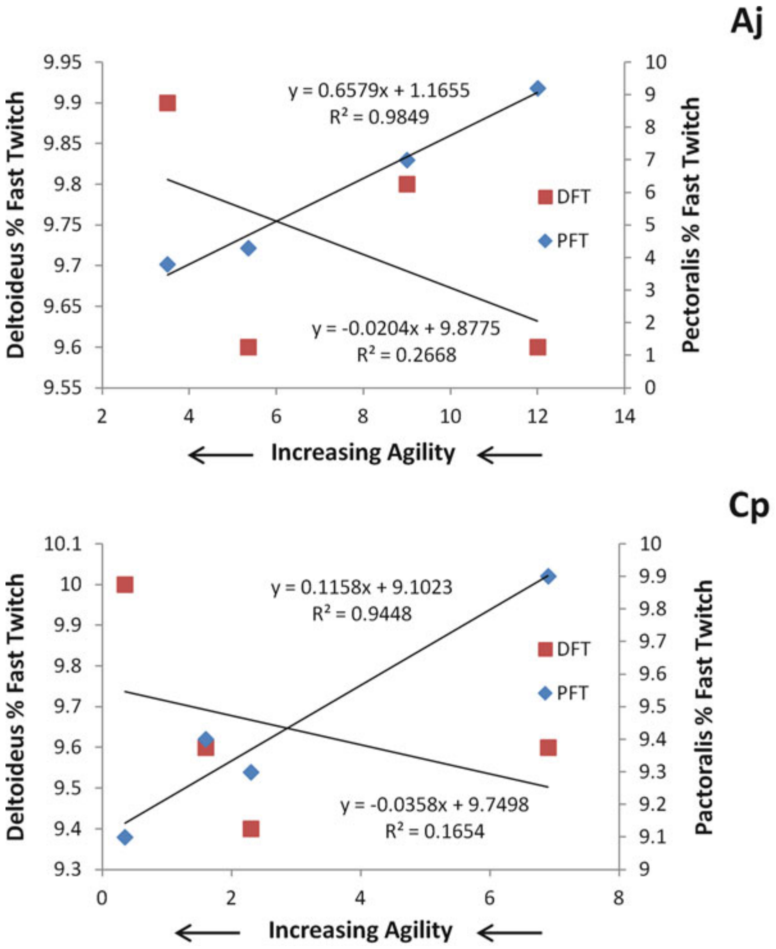


Fig. 2.9 Development of fast-twitch fibers in the major flight muscles as compared to increasing agility of *A. jamaicensis* and *C. perspicillata*. In both species, agility was best correlated with percent maturation of pectoralis fast-twitch muscles. *DFT* acromiodeltoideus fast twitch, *PFT* pectoralis fast-twitch muscles

flight. In fact, *C. perspicillata* begins flying on quite stubby wings compared to *A. jamaicensis* and yet has the same agility (Table 2.1).

2.5 Evo-Devo Modeling of Derived Morphologies

There is a long and deep history between development and evolution that supports the underlying premise that derived morphologies are additive rather than substitutive. Perhaps Garstang's (1922) statement "Ontogeny does not recapitulate

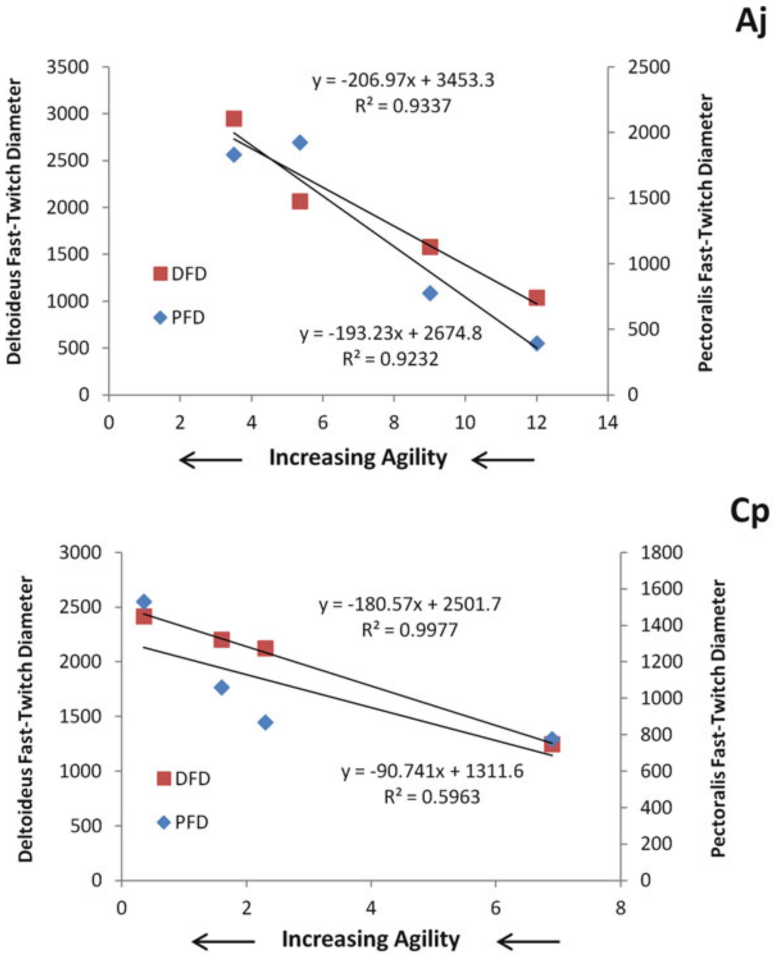


Fig. 2.10 Plots of mean diameters of the pectoralis and acromiodeltoideus muscles as related to agility in *A. jamaicensis* and *C. perspicillata*. Abbreviations as in Fig. 2.9

phylogeny, it creates it" says it best. In kind, Liem and Wake (1985) stated "As far as morphological change is concerned, evolution acts by altering development." Despite the simplicity of these two notions, a coherent understanding has been confounded by the inability to sometimes track the ontogeny of derived morphologies (Swan 1990; True and Haag 2001).

"Deep homology," a product of shared genetic apparatus to form similar structures among phylogenetically disparate organisms (i.e., dipteran wings and tetrapod limbs), shows that historical continuity may reside cryptically within complex regulatory circuitry (Baguñà and Garcia-Fernández 2003; Jablonka and Lamb 1998; Shubin et al. 2009). Innovation, it appears, is a product of modifications of conserved genetic networks rather than the evolution of novel genes or genetic pathways (see reviews in Brakefield 2006, 2011; Shubin et al. 2009). For example, regulatory constraints and co-option of the trunk segmentation collinear mechanism appear to have imposed an anterior-posterior polarity in tetrapod limbs at their first appearance, and, although hard to track, this regulation is still present throughout modern mammals (Kmita et al. 2005; Tarchini et al. 2006; Young and Badyaev 2007). A relatively new focus on how modified developmental pathways result in evolutionary divergence includes the epigenetic aspects of environmental sensitivity (see reviews in Hall et al. 2003; West-Eberhard 2003) wherein increased developmental plasticity produces novel morphologies with environmental selection acting on the new phenotype or even as an inducing mechanism (Young and Badyaev 2007).

Much of skeletal development is epigenetic (Goldberg et al. 2007; see also Chap. 1), and differential expression of bone morphogenic protein (BMP) is a primary mechanism in controlling cartilage and bone formation. Typically these changes are attributed to mutations in the regulatory regions of BMP pathways (Albertson and Kocher 2006; Sears et al. 2006; Terai et al. 2002; Chap. 1); however, we know comparatively little about how extrinsic stimuli affect development (see West-Eberhard 2005 for review).

2.6 Evo-Devo Modeling of Mammalian Locomotor Patterns

Because adaptive behaviors require functional links among morphogenetic modules both developmentally and evolutionarily, quantifying how locomotion behaviors come on-line during ontogeny can act as a surrogate for understanding ancestor-descendent transitions (Bertossa 2011; Müller 1990, 2007; Oster et al. 1988; Raff 2007). The wide diversity of locomotion in mammals all stem from a basal ancestor and develop through the ancestral ontogenetic pathways that stretch back to the origin of terrestrial vertebrates 360 mya. For example, Westerga and Gramsbergen (1990) found that newborn rats crawled with their ventrum in contact with the floor and after day 11, a gradual transition to free walking took place during which paw strikes were initially plantigrade with flexed interphalangeal joints producing wobbly gait with lateral bending of the spine similarly to that of the first terrestrial vertebrates (Ischer and Ireland 2009; Romer 1959; Williams 1981).

During ontogeny, gait kinematics of the adult tree shrew (*T. glis*) was surprisingly similar to the precocial terrestrial cui (*Galea musteloides*) even though as adults kinematic distinctions are easily evident (Schilling and Petrovitch 2006). Even the development of bipedal locomotion in the jerboa (*Jaculus orientalis*) begins with the typical neonatal condition of walking on hind limbs and forelimbs of equal length with bipedal locomotion not arising until day 47 (Eilam and Shefer 1997), and the overall progression of ontogenetic events followed those of less derived quadrupedal rodents (Eilam 1997; Eilam and Shefer 1997). In black-tailed jackrabbits (*Lepus californicus*), neonates have short hind limbs for walking prior to the onset of rapid elongation and quadrupedal saltation (Cretekos et al. 2008; Eilam 1997). A comparison between rodent species that use a specialized half-bounding gait (*Chinchilla lanigera* and *Oryctolagus cuniculus*) and two unspecialized species (*Rattus norvegicus* and *Monodelphis domestica*) showed that all four groups shared common developmental patterns (Lamers and German 2002).

Ferron (1981) compared ontogeny of locomotion in four squirrel species [*Spermophilus columbianus*, *S. lateralis* (both terrestrial), *Tamiasciurus hudsonicus* (arboreal), and *Glaucomys sabrinus* (arboreal/gliding)] and found that all four followed the same patterns of locomotor development, but in the gliding species *G. sabrinus*, climbing behavior occurred 2 weeks earlier than in the others. Indeed, comparative locomotor development across many mammal taxa (Didelphidae, Rodentia, Lagomorpha, Hyracoidea, Artiodactyla, Scandentia, and Primates) shows that ancestral morphologies and gaits consistently precede the appending of derived morphologies and behaviors (Fischer et al. 2002; Schilling 2005; Schilling and Petrovitch 2006; Witte et al. 2002).

2.7 Gliding Versus Direct Flapping Flight Hypotheses

Studies of the ontogeny of flight are central in uncovering how vertebrate wings evolved (Dial et al. 2008). Foundational modular integration is a key attribute for both the ontogeny and evolution of form and function and must be maintained during transitional states (Breuker et al. 2006). Using high-speed videography, we have shown that newborn bats just a few hours old have the reflexive behavior of wing flapping even when their body orientation is not conducive for flight (Fig. 2.11). Clearly then the basic mechanics and neurology for reflexive flapping are laid down before birth, and bats may be similar to rats in which inter-limb coordination occurred at day 20 of a 21-day gestation period (Bekoff and Lau 1980).

Initially, newborn bats only dorsally extended their wings about +45° to the body plane, whereas ventral range of motion was nearly −85°. By day 4, however, the backstroke wing angle was nearly +84°, reflecting the sudden burst in acromiodeltoideus function (represented in Fig. 2.10). When newborn young were dropped

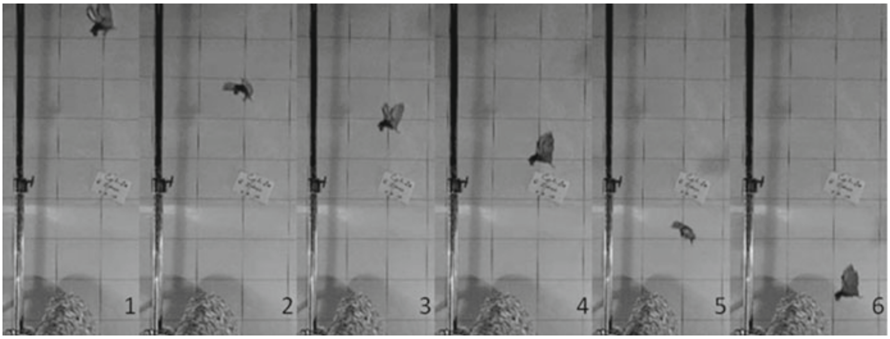


Fig. 2.11 Hand-dropped 6-day-old *C. perspicillata* exhibits flapping immediately upon release

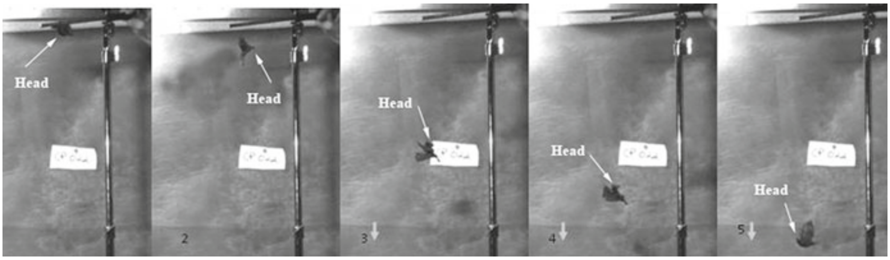


Fig. 2.12 Sequence of 1-day-old postpartum *C. perspicillata* dropped from a drop-stick hanging position. This individual became inverted and unable to reorient its body axis. Although the individual becomes inverted, it flapped its wings throughout the 1 m drop. We interpret this to mean that flapping is an innate reflex

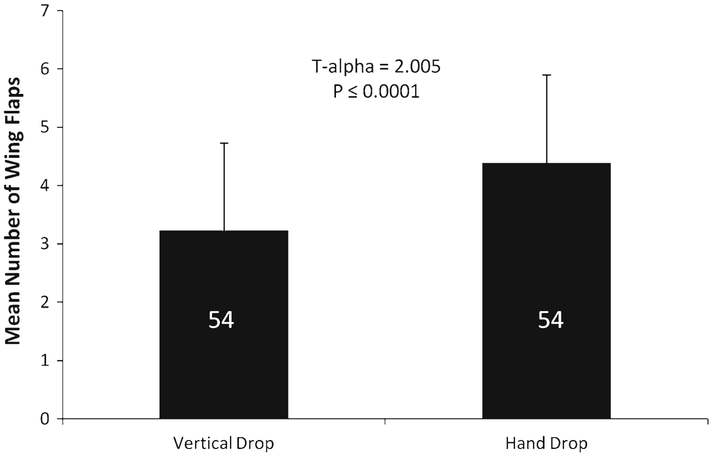


Fig. 2.13 Comparison of number of flaps achieved by preflight juvenile *C. perspicillata* in 54 releases hanging from a rod in a vertical body position versus 54 releases of the same individuals from a handheld horizontal body position. Vertically drop averaged 3.23 flaps (SD = 1.49) versus horizontal drops averaged 4.39 (SD = 1.51)

from a vertically hung position, they had significant trouble controlling body-axis rotations, and on several occasions, individuals that became inverted (were falling upside down) would nevertheless flap their wings until they landed on the soft pad below (Fig. 2.12). We also found that preflight young released with their bodies held horizontally significantly outperformed trials in which the same individuals were released from a hanging position (Fig. 2.13). In addition, flapping performance using the same tests on 18 individuals of *C. perspicillata* from 16.5 to 26.5 days of age showed similar patterns of greater performance when horizontal drops were performed (Fig. 2.14). Thus, control of flapping flight at early developmental stages was gained when individuals did not need to reorient their body axis from vertical to a horizontal flight plane.

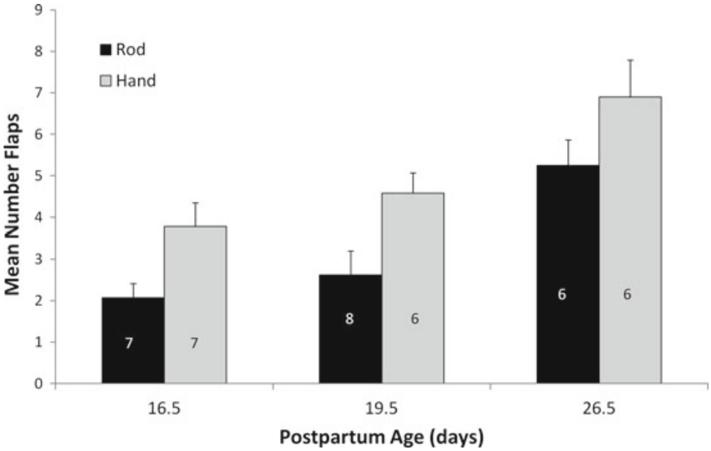


Fig. 2.14 Mean and standard deviations of number of wing flaps during rod- versus hand-drop tests of juvenile *C. perspicillata* at 16.5, 19.5, and 26.5 days of age. *Numbers* in bars indicate number of drops per test. There were significant differences between each category per age, but the degree of significance lessened with age

Fig. 2.15 A 24-day-old *C. perspicillata* lifting off from a horizontal platform on the first day it was capable of flight holds its body axis steady throughout takeoff and subsequent flight. *White lines* represent the horizontal

The first day of sustained flight for juvenile *C. perspicillata* was at 26 days on average, and all were capable of performing horizontal launches from a table top by pushing off at the wrist, resulting in excellent body-axis control during takeoff (Fig. 2.15). However, the same juveniles released from a vertical, hanging position required a free fall of about 0.5 m before orienting the body axis properly (horizontally); struggled to maintain pitch, yaw, and roll control; and commonly did not get to a sustained flight mode. It is certainly conceivable that bats evolved on vertical landscapes such as cliff faces using push-off launches (Caple et al. 1983) from a vertical position (Fig. 2.16). In our tests, we found that push-off launches while hanging on a vertical platform suffer similar mechanical and control challenges to vertical, drop-stick launches (Fig. 2.17).

2.8 From the Cradle to the Air

Much of what we know about the origin of bats is based on fossils from two regions: Messel, Germany, and the Green River Formation, Wyoming, USA. Although it is currently impossible to determine the geographic origin or "cradle"

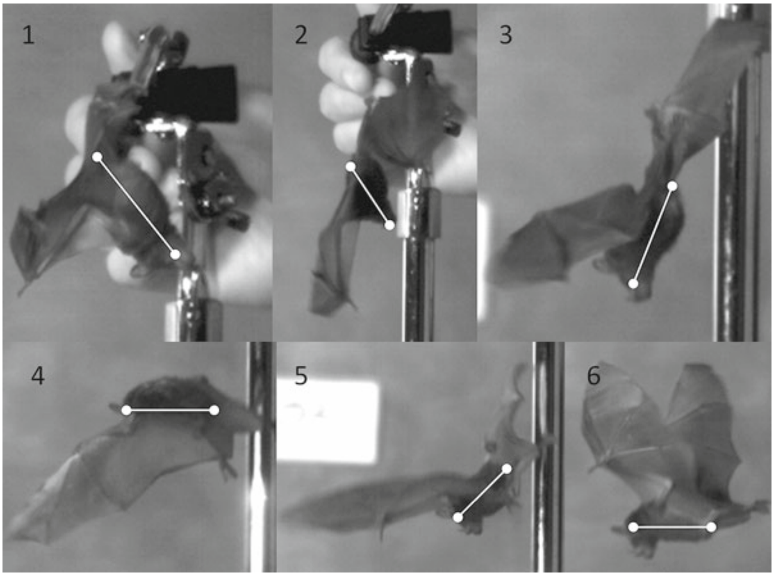


Fig. 2.16 Vertical drop sequence (1–6) of a 31-day-old *C. perspicillata* shows a lack of control in body orientation (pitch, roll, and yaw) when shifting from a vertical to a horizontal flight axis. *White lines* here mark the approximate body axis

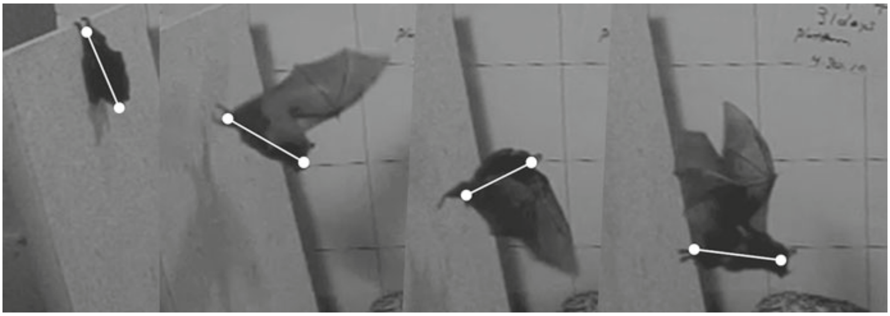


Fig. 2.17 Push-off launch from vertical platform of 31-day-old *C. perspicillata*. Individual struggled to control body-axis pitch. *White lines* mark body axis

of bat evolution, both fossil and molecular data point to a Laurasia origin, possibly North America, sometime near the K–T boundary (~64 mya) (Smith et al. 2012; Teeling et al. 2005). However, molecular analysis has also indicated that a split of Chiroptera into two groups named Yinpterochiroptera (rhinolophids and pteropodids) and Yangochiroptera (all other bats) occurred at about 55–57 mya (Teeling 2009), and this does not appear to align with the early fossil bats. Analysis on the genomes of *Pteropus alecto* and *Myotis davidii* supports a divergence of bats from the Laurasiatheria as early as 88 mya (Zhang et al. 2013). However, the most comprehensive study on the origins of placental mammals and derived groups indicates the origin of bats at or just after the K/Pg boundary at 65 mya (O'Leary et al. 2013). *Icaronycteris index*, described by Jepson (1966), was considered to be the most primitive instance of a bat, but has since been superseded by the discovery of another fossil bat from the same formation. This new species, *Onychonycteris finneyi*, is larger bodied than other Eocene bats, lacks typical fusions in the ribs and vertebrae, and has claws on all digits of the manus (Simmons et al. 2008). Further, *O. finneyi* has limb proportions that are intermediate between other fossil bats and those of nonvolant climbing mammals such as colugos, a more ancestral character state than *I. index* although occurring at about the same time.

More broadly, comparative morphology indicates that bats are descended from the Archonta (dermopterans, primates, and tree shrews); however, molecular analyses indicate that bats originated from Laurasiatheria, appearing either (a) as a sister group or a basal member of a cetferungulate clade (pholidotans, carnivores, cetaceans, artiodactyls, and perissodactyls) or (b) as a sister group of the eulipotyphlan clade (shrews, moles, and possibly hedgehogs). The consensus being that bats rest within the basal laurasiatheres (see Gunnell and Simmons 2005 for review).

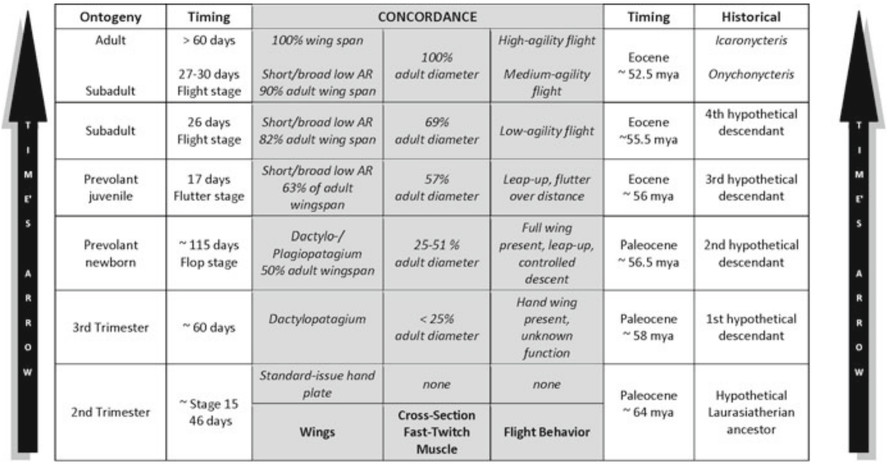
The timing and order in which the major components of the bat wing develop (plagiopatagium, membrane stretching between the body and forearm, i.e., armwing; dactylopatagium, membrane that stretches between digits 2 and 5, i.e., handwing) give insight into the evolutionary sequence of wing evolution. Morphological and molecular investigations of wing formation indicate that the different patagial components that compose a bat's wing develop independently and at different developmental stages in both Microchiroptera and Megachiroptera indicating that the evolution of these structures was also independent and temporally unrelated (Cretekos et al. 2005; Giannini et al. 2006; Weatherbee et al. 2006). Curiously, new research has determined that the wing muscles in bats also have multiple embryonic origins and myogenic sources and that the wing membrane itself regulates wing muscle patterning (Tokito et al. 2012) similar to patagial growth controlling serial length compensation among wing bones (Adams 1998), also shown to occur in *Hipposideros pomona* (Lin et al. 2011).

From a functional perspective, the evolution of flapping flight versus gliding would involve different evolutionary, and thus developmental, arrangement of the patagia (Gardiner et al. 1999). Establishing a gliding morphology would have favored the ontogeny and evolution of a membrane that stretched between the lateral body wall and the forearm (plagiopatagia), thereby producing a flight surface with a small lever arm and turning moment. Conversely, for flapping flight, formation of a dactylopatagium would be favored for maximizing power stroke, turning ability, and orientation control.

2.9 Synthesis

Time's arrow is a concept developed by astronomer Arthur Eddington in 1927 to describe the past to present direction of time (Carroll 2010). Integrating the drastically different arrows of time between ontogeny and evolution has produced

Table 2.2 Concordance hypothesis between developmental stages (based upon data gathered on *C. perspicillata*) and evolutionary stages (based on molecular and fossil evidence)



Ontogeny	Timing	CONCORDANCE			Timing	Historical
Adult	> 60 days	*100% wing span*	*100% adult diameter*	*High-agility flight*	Eocene ~ 52.5 mya	*Icaronycteris*
Subadult	27-30 days Flight stage	*Short/broad low AR 90% adult wing span*		*Medium-agility flight*		*Onychonycteris*
Subadult	26 days Flight stage	*Short/broad low AR 82% adult wing span*	*69% adult diameter*	*Low-agility flight*	Eocene ~55.5 mya	4th hypothetical descendant
Prevolant juvenile	17 days Flutter stage	*Short/broad low AR 63% of adult wingspan*	*57% adult diameter*	*Leap-up, flutter over distance*	Eocene ~ 56 mya	3rd hypothetical descendant
Prevolant newborn	~ 115 days Flop stage	*Dactylo-/ Plagiopatagium 50% adult wingspan*	*25-51 % adult diameter*	*Full wing present, leap-up, controlled descent*	Paleocene ~ 56.5 mya	2nd hypothetical descendant
3rd Trimester	~ 60 days	*Dactylopatagium*	*< 25% adult diameter*	*Hand wing present, unknown function*	Paleocene ~ 58 mya	1st hypothetical descendant
2nd Trimester	~ Stage 15 46 days	*Standard-issue hand plate*	*none*	*none*	Paleocene ~ 64 mya	Hypothetical Laurasiatherian ancestor
		Wings	**Cross-Section Fast-Twitch Muscle**	**Flight Behavior**		

Error bars for estimated evolutionary events may comprise several million years

important insights into how, when, and why key morphological and behavioral innovations transpired (Hall et al. 2003; Smith 2003; Swan 1990), and some would argue that the evolution of adult morphology is little more than the differential success of developmental innovation (Klingenberg 1998; Liem and Wake 1985; Müller 1990).

By evaluating both the timing of significant ontogenetic events in relation to molecular and fossil evidence on bat evolution, we can begin to draw concordances (Table 2.2). Because the fossil record for bats is so poor and in many cases molecular analysis has not been supported by fossil evidence, we use a combination of those aspects of each which do appear to align together. Data from correlations between the rate of speciation and the rate of chromosomal evolution show that extant bats are evolving at a relatively slow rate compared to insectivores, rodents, carnivores, lagomorphs, artiodactyls, and marsupials, but faster than whales. The corrected speciation rate for bats as compared to these other species was determined to be 0.7 new species/lineage per million years (Bush et al. 1977). Of course, speciation rates can vary through space and time, and one would expect key evolutionary innovations to evolve in a punctuated manner with rapid transitional stages (Eldridge and Gould 1977). Furthermore, diversification rates in any lineage likely have not been constant and for bats appear to have responded positively to major environmental events such as shifts in flowering plant diversity rates (Jones et al. 2005).

Because the components of the wing in bats develop separately both in timing and location (Weatherbee et al. 2006), the dactylopatagium, which develops first, was probably the first patagium to evolve in concert with elongation of digits 2–5, forming an incipient wing that appears prenatally on ~day 60 of a ~115-day gestation period. Evolutionarily, this timing would equate to the Middle Paleocene (~58 mya) and represent an initial hypothetical descendant on the pathway to true flight. The adaptive nature of the proposed dactylopatagium remains unknown, but it has been suggested that such a structure would be useful in capturing insects (Speakman 1999). In our scenario, these could be either ground or flying insects. At birth (~115 days), *C. perspicillata* has both a dactylo- and plagiopatagium and is capable of some controlled flapping upon descent (flop stage). This equates to a second-stage descendent with the capacity to leap up with controlled descent living in the Late Paleocene (~56.5 mya). This may be the first time in evolutionary history that protobats attempted to use their wings for a newly found locomotor mode, flight. From this point onward, selection favored adaptations consistent with further flight development and evolution perhaps to avoid predation as observed in birds (see Heers and Dial 2012 for review). Our third hypothetical descendant living ~56 mya equates to a 17-day-old prevolant juvenile with the capacity to flutter over a distance up to 200 cm to or from vertical surfaces or flapping gently to the ground after jumping upward to catch insects as portrayed by Caple et al. (1983). We estimate that such jumping and fluttering behavior would not be sustainable in distance or altitude due to the lack of fast-twitch muscle support, predicted by ontogeny to be only about 25 % of adult capacity. The fossils *Onychonycteris* and *Icaronycteris* appear to be steps along the evolutionary continuum with the former being an ancestral state. These fossils, both living forms ~52 mya, appear to equate with the ontogenetic transition from late subadult to adult flight morphology, mechanics, and agility. Late-stage subadult *C. perspicillata* are capable of sustained flight, but lack some agility, and we hypothesize that this flight behavior is representative of what would be expected in *O. finneyi*, apparently a less agile flyer than was *I. index* due to its higher wing loading and small wing tips (Simmons et al. 2008). As mentioned, *C. perspicillata* began flying on quite stubby wings with underdeveloped flight muscles that seem to represent a more ancestral flight morphology similar to that depicted in *O. finneyi* (Simmons et al. 2008). However, *O. finneyi* had body-size dimensions similar to *A. jamaicensis*, meaning that higher flight speed would be required to stay aloft and thus consequently lower agility. Perhaps *O. finneyi* represents an evolutionary benchmark that depicts a time in bat evolution when flight was entering an innovative phase allowing for aerial insectivory.

2.10 What Bats Inherited Versus Derived

Much focus on the derived characters of bats has involved the serial order in which such innovations came about over evolutionary time (see Gunnell and Simmons 2012 for review). It seems to us that perhaps in some of these cases, the traits that are being assigned as evolving in bats may instead have been inherited in basal form from their common ancestor. We propose that recent hypotheses on the evolution of bats have confused inheritance with derivation of form and function. A prima facie example is the argument concerning which came first in bats, flight or sonar (Simmons et al. 2008; Veselka et al. 2010). Recent research has shown quite unequivocally that the use of ultrasound is quite common among small mammals (Blumberg 1992; Kalcounis-Rüppell et al. 2006). Most relevant to the evolution of bats is the fact that sonar is used by contemporary species in the genus *Sorex* (Siemers et al. 2009) and *Blarina* (Tomasi 1979). In addition, it appears that the use of ultrasound in these shrews has evolved to a state allowing for its use in navigation and potentially the identification of objects in the environment. Thus, it seems more probable to us that bats inherited primitive sonar from their ancestor and built upon this foundation to produce the exquisite echolocation exhibited by extant species, rather than the likelihood that bats reinvented this ability from scratch. Likewise, other aspects commonly associated with bat evolution such as nocturnal activity, high metabolic rates, and reduced visual acuity were inherited from a shrewlike ancestor. Other aspects of bat natural history seem clearly to be derived by bats and may be directly linked to the evolution of flight such as longer gestation periods that facilitate the growth and development of wings and lengthening of life spans hypothesized to be a consequence of the extensive use of torpor and hibernation (see Barclay and Harder 2003 for review).

2.11 Conclusions

The hypothesized concordance between the timing of ontogenetic events and those of important evolutionary innovations we propose in this chapter provides a foundation for further testing. Our ontogenetic analysis in relation to our four basic principles stated in the introduction thus far supports that, similar to other mammals with derived morphologies and locomotion, bats pass through ancestral locomotor stages before acquiring the morphology and functional capacity for flight. We found that the most stable platform for the ontogenetic transition from nonvolant to volant form indicates that push-off launches from a horizontal platform are best for maintaining body-axis control during takeoff (Fig. 2.18). A horizontal takeoff also entails minimizing risky behavior as failures would result in minimal fall distances. Finally, our ontogeny data support the direct evolution of flapping-powered flight because juveniles with wings not yet developed enough to support flight exhibit reflexive flapping behavior when in free fall. The ontogeny and evolution of direct flapping mechanics also embraces Occam's Razor in that the succession from nonvolant to volant form is accomplished by modest transitional stages rather than dramatic specialized adaptations and behaviors associated with transitions from scansorial and/or pendulant arboreal locomotion to gliding ability and then flapping flight. Much more data and understanding about the ontogeny of locomotion in bats including walking, climbing, and early flight kinematics with integration of ecology and behavior (Adams 2008; Adams and Pedersen 2000) are greatly needed to provide empirical evidence for the origin of flight in mammals.

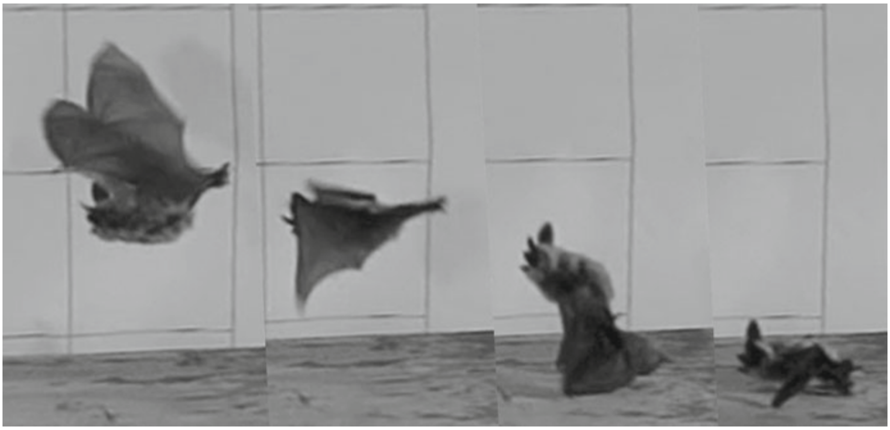


Fig. 2.18 Horizontal platform launch of the vespertilionid bat *Myotis evotis* filmed at 300 frames per second

Our proposed scenario, however, does fall short in understanding why patagia would evolve in protobats if not for gliding first. Speakman (1999) suggested that dactylopatagia evolved initially for insect capture during reach hunting from arboreal perches, and thus it is possible that reach hunting may have evolved in a ground-based or semiarboreal ancestor as well. However, it is also feasible that a semiaquatic ancestor among early insectivores with webbed digits provided a suitable ancestor. Recent findings that "walking" bats, such as mystacinids, had ancestral origins as early as 51 mya, in a time and place where numerous ground-based predators occurred (Hand et al. 2009), offer further evidence that terrestrial locomotion in bats may not be a derived condition, but rather an ancestral condition rooted in all bats.

In addition, new evidence that the tail-membrane in vespertilionid bats may help with thrust during takeoffs from a horizontal position (Adams et al. 2012) might also be part of this evolutionary picture. Because *O. finneyi* had a substantial calcar and thus an extensive tail-membrane, it, and earlier bats, may have used tail-thrust to help during ground launches. The addition of earlier fossil evidence for transitional states will be central to our further understanding for the evolution of bats. However, using ontogeny to bridge the current gaps in time's arrow from past to present, ancestor to descendent, deepens the discussion and refocuses the intellectual path forward.

Acknowledgments We thank the University of Northern Colorado for providing research laboratory space and monetary funding for these projects. We thank David M. Armstrong and Scott C. Pedersen for useful comments on an earlier draft of this chapter.

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Chapter 3
Evolution of Echolocation

M. Brock Fenton

Abstract Basic information about echolocation by bats includes aspects of signal design. Specifically the importance of timing, frequencies in the signals and patterns of frequency change over time are considered along with how bats use harmonics in echolocation signals. Also covered are aspects of signal strength and how bats adjust their echolocation behaviour in areas of clutter. The ways in which bats deal with forward masking (self-deafening) are considered along with differences in duty cycle (low duty cycle, separate pulse and echo in time; high duty cycle separate them in frequency). The importance of echolocation signals in communication also is covered. This information about echolocation is then presented in a phylogenetic context including a discussion of the origin of echolocation and its importance in the diversification of bats.

3.1 Introduction

Echolocation is an astonishing behaviour mainly associated with bats, although neither restricted to nor characteristic of them. Echolocation (biosonar) is an active mode of orientation involving animals using echoes of sounds they produce to collect information about their surroundings (Galambos and Griffin 1942; Griffin and Galambos 1941). The data for the echolocator is the difference between what it said and what it hears. For echolocation to work, the animal must register the outgoing signal in its brain for comparison with retuning echo(es), and so ideally outgoing signals should not mask returning echoes. Echolocation has been documented in some species of birds (Apodiformes, Caprimulgiformes) and mammals (Afrotheria [Afrosoricida], Laurasiatheria [Lipotyphla, Chiroptera,

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_3, © Springer Science+Business Media New York 2013

Cetartiodactyla]) (Thomas et al. 2002). Not all bats echolocate; specifically most of those species belonging to the family Pteropodidae and those few pteropodids (*Rousettus* spp.) that do echolocate use tongue clicks as signals. All other bats echolocate and do so using signals produced in the larynx.

Current research on echolocating bats has revealed the complexity and sophistication of the behaviour. First, echolocating bats adjust their calls according to the situation, whether tongue-clicking pteropodids (*Rousettus aegyptiacus*; Yovel et al. 2010) or laryngeal echolocators (e.g. Jakobsen and Surlykke 2010; Surlykke et al. 2009a, b; Ratcliffe et al. 2011). The echolocation behaviour of tongue-clicking pteropodids is not "unsophisticated" as these bats adjust their flight paths and patterns of call production to maximize information return (e.g. Holland et al. 2004; Yovel et al. 2010). Laryngeally echolocating bats adjust their outgoing signals according to clutter (e.g. Brinklov et al. 2010a, b; Hiryu et al. 2010; Stamper et al. 2009), the presence of conspecifics (Chiu et al. 2008, 2009, 2010) and changes that include the use of harmonics (Hiryu et al. 2010; Stamper et al. 2009), as well as adjustments to the sonar beam (Surlykke et al. 2009a, b). Some also adjust call intensities according to the situation which affects both range and strength of echoes (Brinklov et al. 2010a, b). Still others adjust the frequencies that dominate call components apparently affecting range of operation and conspicuousness to hearing-based defences of prey (Goerlitz et al. 2010). The purpose of this chapter is to consider possible origins of echolocation in bats and the role that echolocation may have played in the diversification of bats.

3.2 Echolocation Signals

A sampling of echolocation calls and relevant features (Figs. 3.1 and 3.2) sets the stage for the information that follows. The general topic of echolocation call design and diversification is well covered in several reviews (e.g. Jones and Holderied 2007; Maltby et al. 2009; Schnitzler and Kalko 2001).

3.2.1 Time and Frequency

Durations and inter-call intervals are measured in ms, the frequencies of echolocation calls (and their components) in kHz. Although most bats use echolocation signals with most energy >20 kHz (by definition, ultrasonic = beyond the range of human hearing), their echolocation signals range in frequency from about 8 to over 200 kHz. Echolocation is not synonymous with ultrasonic. Using humans as reference points for frequency (ultrasonic) or signal strength (dB) is ill-advised. In a group of ten people, it is unlikely that any two will have exactly the same hearing sensitivity, whether response to higher frequencies or threshold.

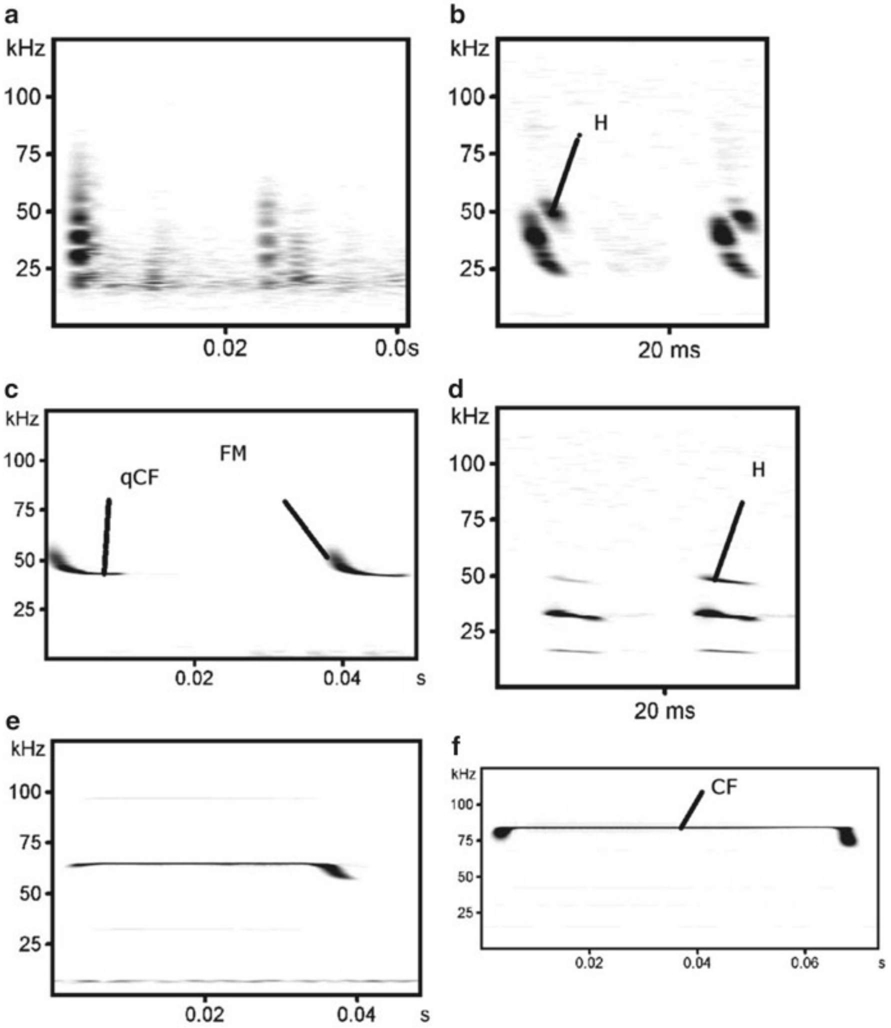

Fig. 3.1 Sampling of echolocation signals, including spectrograms (Part 1) and power spectra (Part 2). Spectrograms include echolocation clicks of *Rousettus aegyptiacus* (**a**); two search phase calls of *Otonycteris hemprichii* (**b**), *Lasiurus cinereus* (**c**) and *Rhinopoma hardwickei* (**d**); and one each of *Pteronotus parnellii* (**e**) and *Rhinolophus capensis* (**f**). Times between pairs of calls have been reduced to show pairs of calls. *H* harmonic, *FM* frequency modulated, *CF* constant frequency, *qCF* quasi-constant frequency. Part 2 shows power spectra, fast Fourier transforms (FFTs) of echolocation calls including *Otonycteris hemprichii* (**a**), *Lasiurus cinereus* (**b**), *Rhinopoma hardwickei* (**c**), *Pteronotus parnellii* (**d**) and *Rhinolophus capensis* (**e**). For *O. hemprichii*, *L. cinereus* and *R. hardwickei*, FFTs are of the first of the two calls shown in Fig. 3.1, Part 1. *FM* frequency modulated, *qCF* quasi-constant frequency, *CF* constant frequency, *H* harmonic

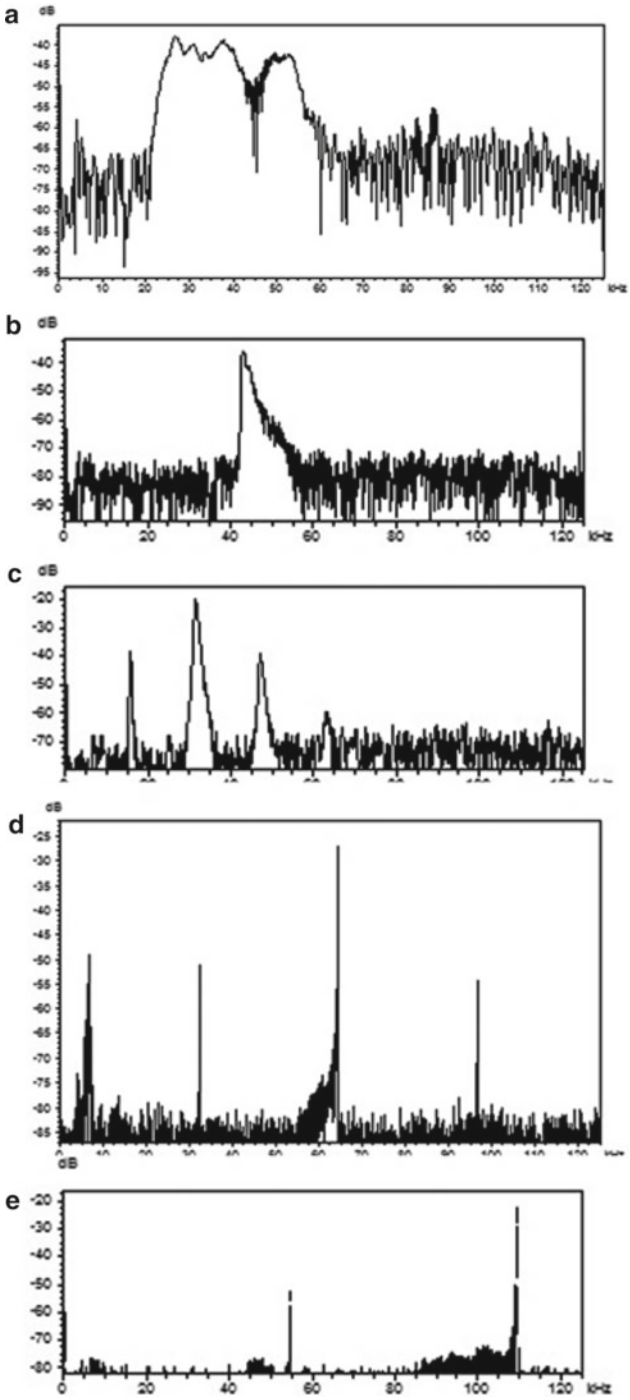

Fig. 3.1 (continued)

3.2.2 Intensity

The strength (intensity) of echolocation calls is usually measured in (dB) sound pressure level (SPL) at a specific distance from the source (e.g. 130 dB SPL at 10 cm; Holderied et al. 2005; Surlykke and Kalko 2008). Intensity (dB) is measured on a log scale where, by definition, 0 dB is the threshold of human hearing at 1 kHz. Griffin (1958) recognized that some bats produced much quieter echolocation calls

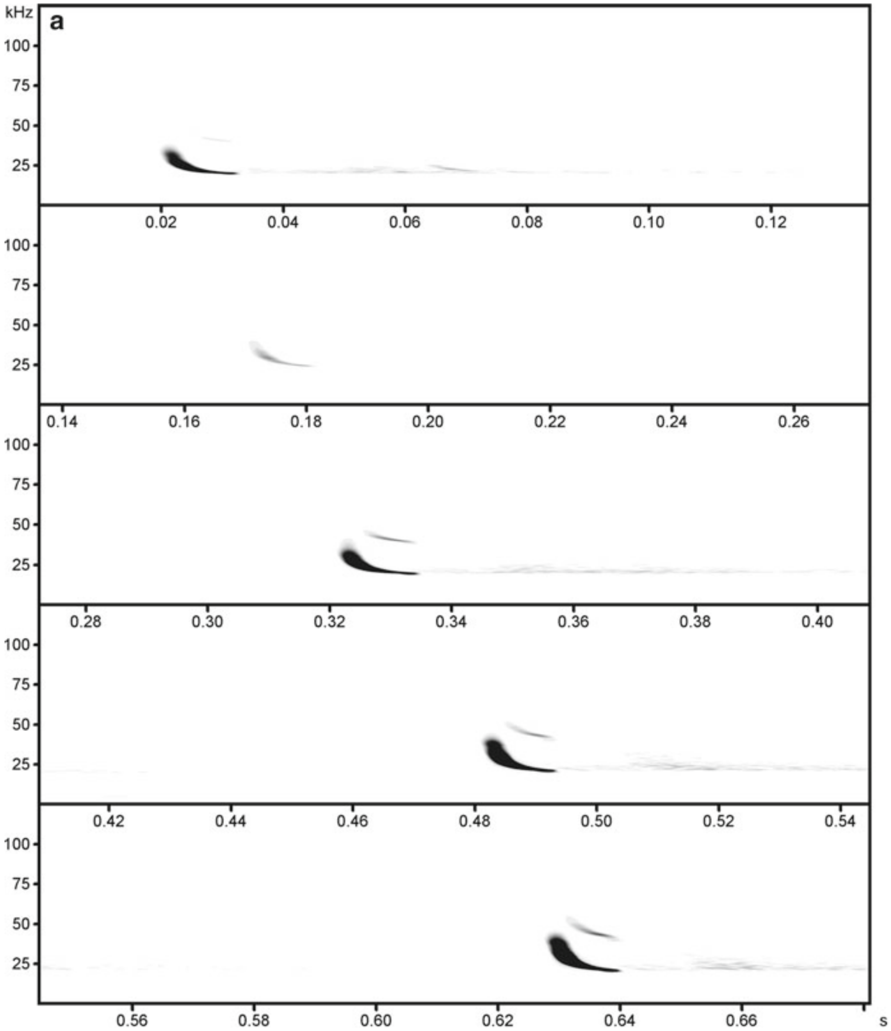

Fig. 3.2 A comparison of low duty cycle (*Lasiurus cinereus*, **a**) and high duty cycle (*Pteronotus parnellii*, **b**) call sequences. Note different patterns of calls, short calls separated by long periods of silence (low duty cycle) versus longer calls separated by short periods of silence (high duty cycle)

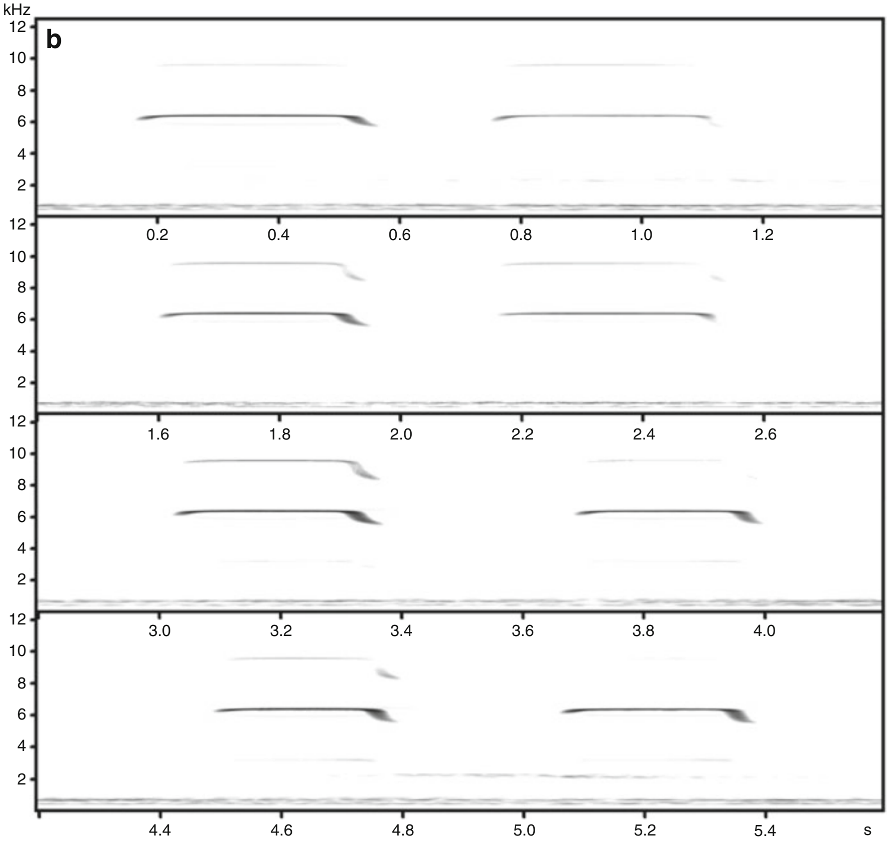

Fig. 3.2 (continued)

than others. He distinguished between whispering bats (e.g. phyllostomids, megadermatids, nycterids) and bats that produced low-intensity echolocation calls (~60 dB SPL at 10 cm) and those using higher intensity signals (>110 dB SPL at 10 cm). But we now know that there is a continuum between "whispering" and other bats and that all echolocating species probably use intensities much higher than early estimates suggested. The situation has been best described in phyllostomids.

The echolocation clicks of *R. aegyptiacus* range in intensity from 85 to 96 dB SPL at 1 m (Holland et al. 2004). Mora and Macias (2007) demonstrated that the phyllostomid *Phyllonycteris poeyi* produces more intense echolocation calls than expected, but they did not provide SPL data. Working with arrays of microphones, Surlykke and Kalko (2008) and Brinklov et al. (2009, 2010) showed that, measured at 2 m distant, other phyllostomids produced calls of 99 dB SPL RMS at 10 cm (*Carollia perspicillata*), 105 dB SPL RMS at 10 cm (*Macrophyllum macrophyllum*) or 110 dB RMS at 10 cm (*Artibeus jamaicensis*) (Surlykke and Kalko 2008; Brinklov et al. 2009, 2010a, b).

3.2.3 *Signals and Frequency*

Bandwidth refers to the range of frequencies in an echolocation signal. Some narrowband signals are CF (bandwidth ~ 0 kHz; Fig. 3.1e, f), but others are broader in bandwidth (quasi-constant frequency—qCF; Fig. 3.1c, d). The echolocation signals of *R. aegyptiacus* (Fig. 3.1a, and presumably other echolocating *Rousettus* spp.) are broadband tongue clicks (Holland et al. 2004). The echolocation signals of all other bats are produced in the larynx and are tonal (Fig. 3.1b–f) showing structured changes in frequency over time. Tonal FM signals can be broadband or narrowband, and some calls include both narrowband and broadband components.

3.2.4 *Harmonics*

Harmonics (overtones; Figs. 3.1b, d and 3.2a, c–e) are obvious and a consistent feature of the echolocation calls of some, but not all bats (Fenton et al. 2011). Harmonics effectively broaden the bandwidth of echolocation signals, potentially providing more details to the echolocator (Simmons and Stein 1980). Bats such as *Eptesicus fuscus* use more harmonics when operating in clutter (Stamper et al. 2009). Detecting harmonics in the echolocation calls of free-flying bats in the field often depends upon on the position of the bat relative to the position of the microphone, so only recordings using arrays of microphones where the bat was in (close) proximity may dependably be used in studies of harmonics (Fenton et al. 2011).

3.2.5 *Clutter*

"Clutter" could simply be defined as echoes from anything other than the target of interest, but as Denny (2006) pointed out, the situation can be complicated. The definition of clutter will depend upon several features of the bats involved, such as their size, flight speed and inter-call intervals. For a bat to deal with clutter, it must detect clutter and negotiate a course through it. This means that both echolocation (design of calls and behaviour) and manoeuvrability in flight (wing design) will influence how a bat adjusts to clutter (Aldridge and Rautenbach 1987; Norberg and Rayner 1987). Some bats adjust their echolocation calls (harmonics, Stamper et al. 2009; intensity, Brinklov et al. 2010a, b) when operating in cluttered areas such as thick vegetation. Tactile sensitivity of flight membranes seems to augment flight performance under similar circumstances (Chadha et al. 2011; Miller 2005).

3.2.6 Range

A combination of spherical spreading loss and frequency-dependent atmospheric attenuation (Lawrence and Simmons 1982a) dictates that echolocation in air is a relatively short-range operation. Kick (1982) demonstrated that using echolocation, *E. fuscus* first detected a 19 mm diameter target at 5 m and a 4.8 mm target at 3 m. In the field, Holderied et al. (2005) used videogrammetry to document that *Eptesicus bottae* flew 3–8 m s^{-1} and first detected large objects at ~40 m. They concluded that the inter-call interval (Fig. 3.3) corresponding to lowest peak in mean wingbeat period provided a useful indicator of maximum range of detection of *E. bottae*-sized targets at about 20 m. Surlykke and Kalko (2008) used an array of microphones and demonstrated that the effective range of detection for insect-sized prey was 20–30 m in many species and more than 60 m in *Cormura brevirostris*. The long detection range in *C. brevirostris* coincides with calls consistently produced in triplets that show consistent changes in frequency as well as prominent harmonics.

Flight speed and effective detection range together have important implications for bats hunting for flying prey. Higher flight speeds mean shorter time from detection to contact and challenge bats accordingly. Several species of bats alternate frequencies between adjacent calls perhaps to thwart insect defences (Goerlitz et al. 2010) or as a means of enhancing the range at which they detect prey (Holderied and von Helversen 2003). Mora et al. (2004) reported neurophysiological specializations in the inferior colliculus of *Molossus molossus* that enhance the effect of alternating frequencies between calls. Ratcliffe et al. (2011) reported that *Saccopteryx bilineata* alternate calls while foraging but not when they are in the vicinity of their roost. They also proposed that *S. bilineata* use call alternation to detect prey at short range in cluttered situations (Ratcliffe et al. 2011).

3.2.7 Self-Deafening (Forward Masking)

Jen and Suga (1977) reported that in *Myotis lucifugus* contraction of the stapedius muscle in the middle ear reduced hearing sensitivity as an echolocation call was produced. Contractions of the muscle significantly reduce the movement of the ossicles, thereby reducing sound transmission to the cochlea (self-dampening), yet allow the outgoing pulse to be registered in the brain. Alternately, separating pulse and echo in time minimizes the chances of loud outgoing pulses masking faint returning echoes, but it also means that most echolocating bats are not expected to be able to effectively broadcast and receive at the same time. Veselka et al. (2010a, b) showed that contact between stylohyal and tympanic bones was a characteristic of laryngeally echolocating bats that may further facilitate registering registration of the outgoing pulse for future comparison with returning echoes.

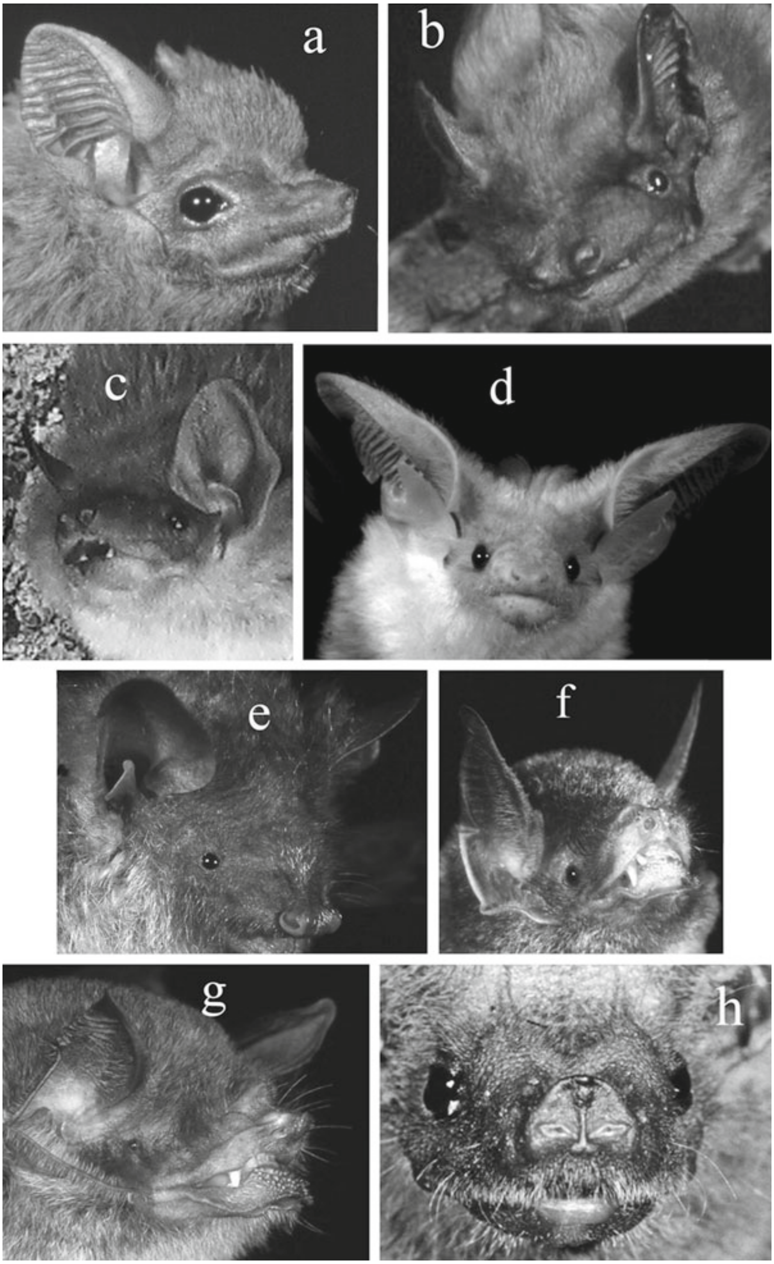

Fig. 3.3 A comparison of the faces of bats with special reference to ears, tragi, noseleafs and related structures. Included are *Balantiopteryx io* (**a**), *Nyctalus noctula* (**b**), *Eptesicus fuscus* (**c**), *Otonycteris hemprichii* (**d**), *Murina leucogaster* (**e**), *Pteronotus personatus* (**f**), *Pteronotus parnellii* (**g**), *Rhinopoma hardwickei* (**h**), *Asellia tridens* (**i**), *Rhinolophus clivosus* (**j**), *Megaderma lyra* (**k**), *Nycteris grandis* (**l**), *Phyllostomus discolor* (**m**), *Anoura caudifer* (**n**), *Desmodus rotundus* (**o**), *Chrotopterus auritus* (**p**) and *Macrotus waterhousii*

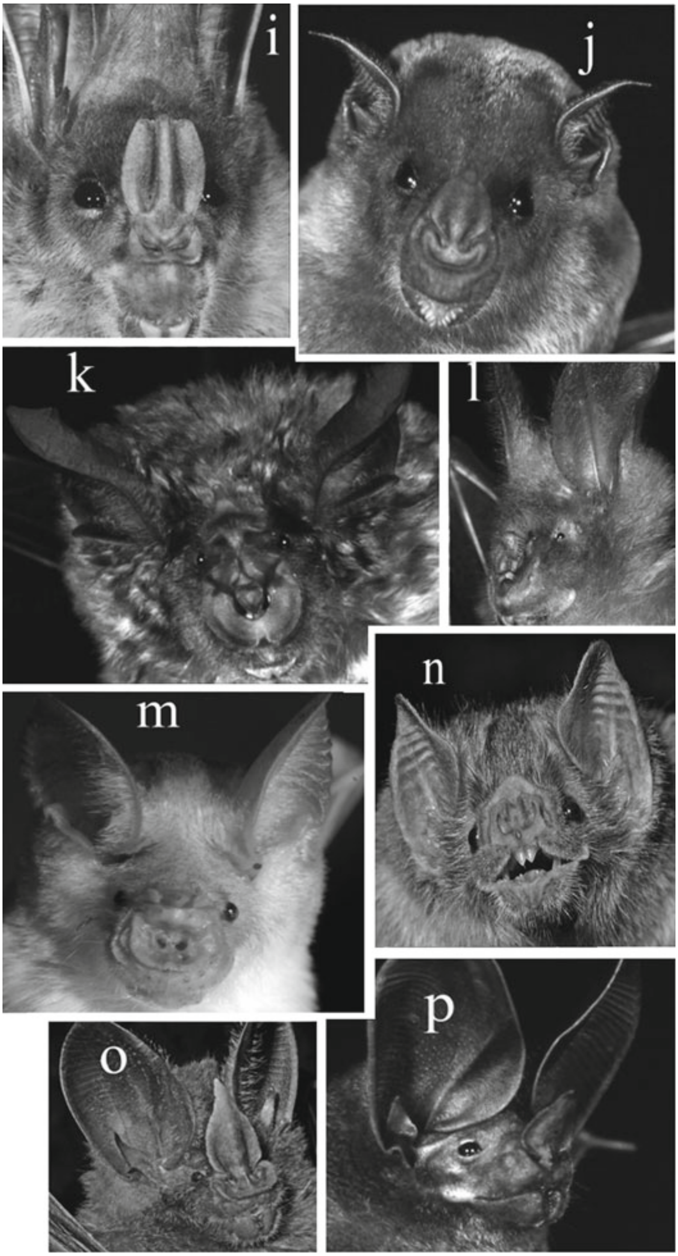

Fig. 3.3 (continued)

Bats in the families Rhinolophidae and Hipposideridae and the mormoopid *Pteronotus parnellii* use Doppler-shifted echolocation calls so as to separate pulse and returning echoes in frequency. These bats can broadcast and receive at the same time.

3.2.8 Duty Cycle

Bats that separate pulse and echo in time produce short echolocation calls separated by long periods of silence (Fig. 3.3a), producing a signal about 5 % of the time they are echolocating—a low duty cycle. Species that separate pulse and echo in frequency produce long calls separated by short periods of silence (Fig. 3.3b). These bats broadcast at a high duty cycle, producing a signal >40 % of the time they are echolocating.

Each echolocation call of a high duty cycle bat is dominated by a single frequency (CF), but most calls begin and end with an FM sweep. Echolocation calls of low duty cycle bats are more variable, being FM signals with a range of bandwidths. Even low duty cycle echolocators producing long, narrowband signals (qCF) echolocate at low duty cycle. The advantage of narrowband signals is that for every ten times reduction in bandwidth, signal strength increases by 10 dB with no additional cost of sound production (Oppenheim et al. 1999).

3.3 Signal Production

With the exception of *Rousettus* species that use tongue clicks, echolocating bats produce their signals in the larynx. Earlier work provided details of pulse production and anatomy of the larynx and associated structures (e.g. Fattu and Suthers 1981; Griffiths 1978). Tracheal chambers and nasal cavities may further modify signals after they leave the larynx (Suthers et al. 1989). In *Rhinolophus hildebrandtii*, emitted echolocation calls have most energy in the second harmonic, and three tracheal cavities are involved in suppressing the fundamental frequency of sounds leaving the larynx (Suthers et al. 1989).

Most bats emit their echolocation calls through their open mouths, but some (rhinolophids, hipposiderids, nycterids, megadermatids and phyllostomids) emit them through their nostrils (Pedersen 1995, 1996, 1998). The difference in call emission influences cranial anatomy, but the role that other cranial features, such as concavities in the basisphenoid and/or basioccipital bones, play in echolocation remains unclear (DeBaeremaeker and Fenton 2003).

Noseleafs and related structures (Fig. 3.4) can further influence the signal leaving the bat. Hartley and Suthers (1987) demonstrated that the dorsal lancet of the noseleaf of *C. perspicillata* directed echolocation signals (short, broadband FM pulses) vertically. Microcomputed tomography reveals that lancet furrows in the noseleaf complex of *Rhinolophus* affect the FM sweep that terminates each echolocation call (Zhuang and Mueller 2006). Vanderelst et al. (2010) used a comparison of *Micronycteris microtis* and *Phyllostomus discolor* to demonstrate that in phyllostomid bats noseleafs can focus energy and help the bats distinguish between echoes from the periphery from those of objects in the area of focus.

Flying bats tend to produce echolocation calls during the expiration phase of the wingbeat cycle (Suthers et al. 1972), so that they do not incur additional costs associated with sound production during echolocation (Speakman and Racey 1991, but see Parsons et al. 2010). Contractions of abdominal wall muscles further assist call production in flying *P. parnellii* (Lancaster et al. 1995; Lancaster and Speakman 2001). Of particular note is the striking difference between high duty cycle *P. parnellii* and low duty cycle *Eptesicus*, *Pipistrellus* and *Myotis* (Lancaster and Speakman 2001) in terms of how the diaphragm contributes to vocalization.

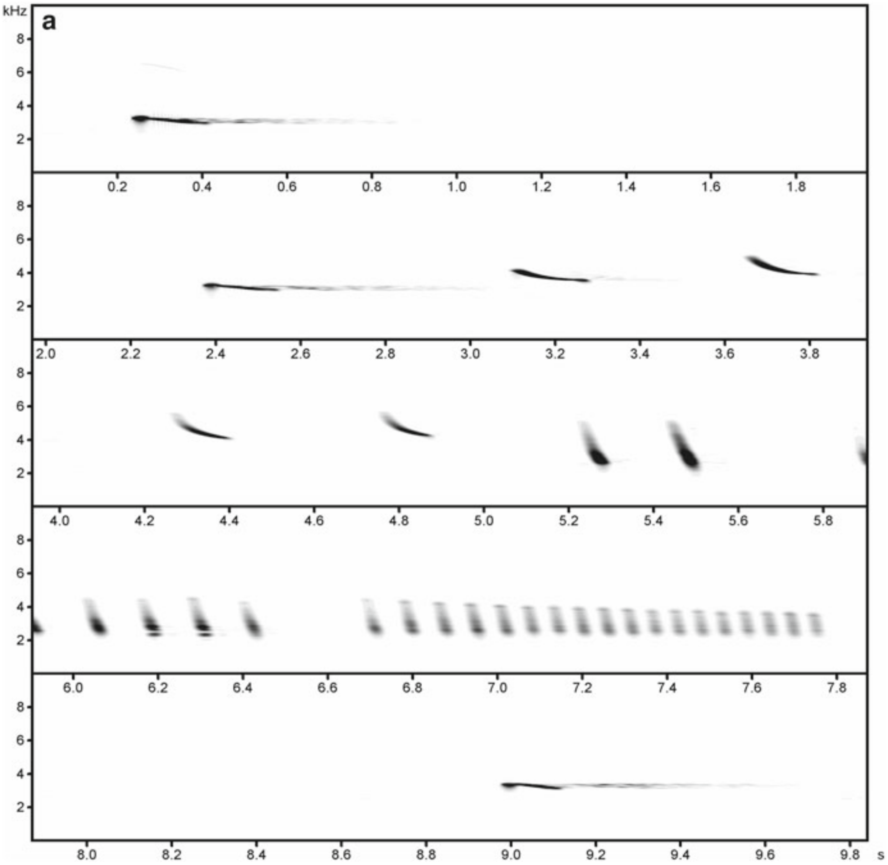

Fig. 3.4 A comparison of a feeding buzz (*Molossus ater*, **a**) and a social buzz (*Tadarida teniotis*, **b**)

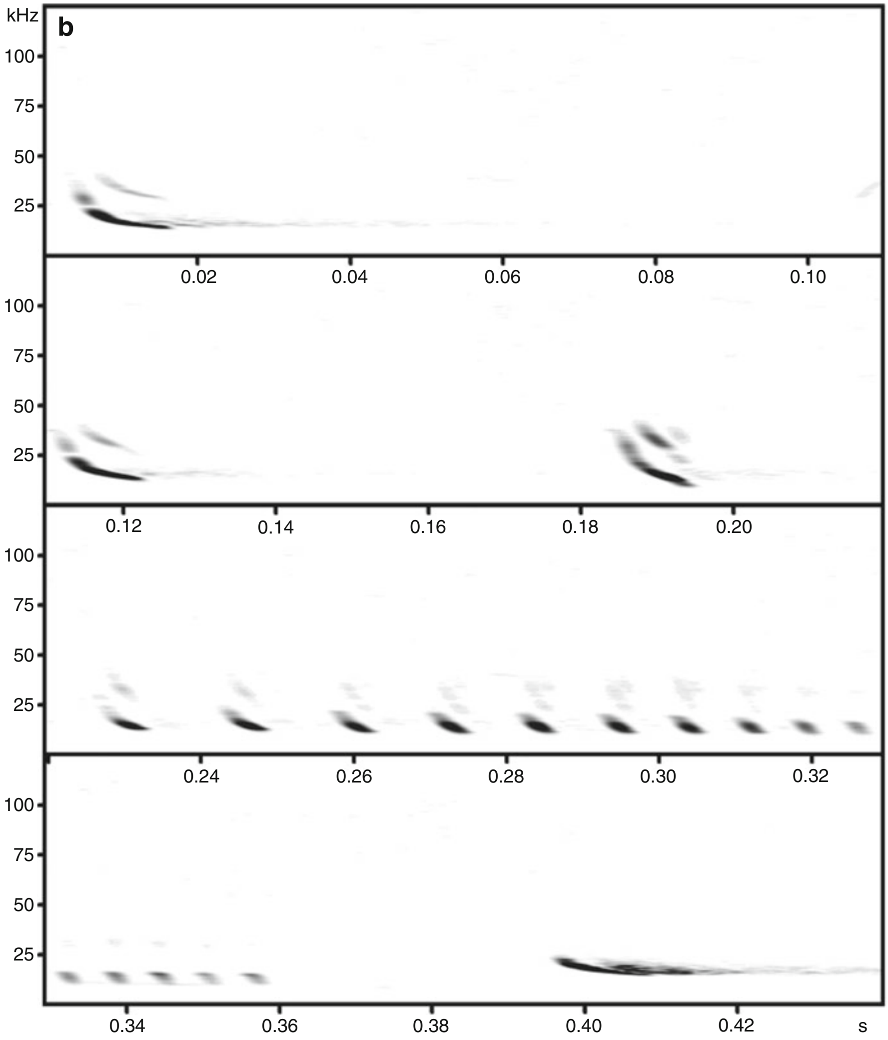

Fig. 3.4 (continued)

3.4 Signal Reception

The pinnae of bats (Fig. 3.4) generally increase the sensitivity of hearing, notably so in high duty cycle species where the pinnae are mechanically tuned to the dominant frequencies of their echolocation calls (Obrist et al. 1993). The degree of specialization of pinnae varies across taxa, and in some species, the ears are better tuned to detecting the sounds of prey than to echolocation signals (Obrist et al. 1993).

Gleaning bats, such as *Megaderma lyra*, detect very low-intensity rustling sounds (Hubner and Weigrebe 2003). Sensitivity arising from tuning of the pinnae is further enhanced by neurological tuning in the inferior colliculus (e.g. Mora et al. 2004).

Some parts of the ear, such as the thickened lower edge of the pinnae in *Nyctalus* spp. combined with the tragus (Fig. 3.4b), reduce extra side lobes in the echoes reaching the bat (Mueller et al. 2006). Early evidence suggested that the tragus affected vertical localization of targets by *E. fuscus* (Lawrence and Simmons 1982a, b). More recent work with microcomputed tomography indicates that the tragus in *E. fuscus* reduces extra side lobes from echoes (Mueller 2004). The variety of tragi and ear structures in bats promises new insights from this emerging area of research.

3.5 Uses for Echolocation

Some species of *Rousettus* may use echolocation only to gain access to dark roosts and little more (Holland et al. 2004); however, many other species of bats use echolocation to detect, assess and track prey, a process that is particularly clear during feeding buzzes produced during attacks on prey (Fig. 3.5a) (e.g. Kalko and Schnitzler 1989; Schnitzler and Kalko 2001). Echolocation calls of foraging insectivorous bats have received considerable attention, providing a rich array of examples of specific behaviour and call design (e.g. Chiu et al. 2010; Goerlitz et al. 2010; Stamper et al. 2009). The role of echolocation in foraging of fruit eaters and blood feeders remains unclear; however, some New World flowers use nectar guides that reflect ultrasonic signals in order to attract flower-visiting bats and thereby increase rates of pollination (Von Helversen and von Helverson 1999, 2003; Yovel et al. 2008).

Möhres (1966) noted that the signals one bat uses in echolocation may also affect the behaviour of other bats, and Barclay (1982) used playback experiments to further explore this aspect of echolocation. More recently, more evidence of the role of echolocation signals in communication has expanded our knowledge (e.g. Bayefsky-Anand et al. 2008; Gillam et al. 2007; Schuchmann and Siemers 2010; Swartz et al. 2007; Ulanovsky et al. 2004; Voight-Heucke et al. 2010). Kingston and Rossiter (2004) proposed that enhanced communication may underlie the taxonomic diversification of some high duty cycle echolocating bats. When several bats fly in the same airspace, they may shift the dominant frequencies in their echolocation calls which suggests jamming avoidance and/or enhanced communication (Ulanovsky et al. 2004; Voight-Heucke et al. 2010). In many situations, foraging bats produce social buzzes (Fig. 3.5b) apparently directed at nearby conspecifics. In some situations *E. fuscus* stop producing echolocation calls apparently to avoid jamming (Chiu et al. 2008).

Information leakage and short operational range are two important drawbacks to using echolocation as a pursuit strategy. Bat-detecting ears have attracted considerable attention (e.g. Pye 1968; Roeder 1967), and their widespread occurrence among insects (Ratcliffe 2009) testifies to the importance of bats as predators. The

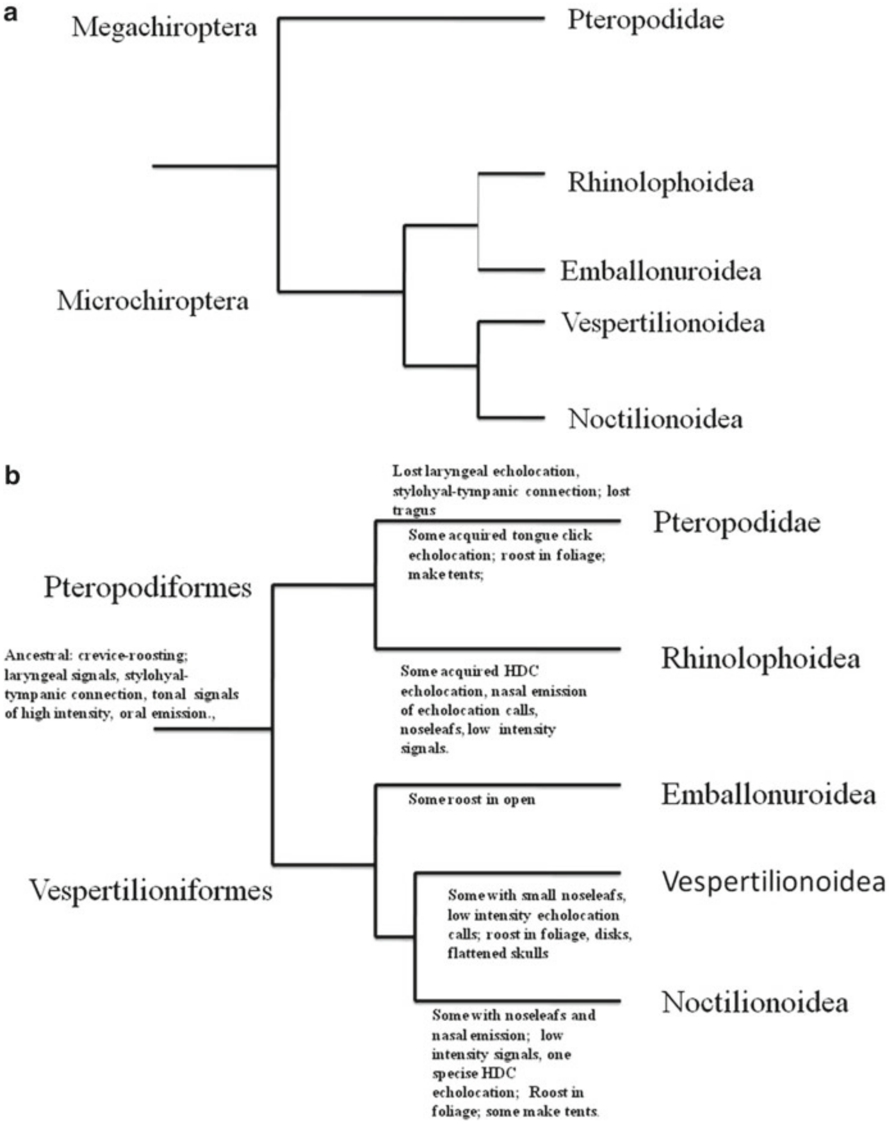

Fig. 3.5 The "old" (**a**) and "new" (**b**) phylogenies of bats

success of hearing-based defences in many insects reflects the problem of information leakage. Bats use various strategies to circumvent insect defences; behaviours ranging from an allotonic approach (broadcast outside the frequencies to which insect ears are most sensitive; Fenton and Fullard 1979), to stealth, are producing calls of lower intensity (Goerlitz et al. 2010). Bats such as *Lasiurus borealis* use echolocation calls that are detected by moths with ears, many of which successfully evade the bats (Acharya and Fenton 1992, 1999); however, *L. borealis* still preys heavily on these moths (Clare et al. 2009).

Laryngeally echolocating bats use morphological specializations of their pinnae that include tuning to a particular frequency or better sound collection (Obrist et al. 1993) combined with neurological adaptations to increase their hearing sensitivity and, thus, their effective target detection range (Neuweiler 1989, 1990). The echolocation-based hunting strategies of many bats are most impressive (Neuweiler 1989), ranging from those detecting, pursuing and attacking flying insects (e.g. Goerlitz et al. 2010; Lazure and Fenton 2011) to those taking prey from the water's surface (e.g. Hartley et al. 1989; Kalko and Schnitzler 1989; Schnitzler et al. 1994). Other bats may or may not use echolocation when gleaning prey from surfaces (Faure and Barclary 1994; Hubner and Weigrebe 2003; Siemers and Schnitzler 2004), while others may use vision (Bell 1985). The tactile sensitivity of small sensory hairs located on the bats' wings has also been shown to augment flight performance during pursuit or gleaning activities (Chadha et al. 2011; Miller 2005).

3.6 Echolocation and the Phylogeny of Bats

Teeling (2009) and Jones and Teeling (2006) have provided excellent overviews of the three hypotheses concerning the origin of echolocation in bats. Simmons and Geisler (1998) proposed that flight was ancestral, providing the ancestors of bats with mobility, and that echolocation evolved later. Simmons et al. (2008) proposed that the Eocene *Onychonycteris finneyi* could fly but not echolocate, also supporting the flight-first hypothesis (Simmons 2008). Fenton et al. (1995) proposed that echolocation was ancestral in bats and was the key innovation that provided the ancestors of bats access to flying nocturnal insects as food. Speakman and Racey (1991) proposed that flight and echolocation evolved simultaneously in the ancestors of bats allowing them to produce intense signals on the downstroke, covering the cost of signal production with the costs of flight. It appears that this coincidence may not apply to all bats, for example, *Mystacina tuberculata* (Parsons et al. 2010) and *S. bilineata* (Ratcliffe et al. 2011).

Morphology, particularly of the shoulder girdle, supports the view that *Onychonycteris* and other Eocene bats could fly (Simmons and Geisler 1998; Simmons et al. 2008). In bats, connections between stylohyal and tympanic bones are unambiguous morphological indicators of laryngeal echolocation (Veselka et al. 2010a). Simmons and Geisler (1998) used features of the proximal end of the stylohyal bone and the relative size of the cochlea to argue that other Eocene bats (e.g. *Icaronycteris*, *Hassianycteris*, *Palaeochiropteryx*) could echolocate. The two specimens of *O. finneyi* are not well enough preserved to assess possible contact between tympanic and stylohyal bones, leaving open the question of whether or not the bat had the capacity for laryngeal echolocation (Simmons et al. 2010; Veselka et al. 2010b).

If laryngeal echolocation is ancestral, then it evolved once in the ancestors of Chiroptera but was lost in the lineage leading to Pteropodidae. Within the pteropodids, echolocation by tongue clicking appears to have evolved secondarily once. If laryngeal echolocation was not ancestral, then it could have evolved twice, once in each suborder of Chiroptera (Yinpterochiroptera and Yangochiroptera, Fig. 3.6). As noted by Teeling (2009), the emergence of the new phylogeny and classification of bats has implications for the evolution of echolocation. In the "old" classification, separation of Pteropodidae as Megachiroptera from all other bats presented a different situation than the "new" classification in which both suborders include laryngeal echolocators. High duty cycle echolocation has evolved independently in both Yinpterochiroptera and Yangochiroptera, along with other features such as low-intensity echolocation calls and the use of prey-generated sounds for finding food (Fenton et al. in press). As of 2012 it appears that only the discovery of additional fossil material would resolve the question of the origin of echolocation among Chiroptera. Data on the incidence of genes associated with hearing and vocalizing (*Prestin* and *FoxP2*—Li et al. 2007, 2008, respectively) does not resolve the situation or provide unambiguous support for either the traditional (Megachiroptera, Microchiroptera) or "new" (Yinpterochiroptera, Yangochiroptera) phylogeny.

Most data about the phylogeny of bats suggest that Chiroptera is monophyletic (Eick et al. 2005; Simmons 1994; Simmons et al. 1991; Simmons and Geisler 1998), but this view is not unanimous (Pettigrew 1991; Dell et al. 2010; Kruger et al. 2010).

3.7 Origin of Bats

Although Jepsen (1970) argued that bats evolved in caves, I prefer the suggestion that they evolved in forests with open understory. The protobat has been depicted as being arboreal (Hill and Smith 1984) and presumed to have glided in pursuit of flying insects (Fig. 3.7). Norberg (1985) proposed a model that demonstrated the feasibility of such a transition from gliding to powered flight. Coordinated elongation of arm, hand and finger bones has been shown to be under genetic control during development (Sears et al. 2006). A related scenario applies to the webbing (patagium) of bat wings but not their hind feet (Weatherbee et al. 2006).

Fenton et al. (1995) argued that to function in detecting and tracking flying insects, echolocation signals had to be more intense to increase the effective range of the call. They contended that the use of high-intensity signals in echolocation constituted a "breakthrough", providing access to a previously inaccessible food source (flying nocturnal insects). Maltby et al. (2009) proposed that the ancestral echolocation call in laryngeally echolocating bats was short, narrowband and multiharmonic. In the initial phase, I think that the protobat would have glided in pursuit of its prey, being a more fundamental step in detecting and tracking targets than the more complicated scenario of when both predator and prey are flying.

This hypothesis makes the prediction that fossil protobats will have laryngeal cartilages robust enough to anchor the cricothyroid muscles that adjust tension on the

Fig. 3.6 J. D. Smith's proposed protobat (Hill and Smith 1984)

vocal cords. These cartilages will match increased subglottal pressures associated with echolocation. Precise control of the vocal cords must have been mirrored by simultaneous development of cricothyroid muscles. The appearance of the stylohyal-tympanic connection may have appeared after development of echolocation, followed by diversification and elaboration of facial and ear structures associated with echolocation. It may be that only exceptionally well-preserved fossils will provide the detail necessary to address and perhaps resolve this prediction.

3.8 Conclusions

The diversity of bats and echolocation behaviour provides as many opportunities for further work as it did 50 years ago; new discoveries open many doors than definitive answers to old questions close. The present array of tools for examining the details of what bats do and how they do it enhances our capacity for better understanding the evolution and diversification of bats. Additional fossil finds may well oblige us to adjust our theories about the origin of bats and echolocation.

Box 3.1 Echolocation: Units, Terms

Attenuation—absorption of acoustic energy as it moves through air, influenced by frequency
Audiogram—frequency sensitivity of audiogram measured as strength of signal to evoke neural (or behavioural) response at different frequencies
Broadband—bandwidth of an echolocation call measured in kHz (Fig. 3.1)
CF—constant frequency call (or call component) (Fig. 3.1)
CF-FM—an echolocation call with CF and FM components (Fig. 3.1)
Click—broadband sound produced by clicking the tongue (Fig. 3.1)
Clutter—echoes from other than the target (see also Denny 2007)
dB—decibels (see dB SPL at x cm)
Duration—the duration of a call
FFT—fast Fourier transform (frequency versus energy) (Fig. 3.1)
FME—frequency with maximum energy (kHz) usually measured from a fast Fourier transform power spectrum (Fig. 3.1)
FM—frequency modulated call (or call component) (Fig. 3.1)
Frequency—pitch measured in kHz
Fundamental frequency—lowest frequency of a periodic waveform, sometimes called the first harmonic
Harmonic—overtone, doubling of frequency over fundamental (Fig. 3.1)
Highest frequency—in an echolocation call (HF)
Intensity—measure of signal strength (dB SPL at x cm from source)
Inter-call interval—time between calls (sometimes called interpulse interval), measured from the start of the first call to the start of the next)
kHz—kilohertz measure of frequency or pitch
Loudness—psychometric measure, how loud a signal sounds (a function of hearing sensitivity—audiogram)
Lowest frequency—in an echolocation call (LF)
ms—milliseconds (measure of time)
Narrowband—bandwidth of an echolocation call measured in kHz (Fig. 3.1)
Power spectrum—distribution of energy in a call by frequency (Fig. 3.1)
qCF—quasi-constant frequency signal (narrowband) (Fig. 3.1)
Spectrogram—changes in frequency over time. a three dimensional display with energy represented by the colour or blackness of the display (Fig. 3.1)
SPL—sound pressure level (usually in the context of dB SPL at a given distance from sound source). Root mean square (rms) values are, for the same signal, 9 dB lower than for peak-to-peak measures
Spreading loss—energy dissipation as sound moves away from a source (en route to and from a target)
Wavelength—λ of a sound

Acknowledgements I thank Beth Clare and Liam McGuire for their comments on earlier versions of this manuscript and the editors for inviting me to contribute to this volume. My research on bats has been supported by Discovery and Research Tools and Equipment Grants from the Natural Sciences and Engineering Research Council of Canada.

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Chapter 4
Nasal-Emission and Nose leaves

Scott C. Pedersen and Rolf Müller

Abstract Despite all other craniodental adaptations, the head of most bats must function as an ultrasonic emitter and receiver. Not all echolocation calls are ultrasonic, but all either are emitted from an open mouth (oral-emission) or are forced through the confines of the nasal passages (nasal-emission), and some nasal-emitting bats alternate between modes as the situation demands. The conspicuous baffles that surround the nostrils of nasal-emitting bats are not ornamental structures; rather, these "noseleaves" serve several important acoustic functions and are considered to be the earmark of nasal-emitting bats. For many readers, the difference between oral- and nasal-emission is viewed as a simple character state, most likely tied to the vagaries of foraging ecology in some tangential manner. However, Pedersen and Timm (Evolutionary history of bats: fossils molecules and morphology. Cambridge University Press, Cambridge, pp 470–499, 2012) reviewed a considerable volume of literature discussing how the advent of nasal-emitting bats required a dramatic redesign of the microchiropteran rostrum and skull base during development. Nasal-emission is therefore a key innovation responsible for two of the most dramatic morphological radiations in the Chiroptera—phyllostomid and rhinolophid + hipposiderid bats. Herein, we summarize and update that review and then discuss recent advances in the numerical analysis of form and function in regard to the beamforming function of noseleaves (Müller, J Acoust Soc Am 128:1414–1425, 2010).

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_4,

4.1 Bat Heads

The vertebrate head is an evolutionary novelty—most of its genes, tissues, and segmental arrangement were cannibalized from old postcranial material. The differential sequence of events that occur during construction of the mammalian head is typically ignored in phylogenetic reconstructions. Instead, the skull is presented as an immutable structure into which the brain, ears, and eyes are squeezed during development. The converse is in fact true—the skull should be seen as a complicated cast of, and not a mold for, craniofacial evolution (Carroll 2005; Chai and Maxson 2006; Creuzet et al. 2005; Fondon and Garner 2007; Hallgrìmsson and Lieberman 2008; Hallgrìmsson et al. 2007, 2009; Marcucio et al. 2011; Radlanski and Renz 2006; Young and Badyaev 2007; Young et al. 2010).

Using cephalometric data collected from skulls of fetal and adult bats, Pedersen (2000) described the inertial and acoustic axes of the chiropteran skull. These data showed that skulls of bats that produce echolocation signals with their larynx (Microchiroptera; sensu Dobson 185) follow one of two mutually exclusive sets of construction rules based on the ultimate use of either the oral cavity or the nasal cavity/facial skeleton as a waveguide. In general, the rostra of oral-emitting bats are stereotypically located well above the inertial axis of the head in flight (dorsiflexed), whereas nasal-emitting bats fly with their rostra at or well below the inertial axis of the head (ventroflexed; Fig. 4.1) and their noseleaves aligned perpendicular to the direction of flight.

This dramatic dichotomy in skull design and head posture is laid down very early in development. The plesiomorphic condition for mammalian embryos is exemplified by mormoopids, emballonurids, and vespertilionids wherein the fetal rostrum and hard palate rotate dorsally about the braincase until they are aligned with or elevated well above the basicranium. In contrast, the palate of nasal-emitting forms is retained ventral to the basicranial axis. This dichotomy between oral- and nasal-emitting baupläne imposes dramatic changes in the subsequent development of the entire head and the sensory organs therein, i.e., rotation of the eyes and otic capsules so as to align them with the inertial axis of the head. These baupläne are well established before the skull begins to ossify and well before the forces of mastication begin to effect other aspects of skull morphogenesis.

This dichotomy is not a simple reversible character state as some would suggest (see below). Rather, it reflects a fundamental renovation of the head and skull—arguably the most important innovation in bats beyond the evolution flight and echolocation.

4.2 Nasal-Emitters

There are, however, two different types of nasal-emitting head: (1) highly derived forms in which the organization of the nasal cavities is effectively dominated by the emission of acoustic signals (rhinolophid and hipposiderid bats) and (2) less-derived

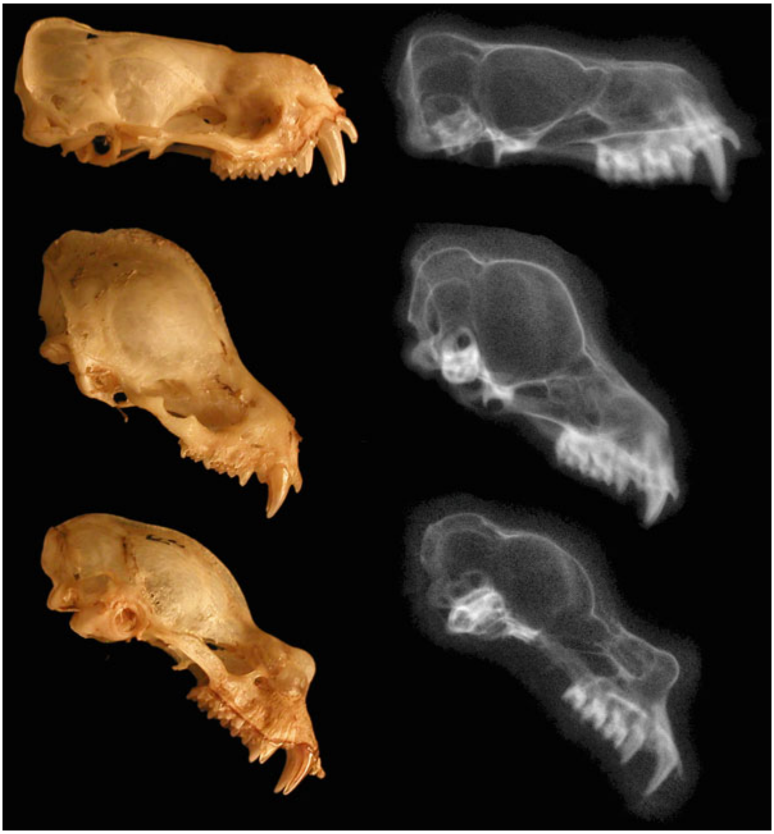

Fig. 4.1 Representative photographs and radiographs (*top* to *bottom*): oral-emitting molossid, *Eumops glaucinus* (male); nasal-emitting phyllostomid, *Lophostoma silvicolum* (male); and a nasal-emitting rhinolophid, *Rhinolophus yunanensis* (male). Each image is oriented such that the plane of the semicircular canals is elevated equally above the horizontal. This orientation is a crude approximation of how the heads are held in flight. Images are not to scale and are for comparison purposes only

forms in which nasal-emission is not the primary modality driving the layout of the head and face. In these bats, other functions may be equally or more important than nasal-emission, e.g., vision, olfaction, and perhaps the suspension of the tongue (phyllostomids). Given that the packaging of the embryonic head is a balancing act between many competing factors (differential growth of various soft tissue elements, morphogenetic plasticity of skeletal elements, dental ontogeny, tongue size, etc.), Pedersen (2000) suggested that there was only enough room in the rostrum of a nasal-emitting bat skull to develop 1–2 sensory modalities at any one time—having either large olfactory fossae or expansive resonating chambers, but not both. This is a readily testable hypothesis. Have phyllostomids retained olfaction at the cost of sound pressure amplitude of the call, whereas rhinolophids and hipposiderids have emphasized sound pressure amplitude of the call at the expense of olfaction?

This superficial dichotomy is misleading because many phyllostomids are not obligate nasal-emitters and the oral-emission of their calls may result in higher pressure amplitudes (Marcías et al. 2005; Mora and Marcías 2006).

4.3 Skull Mechanics

Regardless of their relative position on the neurocranium, opposing jaws, teeth, and muscles function together as components of a well-integrated system (Soukup et al. 2013; reviewed by Pedersen and Timm 2012). During mastication, the rostrum and zygomatic arches brace the palate against the braincase posteriorly, which then transfers these forces to the occiput, cervical spine, and thorax. In oral-emitting skulls that are strongly dorsiflexed (e.g., *Thyroptera*) or in the strongly ventroflexed crania of *Rhinolophus*, the distribution of bone mass suggests that these skulls are poorly designed to resist torsional/bending forces. Thus, soft tissues such as muscle, ligament, and tendons must compensate in part for the significant reduction of boney architecture, or alternatively, these extreme forms are limited to soft-bodied prey items. Despite sharing the nasal-emitting bauplan (and excluding the highly derived nectivorous forms), phyllostomid skulls are considerably more robust than those of most rhinolophids or hipposiderids (Davis et al. 2010; Monteiro and Nogueira 2011; Nogueira et al. 2009; Santana et al. 2012). It would therefore be of interest to see if durophages (Dumont 2004; Dumont et al. 2011; Freeman 2000; Freeman and Lemen 2010) exhibit more moderate skull angulations within their particular oral- or nasal-emitting construct.

4.4 Rostrum as Vocal Tract

Gross modification of the rostrum and midface is related to vocalization in many mammals (Frey et al. 2007). Of interest, the sound produced by the larynx is not necessarily what nasal-emitting bats emit from their nostrils. Differential filtering of the source spectrum is effected primarily by the dimensions of the vocal tract and discontinuities in the walls of the pharynx and trachea. In taxa with minimal modification to the rostrum, the projection of sound through the high impedance of the nasal passages may restrict those taxa to the use of low-intensity multiharmonic calls (nycterids, megadermatids, and many phyllostomids). Conversely, extensive modification of the nasal cavities and pharynx may well restrict the range of craniodental adaptations available to a taxon, e.g., rhinolophids.

Depending on prey type, foraging strategy, and habitat complexity, both oral- and nasal-emitting bats may shift between different types of calls or modulate where they put energy into each call (Fenton, Chap. 3). Within a taxon, this variation in call structure may be best viewed as a behavioral response to clutter and selection of prey type (Surlykke and Moss 2000). Call design has also been mapped onto various molecular and morphological phylogenies but with only limited success (Eick et al. 2005; Jones and Holderied 2007; Jones and Teeling 2006), but this is not surprising given the remarkable behavioral plasticity demonstrated by extant bats. However, what constraints are imposed on call design or call structure as sound is projected through the confines of the nasal passages?

4.4.1 Phyllostomids

The low-intensity calls of many phyllostomids led Griffin (1958) to refer to them as "whispering bats." However, recent work has shown these bats are capable of being much louder than previously believed (Brinkløv et al. 2009, 2011). There is considerable variation amongst phyllostomid bats in terms of which harmonics and the number of harmonics that are differentially recruited into the call under different circumstances (Jennings et al. 2004). The fundamental frequency (f_1) is commonly used by these bats during search and commuting, but they often shift up into the second and/or third harmonics when moving into clutter or running up onto a roost; this pattern is observed in several subfamilies (*Macrophyllum*, Brinkløv et al. 2010; *Phyllonycteris*, Mora and Marcías 2006; *Phyllops*, Marcías et al. 2005).

In contrast to rhinolophids and hipposiderids, the rostra of phyllostomids have not been grossly modified to accommodate nasal-emission, and differential use of the upper harmonics in many of these taxa would seem to be a flexible behavioral adaptation. However, the diversity of noseleaf shapes (see below), in conjunction with the moderate inflation of the rostrum in *Lonchorhina*, and the limited modification of the paranasal sinuses in *Micronycteris* (Vanderelst et al. 2010a) suggest that the acoustics of phyllostomine skulls may be unique amongst phyllostomids. Otherwise, morphological diversity in the phyllostomid rostrum is well correlated with membership in a specific feeding guild and structural allometry (Dumont et al. 2012; Monteiro and Nogueira 2011; Nogueira et al. 2009; Wetterer et al. 2000).

4.4.2 Rhinolophids and Hipposiderids

The unique shape of these skulls is characterized by the gross dilation of the nasal cavities and paranasal sinuses. This modification reduces the impedance of the nasal passages, effectively transforming them into a major component of the vocal tract. In addition, the robust larynx of many rhinolophids (and presumably hipposiderids) is retained within the nasal shunt by a unique arrangement of muscles of the soft palate and the cartilages of the larynx. This arrangement implies that rhinolophids may be obligate nasal-emitters. In concert, these extensive modifications effect a derangement of adjacent musculoskeletal elements associated with the soft palate and pharynx, e.g., pterygoid width, abbreviated hard palate, large-bore choanae, and otic capsule separation. Arguably these are the most extensive structural modifications to the skull within the order.

Ossification of bat skulls follows the common mammalian pattern. The few exceptions to this plan are found in rhinolophids and are related to this unique distortion and packaging of the midface. Shifts in the ossification sequence also include skeletal elements involved in the suspension of the larynx from the basicranium in rhinolophids and hipposiderids. Perhaps future discussion concerning the evolution of echolocation would be well served by evaluating the embryology of the trachea, tracheal diverticula, and basicranial pits in the skull base (Cretekos et al. 2005; Debaeremaeker and Fenton 2003; ten Berge et al. 1998; Veselka et al. 2010). Many oral-emitting bats and nearly 70 % of phyllostomid taxa exhibit these pits; however, they are rare (6 %) in rhinolophids and hipposiderids. It would be interesting to see if there is a reciprocal arrangement between the presence of supra- and subglottal resonators across taxa.

4.4.3 Linear Model of the Rhinolophid Vocal Tract

Simple linear models of the supraglottal vocal tract suggested previously that the composition of the midface and differential development of the paranasal sinuses could be associated with fixed cavity resonances which subsequently affect the sound levels and frequency profiles permitted by the supraglottal vocal tract (Armstrong and Coles 2007; reviewed by Pedersen and Timm 2012). In general, the nasal passages have been viewed as a band-pass filter for the second harmonic, i.e., they suppress the fundamental and several of the remaining harmonics. We reopened this line of inquiry to readdress the physics of the enormous sound levels produced by some species. We used micro-CT to reconstruct the airway of *Rhinolophus ferrumequinum* to quantify vocal tract dimensions (Fig. 4.2). We also evaluated the resonant mechanics of this system, testing both linear and nonlinear numerical models of the sub- and supraglottal vocal tract. The relative position of several landmarks along the length of the vocal tract matches the predictions of the model and those expectations of previous studies regarding the dominant spectral component of the call (f_2 wavelength, 4.45 mm; 77 kHz).

Due to their simplicity, linear models of the vocal tract have been instructive in a variety of studies. Recordings of foraging bats have shown that their foraging ecology is better explained by the dimensions of the nasal cavities than by body size or environmental variables in both *Rhinolophus* (Odendaal and Jacobs 2011) and *Rhinonicteris* (Armstrong 2002; Armstrong and Coles 2007; Armstrong and Kerry 2011), even despite excessive respiratory water loss in arid environments (Nelson et al. 2007). Developmentally, neither the nasal cavities nor the pinnae of *Rhinolophus* seem to exhibit the linear dimensions necessary for sending or receiving the dominant spectral component of the adult call (f_2) until well after birth (Funakoshi et al. 2010; Liu et al. 2007). Similar data were used to model the differential filtering of other harmonics in the developing rostrum (Pedersen 2000).

The geometry of the vocal tract influences the coupling of laryngeal sound and its propagation towards the nostrils; however, utilitarian linear transfer functions

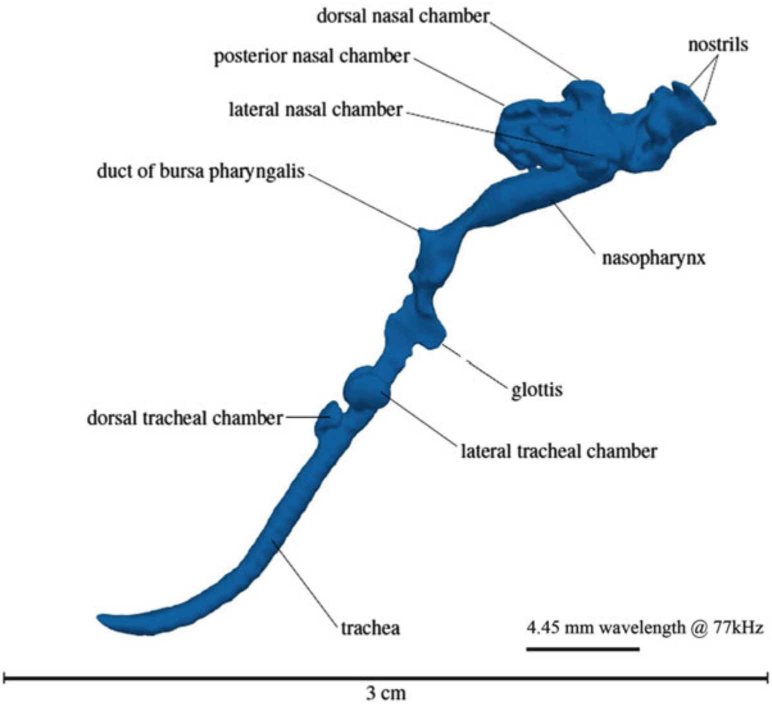

Fig. 4.2 Micro-CT reconstruction of the vocal tract of *Rhinolophus ferrumequinum* (from Pedersen et al. 2009)

alone are insufficient to explain the reactance of the pharynx or the efficiency of the larynx—both of which would clearly benefit from further study.

4.5 Noseleaf Structure

Noseleaf size, shape, and complexity exhibit considerable variation among taxa. Two structural elements can be found in most noseleaves: an anterior leaf that resembles a horn-shaped baffle and a posterior leaf that is often ornamented with a wide variety of cavities, furrows, or ribs. In rhinolophids, a third structure resembling a gnomon-like spike (sella) is found just above and between the nostrils, but it is unclear if and how this element is represented in the closely related hipposiderids. The majority of phyllostomid bats exhibit a noseleaf with a distinct horseshoe and a posterior element referred to as the spear—a diminutive sella appears in a small number of phyllostomines (Wetterer et al. 2000). Phyllostomid spears often exhibit shallow depressions that are oriented vertically along the sides of a central rib (Wetterer et al. 2000). The dramatic spears of megadermatids also exhibit deep vertical furrows (e.g., *Lavia*) and beg further analysis (Göbbel 2002a).

The relative proportions and ornamentation of these various parts of the noseleaf are useful as character states in phylogenetic reconstructions (Csorba et al. 2003; Wetterer et al. 2000).

The homology of noseleaves is still under debate (Göbbel 2000, 2002a; Springer et al. 2001a; Yokoyama and Uchida 2000). However, noseleaf primordia appear before the eyes and external ears are visible in many bat embryos, indicating that such ornamentation is related to deep taxonomic differences in developmental timing and the construction of the head in general (Göbbel 2000, 2002a, b; Chen et al. 2005; Cretekos et al. 2005, 2007; Giannini et al. 2006; Nolte et al. 2008; Wyant and Adams 2007; Yokoyama and Uchida 2000).

Additional work is clearly warranted if we are to understand noseleaf function in (a) taxa where we observe dramatic reductions in the size and complexity of the leaf (*Centurio*, vampires, brachyphyllines, many glossophagines) or (b) oral-emitting taxa that exhibit fleshy masses around the nostrils (plecotines, *Antrozous*, *Craseonycteris*, or *Rhinopoma*). The articulated facial cleft of nycterids is unique, but its components seem homologous to the noseleaves of hipposiderids. Pedersen (1995) was incorrect in thinking that the volume of space within the cleft might function as a "resonating chamber outside the bony nasal cavity." Rather, this cleft and its associated palps function much like a noseleaf—the upper portion of the concavity is a focus-reflecting baffle for the near field but also narrows the far-field beam for the third and possibly the fourth harmonics (Zhuang et al. 2012).

4.6 General Function and Dimensions of a Noseleaf

Sound produced by the vocal folds travels through the nasal passages and is subsequently radiated from the nostrils into a three-dimensional free field around the bat's head. The noseleaf is therefore situated at a critical position in the bats' biosonar system, where it could act as an acoustic horn which will gradually match the high acoustic impedance of the nostrils to the low acoustic impedance of the free field. Beyond a short transition zone (the acoustic near field), the distribution of acoustic energy in the radial dimension is determined by the geometrical spreading losses and the absorption associated with propagation in air. Here again, the noseleaf is in a critical position to determine the distribution of energy as a function of direction and frequency. Hartley and Suthers (1987) argued that without a noseleaf, the directionality of nasally emitted ultrasound is considerably degraded, i.e., the evolution of nasal-emission may have predicated the subsequent evolution of noseleaves.

The beamforming capacity of the noseleaf is currently receiving considerable attention (Fig. 4.3; Müller 2010). Certainly, narrow beams are advantageous for the spatial separation of echoes of interest from those of surrounding clutter—wide beams can be used to search for targets in open space and retain an overall awareness of obstacles in the environment (Müller and Kuc 2000). Models indicate that large noseleaves could potentially be more directional than small leaves. However, there are physical limitations on the overall dimensions of a large noseleaf in terms of structural

Fig. 4.3 Digital models of representative noseleaf shapes (after Müller 2010)

integrity and the risk of deformation in flight, let alone the additional energetic cost associated with pushing a large noseleaf through the air (Bullen and McKenzie 2008; Gardiner et al. 2008). Interestingly, Brinkløv et al. (2011) showed that big noseleaves are not necessarily associated with a narrower beam but rather provide the bat with some degree of flexibility in terms of beam steering as the situation demands.

Nevertheless, a nasal-emitting bat of a given body mass could theoretically improve the directionality of its call by (1) building a bigger noseleaf, or (2) generating higher frequencies in the larynx, or (3) differential use of the upper harmonics, or (4) tuning the nasal passages to enforce specific harmonics with an efficient resonator. Whether deployed singly or in combination, each of these options is physically limited by the allometry of the body and respiratory tract, the atmospheric attenuation of sound, and certainly taxonomy.

4.7 Beamforming

Previous analyses of the functional morphology of noseleaves were limited to measurements of size and shape, but it was difficult to say *a priori* how each measurement might relate to our understanding of the acoustic functions of noseleaves (see Müller 2010). More recently, computational numerical methods have been used to investigate the relationships between baffle geometry, acoustic mechanisms, and resulting beam patterns in nasal-emitting bats. Key advantages of such approaches are the efficient, high-resolution estimation of beam patterns and the malleability of the underlying shape representations. Readers are directed to a great volume of literature concerning the pinnae of nasal-emitting bats (De Mey et al. 2008; Firzlaf and Schuller 2003; Ma and Müller 2011; Reijniers et al. 2010; Vanderelst et al. 2012; Wang and Müller 2009; Zhao et al. 2003). In this context, we will limit our discussion to noseleaves.

In *Rhinolophus rouxi* (Zhuang and Müller 2007), there would seem to be a "division of labor" between the various parts of the noseleaf, in which the primary function of the anterior leaf and sella appears to be an overall focusing of the beam, whereas the sella and lancet introduce a frequency-specific widening. The phyllostomid noseleaf does not seem to be as sophisticated (Vanderelst et al. 2010a, b). The frequency specificity of these various structures would allow the bat to use separate frequency channels for performing tasks that are better served by either wider or narrower beams—narrow beams for targeting and wide beams for landmark identification and contour following. Each component of the noseleaf will be dealt with in turn below.

4.7.1 Anterior Leaf and Nostrils

The horn-shaped baffle that encircles the ventral aspect of the nostrils exhibits considerable variation amongst nasal-emitters. At the extremes, it takes on distinctly conical shapes (e.g., *Rhinolophus*, *Rhinonicteris*, and several phyllostomines). In other cases, it appears as a less distinct dish-shaped baffle (e.g., some stenodermatines and hipposiderids) or is significantly reduced in some forms such that a baffle is not grossly distinguishable (e.g., brachyphyllines, *Desmodus*, *Centurio*).

The tissues of the anterior leaf surround and support the nostrils. However, the nostrils and anterior leaf are not necessarily coplanar, and the connection between each nasal cavity and the plane of the nostril is not a simple tube. Instead, this passage is a very complicated waveguide, the construction and embryology of which differentiate phyllostomid from rhinolophid noseleaves (Göbbel 2000) and those in turn from the unique floor of the facial cleft in nycterids (pers. obs.; Zhuang et al. 2012).

In contrast to the single point source of oral-emitting bats (mouth), the spacing between the two point sources (nostrils) could influence the forward constructive interference and directionality of the nasally emitted sound (Schnitzler and Grinnell 1977). Narial cartilages and their associated musculature are embedded within the body of the anterior leaf and are in a position where they could effect the spacing of the nostrils and thereby affect the beamforming capacity of the noseleaf (Göbbel 2000 and references). In addition, nasal-emitting bats may have the option of steering or otherwise modifying the beam pattern via the differential positioning of the nostrils within the noseleaf (Göbbel 2002a, b; Vanderelst et al. 2010a) or by imposing differential impedance at either nostril via the valvular nature of the nostril itself.

4.7.2 Posterior Leaf: Lancet and Spear

The lancets of rhinolophids and many hipposiderids exhibit unique half-open cavities (furrows and cells) that are oriented transversely across the upper portion of the leaf. Numerical experiments suggest that these furrows act as half-open resonance

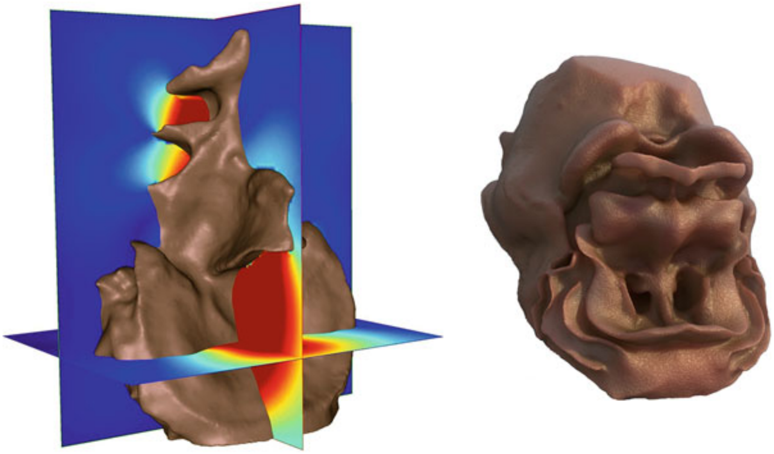

Fig. 4.4 *Left*: digital model of the noseleaf of *Rhinolophus rouxi* that indicates the sound pressure amplitude for 60 kHz in the near field. Note the spatial maxima associated with the lancet furrows (from Zhuang and Müller 2006). *Right*: digital model of the noseleaf of *Hipposideros armiger* (Via Dane Webster)

cavities (Zhuang and Müller 2006, 2007; Fig. 4.4). Sound waves emitted from the nostrils pass across the open face of these furrows and trigger cavity resonances therein. Such resonance effects are typically confined to a very narrow range of frequencies. As such, these furrows are well suited to alter the behavior of a device such as a noseleaf within an already narrow frequency band of operation (CF call). In *R. rouxi*, this effect was most notable in those lower frequencies found in the FM tail of their CF call wherein these furrows effectively widen the biosonar beam in a transient fashion. Horseshoe bats may use these resonances to produce both wide and narrow beam patterns within the confines of a single biosonar pulse. It may be advantageous for a high duty cycle CF bat to illuminate the environment simultaneously through both narrow beams (targeting) and wide beams to retain an overall awareness of obstacles in the environment (Müller and Kuc 2000). Wide beams, even if produced intermittently, are thought to assist in ground tracking while the bat is otherwise focused on targets of interest directly ahead (Ghose et al. 2007; Kuc 2011; Zhuang and Müller 2006, 2007).

In contrast, Vanderelst et al. (2012) found that these same furrows (*R. rouxi*) focus the FM portion of the beam rather than expand it in the vertical dimension (Zhuang and Müller 2007). Vanderelst et al. (2012) also argued that these furrows affect the acoustic near field in the same way that building a taller lancet might—that is, getting a narrower beam from a smaller, albeit highly modified, aperture. These contrasting results could be due to small differences in the specimens used in the construction of their virtual models (e.g., size and sex of the source head) or perhaps be the result of distortion during preservation of the original specimen (Müller 2010) or perhaps in regard to their assumptions about nostril position or leaf orientation in flight. It could also be that the bats can use their noseleaf musculature to reconfigure the leaf shape such that the cells could have a different effect. Clearly, a taller lancet would be a more pragmatic solution (from an embryological perspective) as the elaborate cavities and furrows seem extravagant by any measure (see above; Bullen and McKenzie 2008).

In *Hipposideros*, the posterior leaf does not assume the upright triangular form that characterizes the relatively delicate rhinolophid lancet. Instead, it is considerably fleshier and exhibits a more rounded, arcuate outline (coronet) when viewed anteriorly. Its anterior surface is often concave and may be divided into shallow cells separated by vertical septa. The posterior leaves of other hipposiderids are more delicate and quite ornate (*Rhinonicteris*, *Triaenops*). There are no obvious sellae in these bats. The nostrils lie at the bottom of a central facial hollow surrounded by the horseshoe.

The relative simplicity of the spear in phyllostomid bats has been shown to limit beam spreading in the vertical direction, e.g., *Carollia perspicillata* (Brinkløv et al. 2011; Hartley and Suthers 1987), *Micronycteris microtis*, and *Phyllostomus discolor* (Vanderelst et al. 2010a, b). Beam widths in these three bats are comparable, but not surprisingly, the beam would seem better focused in the gleaning insectivore *Micronycteris* (Vanderelst et al. 2010a). Several aspects of noseleaf structure, size, and shape have been tied to foraging behavior and diet in phyllostomid bats (Wetterer et al. 2000), but the function of the phyllostomid noseleaf may lie equally in the manner in which returning echoes pass around the head to the pinnae, i.e., the head-related transfer function (De Mey et al. 2008; Reijniers et al. 2010; Vanderelst et al. 2010a, b; Feng et al. 2012).

4.7.3 Sella

Morphological variation in rhinolophid sellae is remarkable, but they are apparently absent in hipposiderids and arguably insignificant in those few phyllostomids that posses them.

Since the different components of the noseleaf operate in close proximity to each other to shape the acoustic near field, interactions between their acoustic effects are likely. An example of this was found in the interaction between the cavities of the lancet and the sella in the rufous horseshoe bat (Zhuang and Müller 2007). Due to its position, the ultrasonic waves emitted through the nostrils must diffract around the sella before they can trigger a resonance inside the lancet cavities. The ability of a wave to diffract around an obstacle depends on the ratio between wavelength and obstacle size. Low frequencies with longer wavelengths are better suited for propagation into the space behind an obstacle than higher frequencies associated with shorter wavelengths. This dependence of diffraction and shadowing on wavelength influences the interaction between the lancet cavities and the sella in terms of the beam pattern. Not only is the resonance of the furrows much stronger for the lowest frequencies in the FM portion of the call, but the higher CF frequency band is attenuated by the sella. Hence, the interaction between the two effects sharpens the differences between high and low frequencies (Müller 2010; Zhuang and Müller 2007).

4.8 Movement of the Noseleaf and Head Orientation

Echolocating bats can readily insonify a target by simply aiming the head at the target. However, nasal-emitting bats have the option of steering the beam independent of head movement, via gross movements of the noseleaf or the differential positioning of the nostrils within the noseleaf. Early work done on noseleaf function assumed a static baffle geometry; however, noseleaves are dynamic structures. Voluntary control over the associated facial musculature allows a bat to orient the leaf and to coordinate its movement with pulse emission (Feng et al. 2012; Göbbel 2000; Vanderelst et al. 2010a, b).

4.8.1 Rhinolophids

In rhinolophids, noseleaf displacements are not vibrations at the ultrasonic carrier frequency, i.e., they are not involved with sound radiation per se (contra Kuc 2010). Instead, during the emission of a pulse by *Rhinolophus ferrumequinum*, the outer rim of the horseshoe twitches forward and inward to decrease the aperture of the noseleaf and increase the curvature of its surfaces. This distortion is significant and may amount up to ¼ of a wavelength in the CF portion of the call. These nonrandom motions are not present in all recorded pulses and can apparently be switched on or off. Displacement amplitudes are significant in comparison with the overall size of the horseshoe and the sound wavelengths, but the measured velocities of the noseleaf are too small to induce Doppler shifts of any significance (Feng et al. 2012).

4.8.2 Phyllostomids

Phyllostomid noseleaves vibrate in concert with call emission as well (Hartley and Suthers 1987). At the time of this writing, the authors are not aware of studies being performed on phyllostomids in parallel to those done by Feng et al. (2012). However, numerical models have shown that different positions of the phyllostomid noseleaf may effect significant changes in the outgoing signal (Vanderelst et al. 2010a, b). As such, future attempts at understanding the function of noseleaves will have to incorporate a dynamic component and some novel approaches.

4.8.3 Head Aim

Given the importance of head orientation in terms of effective target illumination and subsequent acquisition of the echo, we can assume that the mutual alignment of the noseleaf, pinnae, inner ear, and general posture of the head should each convey some level of efficiency to the system. Arguably, there should exist some "null" head posture within each emission type (oral or nasal) about which the bat could actively scan. But is there any consistency amongst studies in regard to the quantifying what is meant by head posture, noseleaf orientation, and beam direction?

Using a variety of experimental approaches, several studies have related "head aim" with the subsequent illumination of a target in several species of bat, including *Myotis* (Surlykke et al. 2009b; Surlykke and Kalko 2008), *Eptesicus* (Surlykke et al. 2009a; Ghose and Moss 2006; Ghose et al. 2007), *Carollia* (Brinkløv et al. 2011; Hartley and Suthers 1987), *Macrophyllum* (Weinbeer and Kalko 2007), *Micronycteris* (Vanderelst et al. 2010a), *Phyllostomus* (Firzlaf and Schuller 2003), *Rhinolophus* (Schnitzler and Grinnell 1977), and *Rousettus* (Yovel et al. 2010). Despite their intrinsic value, the results of these various efforts can be difficult to reconcile as each is limited by unique methodological constraints.

In broad strokes, the accurate estimation of the diameter/orientation of the nares can be adversely affected by preservation artifacts in the original specimen or by inaccurate assumptions about the physiology of the head/noseleaf in a live animal. Data from intact (or mostly intact) live animals may in turn be biased by limitations imposed on bat behavior by restraint or sedation. In an attempt to register the axis of an emitted call, several studies have utilized the position of external landmarks as a proxy for the orientation of the vocal tract or head. However, such landmarks are unreliable due to the dynamic nature of the skin and tissue preservation. There is no obvious reconciliation. However, future studies that relate the soft tissues of the vocal tract, noseleaf, and pinnae with specific osteological landmarks of the skull would permit a most welcome integration of several fields of study.

4.8.4 Head Aim and Body Posture

Still photographs and high-speed video recordings of bats in flight substantiate the general dichotomy between oral- and nasal-emitting bats in terms of head posture. Many echolocating bats exhibit gross movements of the head about the body during slow flight, presumably scanning their environment. In directed flight or pursuit, bats keep the "head" aimed directly at a target and the body subsequently follows. Video and photographic data reiterate the great range of body posture exhibited by bats in different flight profiles: commuting, trolling, attack, moving through heavy clutter, on approach, or departure from roost/obstacle/target. Assuredly, body posture is related to flight dynamics (velocity, body size, forearm length, wing shape) and taxonomy. As before, technological difficulties limit our understanding of each of these behaviors, but where possible, a more integrated approach is desirable in the future.

4.9 Evolution of Nasal-Emission and the Yinpterochiroptera

Despite the wealth of morphological and behavioral data at hand, and acknowledging the derivation of powerful numerical and phylogenetic models by which we can process this data, much of what we think we know about the evolution of bats, let alone nasal-emission in bats, relies on a good number of assumptions regarding the *emergence* of form. Fortunately, this last decade has witnessed giant steps forward in the field of developmental genetics. As such, the driving force behind the evolution of morphological novelty in bats will likely not be found in some gene sequence, rather it will be from an understanding of developmental timing and the sequential activity of regulatory genes (Carroll 2005; Cretekos et al. 2005, 2007; Davidson 2006; Göbbel 2000, 2002a; Hallgrìmsson et al. 2007; Hockman et al. 2008; Morsli et al. 1999; Müller and Newman 2005; Radlanski and Renz 2006; Sears et al. 2006; Willa and Rubinoff 2004; Wang et al. 2010).

Nevertheless, recent reconstructions of bat phylogeny continue to rely on an ever-increasing volume of gene sequence data drawn from throughout the genome. This, despite the often considerable discordance between these molecular trees and those generated using morphological/fossil data (Giannini and Simmons 2007; Simmons and Geisler 1998; Springer et al. 2001b). Gene-based phylogenetics has suggested that nasal-emission evolved independently four different times (Rhinolo phidae+Hipposideridae, Megadermatidae, Nycteridae, and Phyllostomidae) and that the non-echolocating pteropodids and the highly sophisticated nasal-emitting rhinolophids and hipposiderids are closely related to each other within the somewhat contentious Yinpterochiroptera. Even if released from the morphological strictures of ultrasonic echolocation (Giannini and Simmons 2012), there is little or nothing about pteropodids (jaw suspension, hyoid suspension, dentition, brains, cranial development, cranial vasculature, neuroacoustic systems, flight musculature, thoracic compliance, or reproductive biology) that would support such a relationship (references in Pedersen and Timm 2012).

The evolution of functional genes that are directly/indirectly associated with vision and hearing in bats is of great interest. Unfortunately, our current understanding of the evolution of color vision in bats (Müller et al. 2007, 2009; Wang et al. 2004; Zhao et al. 2009) and arguably hearing (Li et al. 2007, 2008; Liu et al. 2011) is limited to a relatively small number of taxa which may explain why these data are often inconsistent with the species trees in regard to the status of Yinpterochiroptera.

It may also be that in our search for evolutionary patterns, we often confound correlation with causation/emergence which in turn may exacerbate the discordance between molecular and morphological trees.

4.10 Why Nasal-Emitting Bats?

From an evolutionary perspective, nasal-emission has been a successful innovation—over one-third of the extant microchiropteran species are nasal-emitters; approximately 190 are phyllostomids, 170 are hipposiderids and rhinolophids, and another 20+ from the apparently less successful megadermatids and nycterids. We have made progress in understanding various parts of the nasal-emitting system, yet, as to the question of why there are nasal-emitting bats in the first place, we have little but conjecture to offer.

Could the advent of nasal-emission have been driven by something so simple as the extent to which some early bats used their mouths? After all, an echolocating predator would have difficulty flying and orienting simultaneously with a large prey item in its mouth. Even if nasal-emission was used intermittently, noseleaves would have evolved in quick succession so as to restore directionality to the nasally emitted calls. Oral-emitting bats can increase the directionality of their calls by opening their mouths wider (larger aperture, narrower beam width; Surlykke et al. 2009a). As such, one might predict that directional selection would quickly drive the dimensions of a nascent noseleaf to match the minimum dimensions of the aperture that it was replacing (mouth). This prediction is readily testable, e.g., is the diversity in noseleaf morphology among the animalivorous phyllostomines a derived (Wetterer et al. 2000) or a basal character within the family (Baker et al. 2003)?

Noseleaves seem to be a corequisite of effective nasal-emission, but beyond that, the mechanism of beamforming and the differential application of call dynamics (FM, high duty cycle CF, qCF) seem to be a matter of behavior and foraging ecology (Fenton, Chap. 3). Only in the resonator-equipped rhinolophid and hipposiderid bats do we see significant morphological changes in the rostrum that would impose a relatively fixed effect on the emitted call (i.e., a band-pass filter for the second and third harmonics). Such resonators are intimately tied to the production of loud high duty cycle CF calls. Could it be that by adopting/co-opting such resonators, these bats reduced one aspect of behavioral complexity which then set the stage for experimentation with noseleaf form and function? It remains to be seen as to what extent the organization of the rostrum in fossil bats tell us about the evolution of nasal-emission and noseleaves (Hand 1998; Hand and Archer 2005).

In the future, we face great challenges if we hope to integrate studies of behavior, physics, and embryogeny. It may be even more difficult to determine the extent to which these things augment, compensate, or conflict with each other on the evolutionary stage. However, in the last decade, dramatic increases in computing power have led to rapid advances in X-ray microtomography, access to which has become almost commonplace. The generation of increasingly powerful numerical models has helped us better evaluate the physical limitations and continuity of form—we are beginning to quantify function where once only ornamentation had been perceived.

Acknowledgments We would like to acknowledge the financial support of Rolf Müller from the following agencies: National Natural Science Foundation of China, Shandong Taishan Fund, Shandong University, NSF (Award Id 1053130), US Army Research Office (Grant Number 451069), and National Aeronautics and Space Administration (Grant Number NNX09AU54G). We further acknowledge Dane Webster for his rendering of *Hipposideros* (above). We extend a well-deserved thank you to several graduate students who have worked in the Müller laboratory: He Weikai, Yan Zhen, Lu Hongwang, and Gao Li.

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Chapter 5
Bat Facial and Lip Projections: Unique Integumentary Morphology

Gary G. Kwiecinski, James D. German, and J. Timothy Cannon

Abstract We examined the morphology of chin and lip facial projections of seven species of Phyllostomid and Noctilionid bats utilizing scanning electron microscopy and light microscopy of immunohistochemical and stained paraffin-embedded sections. Results showed that lip and chin facial projections were composed of glabrous skin except for some chin projections of *Noctilio leporinus* that were composed of haired skin. All projections contained a rich and diffuse concentration of nerves that were associated with dermal papillae and epidermal rete pegs that contained Merkel cell–neurite units and a diffuse network of epidermal free nerve endings. The existence of physically extended anatomical structures that contain a variety of receptor sensory units and their associated modalities must be important to be present throughout this diverse group of bats. These facial projections include a unique neural arrangement, but their specific functions remain unknown.

5.1 Introduction

Of the 18 families of bats recognized by Simmons (2005), the family Phyllostomidae (leaf-nosed bats) is by far the largest (55 genera out of 202, 160 species out of 1,116—Wilson and Reeder 2005). The Phyllostomid bats are well known for their nose leaf apparatus (nose leafs and lancet) and patterns of facial projections (aka warts, verrucae, facial projections, bumps, swellings, or excrescences located on lips and chin). The nose leaf apparatus of Phyllostomid bats is a morphological adaptation for enhancing and directing nasally emitted ultrasounds useful in acoustic orientation (Pye 1986). The patterns of facial projections, however, have not been investigated rigorously; their characterization has been minimal anatomically, and

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_5, © Springer Science+Business Media New York 2013

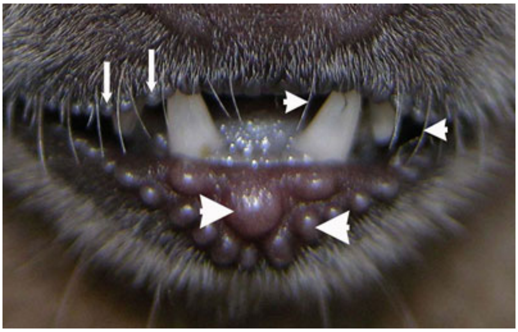

Fig. 5.1 Photograph of *A. jamaicensis* face with prominent chin and lip facial projections. *Thin arrows* distinguish lip projections, *large arrowheads* distinguish glabrous chin projections, and *small arrowheads* distinguish long sinus hairs (vibrissae) from numerous surrounding, shorter, terminal hairs of fur

no function has been attributed to these structures. The existence of these facial projections has been recognized by natural historians, for example, as identification features (e.g., the original description of *Phyllostomus verrucosum* by Elliot 1905), and they have been used as taxonomic characters in Phyllostomid taxonomy (Wetterer et al. 2000). The purpose of this investigation was to characterize light microscopic analyses of these unique structures for the first time.

The facial projections in question are paired chin pads or single chin pads with or without clefts, chin papillae, chin flaps, and upper and lower lip papillae (Fig. 5.1) at the vermillion border with haired skin. For representative figures of variations in facial projections among Phyllostomids, see Wetterer et al. (2000). There is no known sexual dimorphism associated with the chin and lip projections, and these features are very similar within a genus but vary between genera (Wetterer et al. 2000). In the past, the facial projections had been characterized as glandular structures and pads of connective tissue and muscle without discreet morphological characterization (Dalquest and Werner 1954; Harrison and Davies 1949; Quay 1970). Facial projections, which are widely distributed (known to be present in the Phyllostomidae, Noctilionidae, and Mormoopidae), are apparently conserved, albeit variable, structures based on their presence in many species. These structures have not yet been associated with any particular function; they may allow bats to better exploit resources or amplify aspects of sociality. Interestingly, Silva-Toboada and Pine (1969) noted internal labia papillae from specimens preserved in alcohol as characteristic of the Stenodermines. These internal labial papillae were further characterized as limited to the internal lip line or as covering most of the inside of cheeks (Wetterer et al. 2000). Here, we show that the internal labial papillae are morphologically continuous with the external lip projections, and together they form a unistructural anatomical entity.

Weiss (1990) investigated facial projections in routine histological, light microscopic immunohistochemical, and scanning electron microscopic studies of three Phyllostomid species. The unpublished results indicated the projections consisted of glabrous skin with numerous nerve fibers and free nerve endings. Nerve fibers in projections were found to terminate freely in the epidermis and at the dermal–epidermal junction, many in association with epidermal Merkel cells juxtaposed at this junction. Since nerve fibers and Merkel cells were prevalent in facial projections, it was the conclusion of Weiss (1990) that these projections most likely provide an increased surface area for sensory reception. In this project, we attempt to characterize morphologically the nature of facial projections in six Phyllostomid bats and one Noctilionid bat with the ultimate goal being to attribute function to these conserved and widespread features. We report characterization of various facial projections by routine paraffin-embedded light microscopic histological, by light microscopic immunohistochemical, and by scanning electron microscopic methods. Our methods were similar to those of Weiss (1990), but whereas Weiss examined three Phyllostomid species, we examined six Phyllostomid species and one Noctilionid species. *Artibeus jamaicensis* was the only species in our study in common with the study by Weiss. Our working hypothesis was that the facial projections have a sensory function and that the projections provide increased surface area for concentrating epithelial-associated sensory structures and free nerve endings instead of typical epidermal content and epidermal derivatives.

5.2 Materials and Methods

Tissues from 21 bats were examined: *A. jamaicensis* ($n=7$), *Ardops nichollsi* ($n=2$), *Brachyphylla cavernarum* ($n=2$), *Carollia perspicillata* ($n=1$), *Noctilio leporinus* ($n=2$), *Phyllostomus discolor* ($n=3$), and *Sturnira lilium* ($n=4$). With the exceptions noted below, ongoing field studies in West Indian islands by Gary Kwiecinski provided field-caught bats that were euthanized, preserved, and transported according to approved University of Scranton IACUC protocols #8-03 and #1-08. The remaining tissue samples were obtained from specimens of captive colonies (*C. perspicillata* obtained from Rick Adams, University of Northern Colorado, and *P. discolor*, The University of Scranton).

Whole bats were fixed in neutral phosphate-buffered formalin. Excised tissues were fixed in either 4 % glutaraldehyde in 0.05 M cacodylate buffer with 1 mM $CaCl_2$ (Phillips 1985) or 0.05 M cacodylate-buffered 5 % glutaraldehyde/4 % paraformaldehyde (Karnovsky's fixative—Electron Microscopy Sciences, Hatfield, PA). After being held in fixative for 1–7 days at 4 °C, the bats or tissues were washed in running tap water and transferred to 70 % ethanol or 0.05 M cacodylate buffer for shipping to the laboratory if field collected. All specimens for microscopic studies were stored at 4 °C, in 70 % ethanol or in cacodylate buffer.

Tissue samples from the lips and chins of the bats obtained were subsequently processed for examination using routine paraffin histology, immunohistochemistry, and scanning electron microscopy techniques. Observations of whole or half lips and chins were made using a scanning electron microscope (SEM) from stored fixed tissues. Fixed tissues were dehydrated to 100 % ethanol for 24 h. Dehydrated tissue samples were critical point dried and sputter coated (SC7620 Mini Sputter Coater/Glow Discharge System, Quorum Technologies, Kent, Great Britain) with gold under 100 mTorr for 120 s in an argon chamber prior to observation with the SEM. An ISI-ABT Scanning Electron Microscope (Model SX-40A, ISI, Brno, Czech

Republic) with a PGT Omega Digitizer (Model 0S16-I011, Omega Engineering, Inc., Stamford, CT) was used to obtain digital images. We utilized GW Electronics, Inc. Printerface for Windows (Oconomowoc, WI) to capture images.

For routine histological processing, selected areas of upper and lower lips and chins were removed from whole animal specimens, being careful not to damage the facial projections, and these removed areas were placed into fresh 70 % ethanol, while the remaining tissues were replaced into their respective solutions and stored at 4 °C until needed. Excised tissues were dehydrated to 100 % ethanol, cleared in chloroform, and infiltrated with melted paraffin (melting point 56 °C, Fisher Scientific, Pittsburgh, PA). Tissues in paraffin blocks were mounted on wooden blocks designed to fit a chuck/block holder for an AO 820 (American Optical Co., Buffalo, NY) rotary microtome fitted with a steel blade. Sections (6–8 μm) were mounted serially on glass slides subbed with VECTABOND (Vector Labs, Santa Cruz, CA), dried on a hot plate at 40–45 °C, and stained.

Histological stains employed included hematoxylin and eosin (H&E), Masson's trichrome, toluidine blue, eosin and methylene blue, cresyl violet, luxol fast blue, Fontana–Masson, orcein and methylene blue, periodic acid-Schiff, or aldehyde fuchsin (Bancroft and Stevens 1977; Presnell and Schreibman 1997), and Bielschowsky's silver stain for nerve fibers, Bielschowsky's silver stain for nerve fibers with a nuclear fast red counterstain for nuclei, and Bielschowsky's silver stain with a light-green counterstain for collagen (Luna 1960). Stained slides were preserved permanently by cover slipping with Permount (Sigma Chemical, St. Louis, MO) mounting medium. Examination of the slides included the use of an Olympus BH2 light microscope, and digital images were captured with a fitted SPOT 7.2 Color Mosaic camera and accompanying software (Spot RTKE, Diagnostic Instruments, Sterling Heights, MI).

Selected tissue samples stored in 70 % ethanol or 0.05 M cacodylate buffer were embedded in paraffin, sectioned, and analyzed with a modified immunoperoxidase method (Naish 1989) utilizing VECTASTAIN Elite ABC Kits (Vector Labs, Santa Cruz, CA). As described above for histological preparations, microtome cut sections were mounted on glass slides and allowed to dry. Dried slides with sections were deparaffinized, hydrated, and washed with a 0.1 % hydrogen peroxide/pH 7.4 phosphate-buffered saline (PBS) solution (Fisher Scientific, Pittsburgh, PA) for 30 min. The slides were then washed in PBS three times for 5 min each, followed by incubation for 30 min in blocking solution from the VECTASTAIN Elite ABC Kit following the manufacturer's instructions. The slides were subsequently washed in PBS three times for 15 min each and incubated in primary antibody solution made in rabbit [anti-neuron-specific enolase (a-NSE), at a dilution of 1:100 to 1:200 (Calbiochem, Santa Cruz, CA); anti-vasoactive intestinal peptide (a-VIP), at dilutions of 1:100 to 1:200; and anti-neurofilament 200 (a-NF), at dilutions of 1:50 to 1:1,000 (Sigma-Aldrich, St. Louis, MO)] for 2–7 days at room temperature. Slides were then washed in PBS three times for 15 min each, followed by incubation with the secondary antibody solution (anti-rabbit, VECTASTAIN Elite ABC Kit, following the manufacturer's instructions) for 1 h. Slides were then rinsed and incubated in the ABC peroxidase reagent solution (VECTASTAIN Elite ABC Kit) for 60 min and washed in PBS three times for 15 min. A solution of diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) was added until a sufficient brown color was attained (approximately 4 min). The slides were then washed in PBS three times for 15 min, rinsed twice in distilled water, and mounted with VectaMount water-soluble mountant (Vector Labs, Santa Cruz, CA). Acting as negative controls, normal rabbit serum (pre-immune) was used at dilutions of 1:100 in place of immunized primary antisera on adjacent slides of serial sections. Slides were examined with an Olympus BH2 light microscope, and digital images were captured with a fitted SPOT 7.2 Color Mosaic camera and accompanying software (Spot RTKE, Diagnostic Instruments, Sterling Heights, MI).

5.3 Results

Lips and chins of all species, with the exception of *P. discolor*, were examined by scanning electron microscopy (SEM). The extent and shapes of facial projections and presence or absence of internal papillae were revealed by these analyses. Facial projections for all bats lacked hairs and were without any obvious external evidence of epidermal appendage, except for *N. leporinus*. The SEMs revealed the cornified nature of epithelial surfaces of glabrous projections by the presence of sloughing keratinocytes. Some projections had concavities that possessed a reticular network appearance (Fig. 5.2a, b), but these were not consistent from specimen to specimen or between species. Higher magnifications revealed various types of holes and portals in facial projection surfaces, but they were not consistent from one projection to the other or between individuals of the same species. All other bats including *A. jamaicensis*, *A. nichollsi*, *B. cavernarum*, *C. perspicillata*, *P. discolor*, and *S. lilium* had lip and chin projections that were glabrous. The surfaces of posterior chin projections of *N. leporinus* were not comparable to those of the Phyllostomid bats investigated in this study. For *N. leporinus*, the chin consisted, in part, of glabrous projections appearing as flaps and folds that were contiguous with the lower lip and, in part, by haired chin folds or projections, posterior to those connected to the lower lip. The posterior projections were covered with a sparse population of terminal hairs (predominant, contain hair and follicular sheath), and each projection contained vibrissae (elongated hair and follicular sheath surrounded by an encapsulated blood sinus). Low-magnification SEMs demonstrated clearly the continuous nature of external lip projections and internal labial papillae (Fig. 5.2c, d) for those species that possessed internal labial projections that were contiguous with external projections (all species examined except *B. cavernarum* and *N. leporinus*). The internal labial papillae were conical, triangular, or rectangular. There were no internal projections associated with lips or cheeks in *B. cavernarum*. Sparsely located, rectangular-shaped internal labial papillae of *N. leporinus* were found inside the lower cheek and were not associated with the lips.

Through routine paraffin-embedded histological analysis of cut sections, the glabrous-appearing facial projections for all species (except *N. leporinus* chin

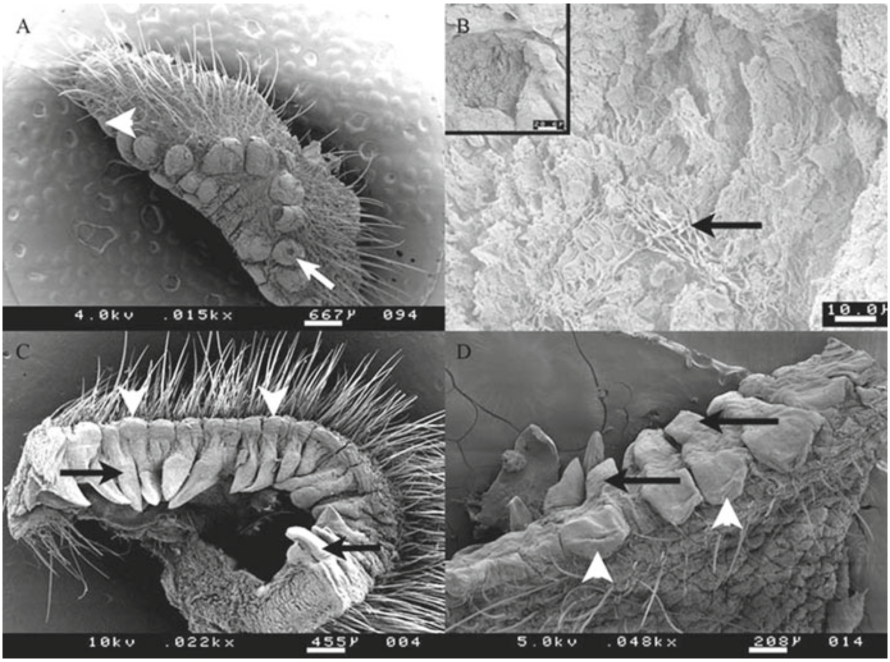

Fig. 5.2 Scanning electron micrographs of lips from *B. cavernarum* (**a**, ×15, and **b**, ×1,000 with inset ×350) and *A. jamaicensis* (**c**, ×22 and **d**, ×48). Note lip projection (*arrowhead*) and chin projection at *arrow* in (**a**) have a concavity; that concavity is magnified in inset of (**b**) and further magnified in (**b**) to reveal a reticular-like network (*arrow*) at the surface of concavity. Buccal internal papillae (*black arrows*) and lip projections (*white arrowheads*) and their continuities are shown in (**c**) and (**d**)

projections) were confirmed as being hairless skin with little epidermal variation between lip, chin, and internal papillae. The posterior portions of chins from *N. leporinus* contained a series of complex folds of haired skin, with each fold containing at least one vibrissa (Fig. 5.3). Since the posterior chin projection skin of *N. leporinus* was not different from the haired skin of all other bats examined, except there being fewer follicles and they contained vibrissae, the results that follow pertain to all projections from all bats examined except the posterior chin projections of *N. leporinus*. The glabrous lip projections of all bats examined were composed of a keratinized stratified squamous epidermis and a collagenous-rich and cellular dermis (Fig. 5.4a). The epidermal stratification clearly displayed a stratum basale resting on a PAS+ basal lamina. A stratum spinosum, a stratum granulosum, and a keratinized stratum corneum were consistently present. A sloughing stratum corneum was commonly found, especially on chin pads. The epidermal thickness of projections was greater than adjacent haired skin region by four to six cell layers or more. The junctions between the epidermis and dermis of facial projections were scalloped with well-developed epidermal rete pegs and dermal papillae (Fig. 5.4b). The epidermal portions of the projections also contained other cellular elements

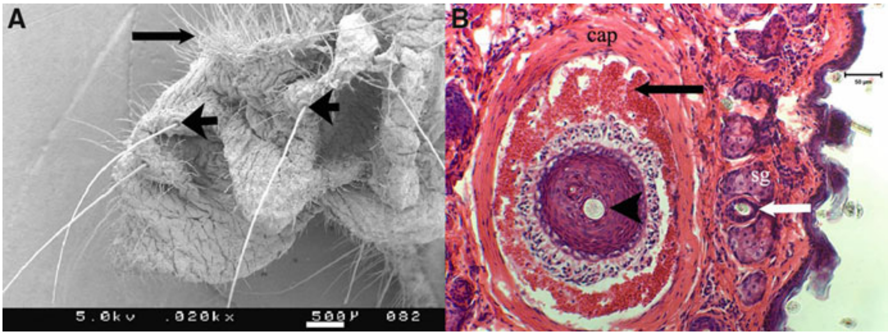

Fig. 5.3 (**a**) Scanning electron micrograph of chin projections from *Noctilio leporinus* (×20) distinguishing sinus hairs (*arrowheads*) from terminal hairs (*thin arrow*). (**b**) Histological section of skin contrasting a sinus hair and its follicle (*black arrowhead*) from a terminal hair (*white arrow*) with its follicle and associated sebaceous glands (sg). Note the much larger size and complexity of sinus hair follicle and its associated structures including the blood sinus (*black arrow*) and sinus capsule (cap)

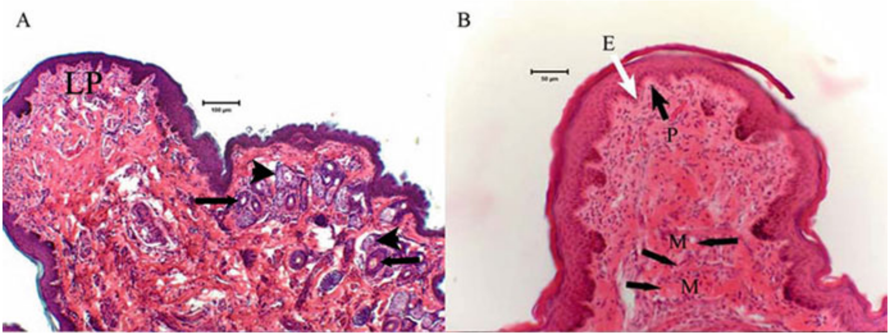

Fig. 5.4 Light photomicrographic representatives of lip and chin projections. (**a**) Low magnification (×143) of lip projection and haired skin junction from *A. jamaicensis*. Note lip projection on *left side* of micrograph lacks hair follicles (*thin arrows*) and sebaceous glands (*arrowheads*) present in haired skin on *right side* of micrograph, H&E stain. (**b**) Higher magnification (×286) image of chin projection from *A. jamaicensis* highlighting aglandular and glabrous nature of papillae, with an epidermal rete peg (E *arrow*) and dermal papillae (P *arrow*) labeled. *Smaller arrows* at M point to skeletal muscle elements, H&E stain

commonly found in mammalian epidermis, including melanocytes and their extracellular product melanin between keratinocytes, Langerhans cells (wandering immune cells), and Merkel cells (somatosensory-associated cells). In H&E- or toluidine blue-stained sections, Merkel cells were concentrated in the stratum basale; particularly dense populations were in the epidermal rete pegs along with melanocytes and pigment, while halo-appearing Langerhans cells could be found within epidermal layers above the stratum basale.

A constant, readily observed feature of all projections was the absence of epidermal appendages: no hair follicles, no sebaceous glands, and no sweat glands. Hair follicles with sebaceous glands, but no sweat glands, were found in skin adjacent to projections (Fig. 5.4a) in all species examined. The dermis of projections (lip, chin, and internal papillae) consisted of a rich collagenous matrix, and an elastic fiber network was found in the reticular layer, but not the papillary layer. The dermis contained an unusually rich cellularity compared to adjacent haired skin areas, containing cellular profiles typical of dermal fibroblasts, but many more cells with a rounded or oblong profile, unlike typical flattened fibroblasts. An especially rich dermal network of nerve bundles were observed in routine H&E-stained sections, were confirmed by silver and immunohistochemical staining, and are discussed further below. The dermis of chin projections contained skeletal muscle fibers, with many oriented parallel or oblique to the skin surface (Fig. 5.4b). The collagenous matrix of the dermis contained the usual network of blood vessels and other typical connective tissue elements, including mast cells, lymphocytes, and polymorphonuclear leukocytes, but a paucity of adipocytes, except for the internal papillae of *N. leporinus*. Adipocytes were routine dermal constituents of *N. leporinus* internal labial papillae. Within the reticular dermis, there was an unusual appearance of some small arteries, similar to periarterial lymphatic sheaths (sheathed arteries) typical of the spleen (Fawcett 1994; Sasou and Sugai 1992). Meissner's corpuscles were not observed in the dermal papillae of projections nor were Pacinian corpuscles or any other encapsulated sensory organs found in the dermis of any species examined.

Analysis of the facial projections by various methods demonstrated a rich dermal nerve fiber content and many intra-epidermal nerve fibers originating from the dermis. The fibrous array of nerves was demonstrated further by immunohistochemical staining with antibodies specific for nerve cells. Epidermal and dermal nerve fibers and cell bodies (or cells with nuclei) were positively stained for immunoreactivity in facial and chin projections and were greater in number than those observed in surrounding haired skin or oral mucosa. In the dermal papillae of facial projections, many free nerve endings were found, and no corpuscle-like endings were located. In silver-stained and a-NSE preparations, many nerve endings appeared to abut the basal lamina, while others appeared to penetrate into the epidermis. The nerve fibers in the dermis appeared to be ensheathed by supporting cells (Fig. 5.6a), which also appeared to be stained by a-NSE (Fig. 5.5c).

Individual facial projections contained an array of nerve fibers (Fig. 5.6a), some of which terminated at the basal layer of the epidermis, while others were observed coursing through the epidermis (Fig. 5.6b). The array of intra-epidermal nerve fibers was a consistent finding in projections from all species examined. These fibers were not visible in routine H&E-stained sections but were demonstrated by silver and immunohistochemical staining. Silver staining showed some nerve fibers traversing the entire epidermis to the surface (Fig. 5.6b); this was confirmed by immunohistochemical staining with a-NSE that also demonstrated trans-epithelial nerve fibers terminating at the surface (Fig. 5.5b) as well as many nerve fibers terminating at various levels within the epidermis. Some intra-epidermal nerve fibers were observed to intertwine with varicosities, while other fibers terminated within the epidermis with knobbed or expanded endings (Fig. 5.5a, d). The intra-epidermal fibers with knobbed terminals were most prominent in strata nearer the surface rather than in basal strata.

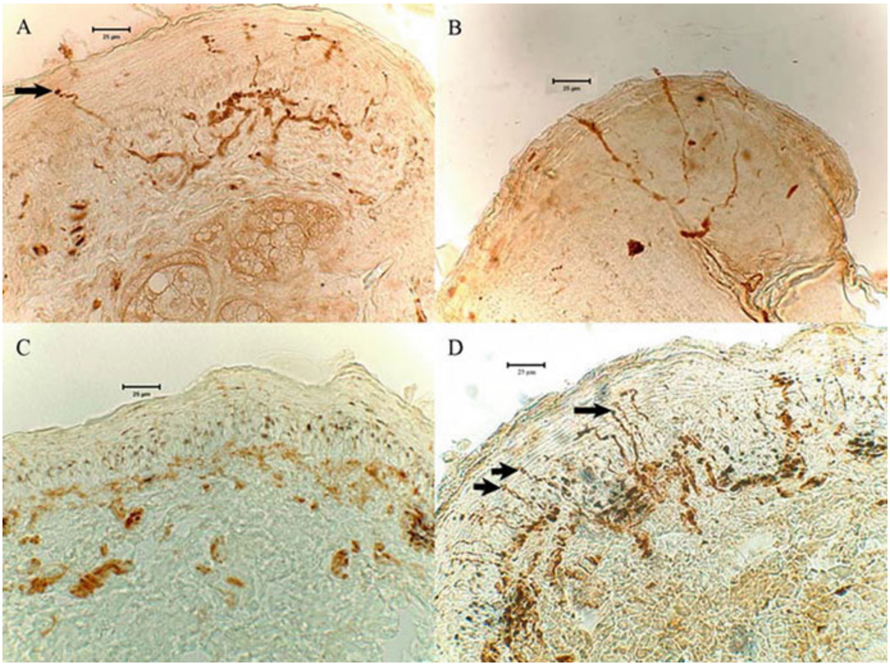

Fig. 5.5 Staining of neuronal elements by DAB (gold/brown color) localization of antineuron-specific enolase (a-NSE) as described in methods, ×572. (**a**) Section through facial projection of *Brachyphylla cavernarum* with a-NSE activity in intra-dermal and intra-epidermal nerve fibers. Note knobbed nerve fiber terminus (*arrow*). (**b**) Section from facial projection of *Sturnira lilium* showing trans-epidermal nerve fibers. (**c**) Section from facial projection of *Artibeus jamaicensis* showing abundant a-NSE activity at the epidermal–dermal junction. (**d**) Section from facial projections of *Artibeus jamaicensis* with epidermal and dermal a-NSE activity. Note knobbed nerve fiber termini (*arrows*) in epidermis and abundant a-NSE activity at epidermal–dermal junction associated with epidermal rete pegs

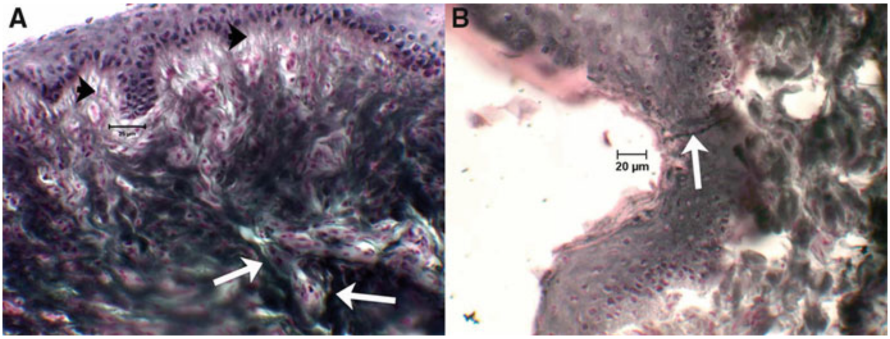

Fig. 5.6 (**a**) Bielschowsky's silver stain for nerve fibers highlights rich dermal innervation (*white arrows*, nerve bundles), particularly free nerve endings at the epidermal–dermal junction (arrowheads), ×243. (**b**) Trans-epidermal nerve fiber (*arrow*) from *Phyllostomus discolor*. Bielschowsky's silver stain, ×572

Merkel cells were also observed repeatedly in routine sections of facial projections and were stained positive with a-NSE but not with a-VIP. Merkel cells associated with external root sheaths of vibrissae and terminal hairs in haired skin were stained positive by a-VIP. Merkel cells of facial projections were distinctly concentrated in the stratum basale layer of the epidermis, primarily located in epidermal rete pegs. The failure to observe cytoplasmic staining in either H&E- or toluidine blue-stained sections does not validate the recognition of the Merkel cell. Subsequent positive immunohistochemical staining and demonstration by the Fontana–Masson method (Luna 1960) of neurosecretory granules inside the cell cytoplasm (Negri bodies) support our identification of these cells as Merkel cells. Because neurosecretory granules in Merkel cells when stained by the Fontana–Masson method are indistinguishable from melanin granules within melanocytes, it is difficult to discriminate between these two cell types. To resolve this problem, Merkel cells with associated Negri bodies were identified by a melanin bleaching treatment with 0.25 % potassium permanganate and 5 % oxalic acid prior to Fontana–Masson staining (Luna 1960) to eliminate confounding melanin.

5.4 Discussion

The results show that facial projections are unique structures consisting of cornified epidermis lacking epidermal derivatives/appendages but rich in nerve networks, compared to surrounding haired skin. The epidermis of the facial projections is arranged with epidermal rete pegs, forming a scalloped junction with the dermis, and rich in free nerve endings. Both dermis and epidermis contain numerous nervous elements without typical epithelial sensory structures but an abundance of free nerve endings. The dermis of facial projections was unusual in lacking glands and other epidermal derivatives and in containing many round and oblong cells in histological profiles. These dermal cells deserve further characterization by ultrastructural and cytochemical methods. The dermis of chin projections contained skeletal muscle fibers, and although we did not characterize these muscle fibers further, they may be important; for example, they may move, spread, elongate, or further elevate the projection away from the skin surface. Such skeletal muscle-powered movements may increase sensitivity or contact range of the projection. These muscle fibers need further characterization to define their role(s) in facial projection structure and function. We also noted an unusual appearance of some small dermal arteries, similar to sheathed arteries in the spleen (Fawcett 1994). These arteries, although noted, were not characterized further in this study. Although sheathed arteries are considered immunological structures (Fawcett 1994; Sasou and Sugai 1992), they are also in need of further attention in the facial projections of bats. For example, do these arteries play a role in hydrostatic control mechanisms associated with elevation/retraction of facial projections or are they associated with the integumentary immune system?

Mammalian cutaneous innervation involves a variety of morphologically distinct sensory receptors that include encapsulated mechanoreceptors, free nerve endings, and Merkel cells (Delmas et al. 2011; Iggo and Andres 1982; Oaklander and Siegel 2005). Many skin receptors are mechanoreceptors, but thermo-, chemo-, and nociceptors are also part of the sensory innervation formed by peripheral terminals of afferent nerve fibers in both haired and non-haired (glabrous) skin. Cutaneous sensory neurons also deploy a variety of specialized transducers with highly specific sensory functions and differing distributions and densities at various regions of the skin (Oaklander and Siegel 2005). The pattern of innervation within the glabrous facial projections of the Phyllostomid and Noctilionid bats examined in this study is organized around epidermal rete pegs, dermal papillae, and intra-epidermal free nerve endings. No encapsulated mechanoreceptors were found in any of the bats examined. Within these facial projections, intra-epidermal free nerve endings and clusters of epidermal rete pegs with Merkel cell concentrations appear to aggregate, forming a unique sensory organ.

Based upon SEM and histological analyses, the lip and chin facial projections of the bats examined were similar to glabrous skin without any epidermal appendages but instead contained an abundance of nerve fibers. Standard histological methods and immunoreactivity to anti-neuron-specific enolase (a-NSE) revealed that facial projection skin contained an abundance of nervous elements that were more numerous than adjacent haired skin. The pattern of innervation within the facial projections was organized around the dermal papillae, the epidermal rete pegs, and including free nerve endings that projected into the layers of the epidermis. These observations were similar to those of Weiss (1990). The neural elements were not associated with epidermal appendages, since they were absent in the glabrous facial projections. Although there were some skeletal muscle fibers in projections, there was not a rich nerve plexus associated with them. Based on the profusion of afferent terminations at their tips in the epidermis and at the epidermal–dermal junction in association with Merkel cells in epidermal rete pegs and the lack of other epidermal appendages or effectors, it is most likely that facial projections have a sensory function.

In routine paraffin-embedded tissue, we stained sections with Bielschowsky's silver stain, a technique relatively specific for nerve fibers. But this technique might also stain reticular fibers, which could be misleading. Our staining, however, appears to be neural for several reasons. Reticular fibers are shorter than nerve fibers (Fawcett 1994) and are stained purple instead of being black as is typical for nerve fibers stained by Bielschowsky's silver stain (Luna 1960). Furthermore, the extracellular fibrous matrix of the papillary dermis is normally devoid of reticular fibers except those of the basal lamina. We further confirmed the specificity of Bielschowsky's silver stain for nerves by observing a similar pattern of nerve networks by positive immunoreactivity to a-NSE.

It is difficult to differentiate Langerhans cells and Merkel cells in isolation due to their similar appearance in the epidermis when stained with H&E or toluidine blue. Although both cell types appear with a cytoplasmic halo (perinuclear negative image), they are site specific (Lacour et al. 1991). Merkel cells were found in the stratum basale of the epidermis, while the Langerhans cells could be located in all strata of the epidermis (Fawcett 1994; Satthaporn and Eremin 2001). Although some of the cells we call Merkel could be Langerhans cells in the stratum basale, the numbers of Merkel cells we observed are consistent with clusters of Merkel cells at the bases of epidermal rete pegs in lips, oral mucosa, and digital skin observed in other mammals (Halata and Munger 1983; Munger and Halata 1983).

The innervation pattern of the facial projections examined in the bats surveyed seems to be typical for glabrous skin, in that somato-neural arrangements around epidermal rete pegs and dermal papillae have been noted previously (Halata and Munger 1983; Montagna et al. 1975). Also, free nerve endings penetrating the epidermis and clusters of epidermal rete pegs as observed in this study have been observed in the noses of other mammals (Montagna et al. 1975). In tree shrews (*Tupaia glis*), moles (*Scapanus townsendii*), and opossums (*Didelphis virginiana*), expanded nerve endings and myelinated fibers form nerve nets that penetrate the epidermis up to the stratum corneum. Although reminiscent of Eimer's organs (Catana 1995) of star-nosed moles (*Condylura cristata*), the epidermal neurites of bats and other mammals tend to be longer and are densely concentrated in the center of epidermal columns that form epidermal rete pegs, and they are not as distinct or morphologically organized as Eimer's organs. Interestingly, at the central base of Eimer's organs in the star-nosed mole is a single Merkel cell–neurite complex. In the bats we examined, the Merkel cell–neurite complexes seem to be structured sensory end organs that resemble Eimer's organs. Eimer's organs consist of stacks of epithelial cells, intra-epidermal neurites, a single Merkel cell–neurite complex, and a single lamellated corpuscle (Catana 1995). The bat epidermal rete pegs contain all the elements of an Eimer's organ except the bats lack a lamellated end organ, the bat epidermal rete pegs contain numerous Merkel cells instead of one, and the bat free nerve endings are not encased within keratinocytes. We suggest that bat epidermal rete pegs with Merkel cells and free nerve endings are a variation of the Eimer's organ that is structured to increase cutaneous sensibility. Such epidermal rete pegs would be advantageous to nocturnal, routing animals whose movements are limited by vision; they would make it more aware of disturbances and obstacles that might be injurious as well as aid in food selection and transport.

Merkel cells have been extensively characterized by means of light and transmission electron microscopy in the integument of many vertebrates (e.g., Catana 1995; Merkel 1875; Tazaki and Sakada 1989; Toyoshima and Shimamura 1988). Clusters of Merkel cells have been observed in epidermal rete pegs in primate eyelids, lips, oral mucosa, and digits (Halata and Munger 1980b, 1983; Munger and Halata 1983, 1984). Merkel cells have also been found in the external root sheaths of regular and sinus hairs (Halata and Munger 1980a, b). Anti-NSE is an accepted marker of Merkel cells and has been utilized in a variety of animals to identify this cell type (Gu et al. 1981; Smoller 2002). In the present study, Merkel cells were most abundant in the bases of epidermal rete pegs in facial projections, while few, or none, were located in haired skin adjacent to facial projections as evinced by intense immunoreactivity to a-NSE. Only weak, if any, immunoreactivity by a-NSE was observed in the external root sheaths of hair follicles. The Merkel cells of external root sheaths were clearly demonstrated by immunoreactivity to a-VIP, while Merkel cells of epidermal rete pegs were weakly, if at all, immunoreactive to a-VIP. Localization of neuropeptides in mammalian Merkel cells seems to be highly variable, and their locations are also variable (Hitchcock et al. 2004; Lucarz and Brand 2007; Tachibana and Nawa 2005). For example, calcitonin gene-related peptide has been immunolocated in Merkel cells of human, pig, dog, and cat (Alvarez et al. 1988; Garcia-Caballero et al. 1989; Cheng Chew and Leung 1993), while met-enkephalin immunoreactivity has been observed in mice but not human, pig, dog, and cat (Cheng Chew and Leung 1991, 1992; Hartschuh et al. 1979, 1983). It is possible that the varied immunoreactive staining with the antibodies selected in this bat study reflects functional divergence in different groups of Merkel cells.

It is known that Merkel cells can be visualized immunohistochemically with a variety of cytokeratin and neuroendocrine markers. Controversial results have been obtained with neurofilament protein 200 kDa, which was detected in only a small percentage of Merkel cells or not at all (Moll et al. 1995; Narisawa et al. 1994; Sauret and Didierjean 1984). The cytoplasm of Merkel cells contains cytoskeletal intermediate filaments, and the low molecular weight cytokeratin CK20 is known as a specific marker for cutaneous Merkel cells (Moll et al. 1995). Our negative results of no immunoreactivity with bat tissues when reacted with a-NF, the anti-200 kDa neurofilament protein, could be because a-NF is not a consistent marker of Merkel cells or rabbit-derived antibodies for a-NF do not cross-react with bat tissues. Future identification of Merkel cells and their distribution might be better served with other Merkel cell markers such as anti-cytokeratin CK-20 and/or the neuronal cytoplasmic enzyme, protein gene product 9.5 (PGP9.5).

The Merkel cell is a neuroendocrine cell that also functions as a slowly adapting mechanoreceptor and is often observed in association with nerve endings, collectively known as the Merkel cell–neurite complex (Hitchcock et al. 2004). This complex is made of clusters of 50–70 cells in contact with the terminals of a single myelinated Aß axon (Delmas et al. 2011). They function as slow-adapting low-threshold mechanoreceptors, respond to indentation depth of skin, and have the highest spatial resolutions of the cutaneous mechanoreceptors. As such, they are purported to transmit a precise spatial image of tactile stimuli and are responsible for form and texture perception (Delmas et al. 2011; Iggo and Muir 1969; Maricich et al. 2009). Such structures would seem to fit the conditions that specialized diets of nocturnal, volant animals would require. The Phyllostomid bats as a group exploit and consume a variety of food items: plant fruits, flowers, and leaves; insects; and a variety of vertebrates including fish, frogs, and mammals (even other bats). As individuals, these bats are unusual in that their food choices are opportunistically flexible as food resources change. To be able to evaluate the safety and nutritional value of a food item quickly is essential for survival. They must evaluate, grasp, remove, and transport a prey item in a short period of time and be able to secure their investment until relocation for ingestion to avoid predation themselves. Feeding behavior would be facilitated by the precise spatial and textural characterizations that clusters of Merkel cells seem able to provide. Observing feeding behavior by high-speed cinematography may facilitate understanding the function(s) of these facial projections.

The absence of corpuscles (e.g., Meissner's) and the presence of high concentrations of free nerve endings in the facial projections of these bats are unusual. This pattern may be unique in these bats. Sensory corpuscles have been described in connective tissues of the tongue, urethra, rectum, and anus of some bat species (Quay 1970), and the sensory innervation in the bat facial projections differs from the innervation that has been typically found in other mammalian glabrous skin. The general principle that morphologically distinct receptors occupy typical and regular locations in skin has been noted in a variety of mammalian species other than bats. Meissner's corpuscles are coiled mechanoreceptors and the predominant sensory corpuscle found within dermal papillae of glabrous skin, for example, in human and primate fingers, ridged skin of rodents, primate palatal mucosa and tongue, and glabrous skin of other mammals (Delmas et al. 2011; Halata and Munger 1983). Meissner's corpuscles were also the most numerous sensory structure of the monkey lip vermilion border (Halata and Munger 1983). In light of these commonly observed innervation patterns, the absence of Meissner's corpuscles and the presence of free nerve endings in close adjacent areas and in close association with clusters of Merkel cells in epidermal rete pegs are noteworthy.

Why are there so many free nerve endings and an absence of sensory corpuscles in these bat facial projections? The meandering properties of highly arborized nerves and the small size and inaccessibility of sensory nerve endings have hampered investigations of morphological and functional studies. Several assays of cellular responses to mechanical stimulation have been developed in recent years and have begun to uncover the molecular basis of mechanotransduction (Delmas et al. 2011). The best-known mechanoreceptors in mammals are located in the skin. Cutaneous somatosensory receptors detect a wide range of mechanical stimuli, including light brush of the skin, texture, vibration, touch, and noxious pressure (Delmas et al. 2011). This variety of stimuli is matched by a diverse array of specialized or encapsulated sensory nerve endings that respond to cutaneous motion and deformation in specific fashions. Some cutaneous nerve endings are classified as low-threshold mechanoreceptors because they respond preferentially to innocuous mechanical forces, while others are considered high-threshold receptors because they are excited by injury (Delmas et al. 2011). In general, for some specialized or encapsulated nerve endings, there is a strict correlation between morphology and functional characteristics, and the complex morphology of corpuscles can be regarded as facilitating their specific mechanoreceptive function (Iggo and Andres 1982). In mammals, free nerve endings represent afferent terminals of both small, myelinated A delta fibers and unmyelinated C fibers that typically terminate in the subepidermal skin. The perception of painful stimuli is initiated by high-threshold C fibers that can be mechanosensitive or polymodal in nature (Perl 1996). Some C fibers are low-threshold mechanoreceptors that respond preferentially to innocuous tactile mechanical forces and signal a pleasant sensation in associative social body contact in humans (Loken et al. 2009; Valbo et al. 1999). It has been proposed that inflammation or trauma may change the sensation conveyed by C fiber low-threshold mechanoreceptors from pleasant touch to pain (Seal et al. 2009). Such information indicates that the functional implications of a high concentration of free nerve endings in bat facial projections do not delimit morphological constraints on their functional potentials. Without morphological characteristics that covary with the diversity of modality transductions exhibited by free nerve endings, discrete functions of free nerve endings in bat facial projections need to be determined by other means such as neurophysiological recordings.

Cutaneous sensory units with unmyelinated fibers exhibit great variation in their sensitivity to different stimuli. Sensory units can be highly responsive (low threshold) to either gentle mechanical stimuli or innocuous thermal stimuli or have a high threshold requiring a strong stimulation for activation, such as noxious heat and irritant chemicals, for example, dilute HCl or acetic acid (Delmas et al. 2011; Bessou and Perl 1969). The epidermal free nerve endings, which would fall into the category of C fibers, may be composed of different types of unmyelinated axons projecting to different locations and may be associated with different modalities. That free nerve endings offer a potentially wide range of modalities within a given skin region creates a potential site for selection of specific receptors that would be useful to the life-history constraints on an animal.

The function of the nerve associations in the facial projections of these Phyllostomid bats appears to be sensory, but the modalities cannot be determined from morphological studies. Without transmission electron microscopic, biochemical, physiological, and behavioral studies, it is impossible to discern if the nerve endings in facial projections are homoplastic or heteroplastic populations. Heterogeneous populations of neurons and receptor modalities would be advantageous for cutaneous sensation in the faces of nearly all Phyllostomid and Noctilionid bats, which are species of bats that occupy a diversity of biomes and exploit variable resources. The elevated concentrations of neuron sensory endings in facial projections allow for heterogeneous populations of neurons that would potentially be important in chemoreception and thermoreception as well as in mechanoreception. The fact that facial thermoreceptors have been identified in the common vampire bat (*Desmodus rotundus*) that function as an aid in detecting warm body prey and surface vasculature (Kürten and Schmidt 1981) is testimony to the importance of receptor diversification and plasticity in this group of bats and to the importance of behavioral and physiological correlation. We hope that these initial characterizations of bat facial projections provide a foundation for future research that will examine the interplay between form, function, and selective adaptations in neuronal plasticity.

Acknowledgements We wish to acknowledge and thank Hugh H. Genoways, Roxy J. Larsen, and Scott C. Pedersen for their help in the field collecting and preserving specimens. We thank Giuseppe Bongiorno, Daniel Herr, Paul Homnick, Jason Mannion, Rachel Metzger, Karl Oberg, and Winona Weimann for the technical assistance in the histology lab at The University of Scranton. The University of Scranton provided financial assistance through Faculty and Student Development Grants.

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Chapter 6
Bat Molecular Phylogenetics: Past, Present, and Future Directions

Ronald A. Van Den Bussche and Justin B. Lack

Abstract With the development of techniques for the isolation, amplification, and sequencing of DNA, studies addressing phylogenetic relationships among bats moved in the 1980s and early 1990s from restriction fragment length polymorphisms and DNA hybridization to examination of changes at the level of individual nucleotides via DNA sequence analysis. Coinciding with these molecular advances were increases in computational capacity and the development of sophisticated analytical models of the nucleotide and amino acid substitution processes. Thus, molecular phylogenetics moved primarily from distance- and parsimony-based algorithms to complex optimality criterion, such as maximum likelihood and Bayesian phylogenetics. These advances helped to clarify our understanding of the evolutionary relationships and biogeographic history of bats as well as the evolution of echolocation and flight. We have now entered the age of phylogenomics, where phylogenetic datasets represent genome-scale variation. With the recent explosion in available data and the ever-expanding ability to inexpensively produce massive datasets for any organism, we now have the ability to extend phylogenomic approaches to non-model organisms such as bats, which have been historically neglected in studies of molecular evolution. Bats represent an extraordinary group characterized by evolutionary novelty (i.e., echolocation and powered flight) and adaptation (e.g., seven distinct feeding strategies within the family Phyllostomidae) to an extent arguably paralleled by no other group of mammals. As we move into the era of phylogenomics, it is time for bats to move to the forefront of the study of evolutionary novelty and adaptation, which for mammals has been dominated by rodents due to the mouse and rat models being so ubiquitous in all aspects of biology.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_6, © Springer Science+Business Media New York 2013

6.1 Bat Phylogenetics from Linnaeus to 2011

Debates over the origin of bats (Chiroptera) have existed since Linnaeus (1758) placed bats within the order Primates and subsequent recognition of the superorder Archonta consisting of Chiroptera, Dermoptera, Primates, and Scandentia (Gregory 1910). Although this superordinal group was recognized by taxonomists and systematists for nearly 100 years, in the early 2000s, independent molecular studies examining mammalian ordinal relationships provided support for placement of Chiroptera within the superorder Laurasiatheria, along with Cetartiodactyla, Perissodactyla, Carnivora, Pholidota, and Eulipothypla (e.g., Lin and Penny 2001; Murphy et al. 2001a, b; Van Den Bussche and Hoofer 2004).

Even prior to this order-level work, the monophyly of Chiroptera itself was questioned. Smith and Madkour (1980) and Pettigrew (1986, 1994) proposed that the chiropteran suborder Megachiroptera was more closely related to Primates than to the suborder Microchiroptera, thereby making Chiroptera paraphyletic. Smith and Madkour's (1980) argument against chiropteran monophyly was based on penile morphology, whereas Pettigrew's (1994) arguments were based on neural anatomical pathways, and that chiropteran monophyly based on molecular data (Adkins and Honeycutt 1991; Ammerman and Hillis 1992; Bennet et al. 1988; Mindell et al. 1991; Stanhope et al. 1992) was due to higher metabolic rates in bats, relative to other mammals, and therefore a base compositional bias toward adenine and thymine in the bat genome. Pettigrew (1994) proposed the "flying DNA" hypothesis to explain the monophyly of Chiroptera based on molecular data. Subsequent examination of the level of base compositional bias in two nuclear and three mitochondrial genes, detailed statistical analyses, and simulation studies confirmed bat monophyly and illustrated that even extreme base compositional bias would not be sufficient to explain the observed levels of support for bat monophyly (Van Den Bussche et al. 1998). Subsequently, examination of about 3 kb of mitochondrial DNA sequence data from 162 taxa, representing 13 orders of mammals and most all families of bats, illustrated that support for the "flying DNA" hypothesis could only be detected under the constraint of archontan monophyly, a hypothesis not supported by their data (Van Den Bussche and Hoofer 2004).

The partitioning of chiropteran families into the suborders Megachiroptera and Microchiroptera was based on morphological characters and the assumption that bats belong to Archonta. With the generation of new molecular techniques in the mid- to late 1990s, several researchers began to question the monophyly of Microchiroptera (Hutcheon et al. 1998; Kirsch 1996; Kirsch and Hutcheon 1997; Kirsch and Pettigrew 1998). These studies detected close relationships between the microchiropteran rhinolophoids and the megachiropteran pteropodids; however, due to the perceived strength of microchiropteran monophyly, these researchers attributed the rhinolophoid-pteropodid association to some type of analytical artifact.

Teeling et al. (2002) addressed the question of microchiropteran paraphyly by phylogenetically analyzing about 7 kb of nuclear DNA sequence data from representatives of 11 chiropteran families and detected strong support for the traditionally recognized microchiropteran families Megadermatidae, Rhinolophidae, and Rhinopomatidae being more closely related to the megachiropteran Pteropodidae than to other microchiropterans. Based on these results, Teeling et al. (2002) proposed recognition of the suborders Yinpterochiroptera, comprised of the superfamilies Pteropodoidea (Pteropodidae) and Rhinolophoidea (Rhinolophidae, Rhinopomatidae, and Megadermatidae), with all remaining families comprising the suborder Yangochiroptera [but see Hutcheon and Kirsch (2006) for a discussion regarding the validity of these subordinal names].

In addition to ordinal and subordinal relationships, molecular data have also provided phylogenetic resolution among and within families of bats. For example, molecular data strongly support the close evolutionary relationship of the Old World Mystacinidae and Myzopodidae with the New World Furipteridae, Mormoopidae, Noctilionidae, Phyllostomidae, and Thyropteridae (Teeling et al. 2003, 2005; Van Den Bussche and Hoofer 2000, 2004). In addition to providing clarity to higher level taxonomic relationships, these studies have increased our understanding of the evolution of echolocation (Eick et al. 2005; Jones and Teeling 2006; Teeling 2009; Teeling et al. 2000) and the biogeographic history of these taxa (Eick et al. 2005; Teeling et al. 2005).

An excellent example of the phylogenetic resolution provided by molecular data regards relationships within the family Phyllostomidae. Miller (1907) partitioned the 51 genera of phyllostomid bats into seven subfamilies, and this classification remained fairly well accepted until the mid-1970s when considerable systematic work based on allozymic, karyological, and morphological data began on this family [see Wetterer et al. (2000) and citations therein]. Over the next 40 years, considerable disagreement existed regarding relationships within Phyllostomidae, and the number of proposed subfamilies ranged from three to eight. In the early 2000s, new morphology and molecular-based phylogenies were produced for Phyllostomidae that began to provide resolution, and possibly consensus, as to the number of genera and partitioning of genera into subfamilies (Baker et al. 2000, 2003; Wetterer et al. 2000). The most recent phylogenetic analysis based on DNA sequences from both nuclear and mitochondrial genomes provided a well-resolved and well-supported phylogeny for phyllostomid bats, statistically supporting 48 of the 55 clades, and resulted in a classification recognizing 11 subfamilies (Baker et al. 2003).

Although the examples provided thus far illustrate the ability of current molecular approaches for resolving phylogenetic relationships, it is important to note that similar approaches have not been able to resolve some long-standing issues in bat phylogenetics. Vespertilionidae is the second most specious mammalian family, with about 407 primarily insectivorous species with a nearly worldwide distribution (Simmons 2005). The fact that these bats possess little morphological variation, even among the most distantly related members of the family, has caused problems for systematists attempting to elucidate their evolutionary history based on morphological data (Jones et al. 2002). In addition, they represent an interesting case in evolutionary diversification in that they have accrued incredible species diversity distributed across 6 continents with essentially no morphological diversification.

DNA sequence data have provided clarity to the taxonomic composition of Vespertilionidae by supporting elevation of two traditionally recognized vespertilionid genera (*Miniopterus* and *Cistugo*) to distinct family lineages, Miniopteridae (Hoofer and Van Den Bussche 2003; Miller-Butterworth et al. 2007) and Cistugidae (Lack et al. 2010), respectively. Moreover, DNA sequence data have provided resolution to the composition and relationships among subfamilies (Hoofer and Van Den Bussche 2003; Roehrs et al. 2010, 2011). As currently understood, genera of vespertilionids are partitioned into the subfamilies Antrozoinae, Kerivoulinae, Murininae, Myotinae, and Vespertilioninae. Of these subfamilies, not only is Vespertilioninae the most taxonomically diverse, being represented by about 42 genera and 240 species, but it is also the taxonomically most problematic with little consensus regarding the composition or evolutionary relationships of genera and tribes (Hoofer and Van Den Bussche 2003; Lack and Van Den Bussche 2010; Roehrs et al. 2010, 2011; Volleth and Heller 1994).

6.2 Promise and Potential Pitfalls of Recent Technological Advances

As the field of phylogenetics has progressed, there has been a push for increasingly diverse sources of data, resulting in progressively larger and more complex datasets (Boore 2006). This has been especially important as we have become aware of the shortcomings of the single-locus approach, or even a small number of loci, to infer phylogenetic relationships (Brinkmann and Philippe 2008; Rodriquez-Ezpeleta et al. 2007). This push for more complex datasets has now thrust systematists into "The Age of Phylogenomics." Phylogenomics is the interplay between molecular biology and evolutionary biology to infer evolutionary relationships (Philippe et al. 2005a) and can be distinguished from molecular phylogenetics, as described above, due to the utilization of genome-scale datasets or at least a large number of genes (>100 genes; Dunn et al. 2008; Philippe et al. 2005a). Because of the large number of independent genes or noncoding regions utilized in phylogenomic studies, several controversial evolutionary relationships or unresolved phylogenetic relationships in other taxa now appear to be resolved (Dunn et al. 2008; Li et al. 2006; Murphy et al. 2004). Moreover, as genome-scale datasets have become more prominent, empirical evidence has confirmed the predictions of theory—the evolutionary histories of individual loci can vary substantially and for a variety of reasons (Edwards 2009; Maddison 1997; Neigel and Avise 1986; Nichols 2001; Rosenberg 2003). In addition to the systematic error inherent in phylogenetic reconstruction, these gene tree incongruencies result from one or a combination of two general processes (1) lineage reticulation and (2) deep coalescence (incomplete lineage sorting). Because topological incongruence among gene trees has been addressed in detail in several reviews (Edwards 2009; Maddison 1997; Slowinski and Page 1999), we will only briefly discuss it here and how it may relate to some of the phylogenetic issues remaining in bats.

Lineage reticulation, where gene flow among lineages occurs through hybridization or horizontal gene transfer, is a mechanism that appears to vary in importance among different taxonomic groups. For plants, hybridization is common (at least 25 % of plant species; Mallet 2005), has been important in plant evolution, and can lead to significant variation in gene tree topologies. Similarly, horizontal gene transfer among microbes is so common; it has led some to suggest that prokaryote evolutionary history cannot be represented by a hierarchical, bifurcating tree (Bapteste et al. 2009). For mammals, it is typically assumed that hybridization is rare and therefore of little importance in estimating species relationships; however, recent work suggests hybridization is much more common among mammals than previously thought and may play an important evolutionary role (Baack and Riesberg 2007; Dowling and Secor 1997; Mallet 2005, 2007; Schwenk et al. 2008; Seehausen 2004).

For bats, documented hybridization events are rare. Nonetheless, as comparative genetic and morphological studies have become more numerous, more examples of hybridization in bats are being identified (e.g., Artyushin et al. 2009; Bachanek and Postawa 2010; Berthier et al. 2006; Hoffman et al. 2003; Larsen et al. 2010; Sztencel-Jabłonka and Bogdanowicz, in review). The discovery of hybrid speciation in fruit-eating bats of the genus *Artibeus* illustrates the potential role hybridization can play in mammalian evolution and diversification (Larsen et al. 2010). Previous examinations of hybridization in bats that we are aware of utilized only a few mitochondrial and nuclear loci (typically combining a single mtDNA locus and several nuclear loci), with the exception of the amplified fragment length polymorphism (AFLP) dataset of Larsen et al. (2010). Although these approaches may be adequate for identifying instances of recent hybridization, a much more detailed understanding of the extent, directionality, and evolutionary significance of hybridization can be obtained with genome-scale datasets. For example, phylogenomic approaches can identify introgressed loci, assess their significance in an evolutionary context, and identify functional relationships among these loci (Baack and Riesberg 2007; Pisani et al. 2007; Richards et al. 2009; Sjolander 2010). Many of the current approaches used to identify hybridization and differentiating hybridization from other sources of gene tree incongruence (i.e., incomplete lineage sorting) rely on measuring genetic divergence and/or quantifying variation in gene tree topology. For these techniques to be accurate, they require phylogenomic datasets consisting of many loci to produce a distribution of phylogenies representative of gene tree variation (Holder et al. 2001; Holland et al. 2008; Joly et al. 2009; Syvanen 1994).

Although hybridization appears to be rare in bats, multiple instances of hybridization have been detected within the genus *Myotis* (e.g., Bachanek and Postawa 2010; Berthier et al. 2006; Bogdanowicz et al. 2009). The ecologically diverse nature of *Myotis* and its impressive radiation make it an excellent model system to investigate the role of hybridization during the evolution of the genus. Genome-scale datasets are now becoming feasible for *Myotis* with the release of a draft version of the *Myotis lucifugus* genome (Pritham and Feschotte 2007; Ray et al. 2008), and this system may hold exciting possibilities for the study of hybridization and its role in bat evolution.

Another source of gene tree heterogeneity is deep coalescence. Following the divergence of two lineages, polymorphisms in each lineage are subject to random genetic drift. The rate with which genetic drift occurs is a function of population size, with drift occurring more rapidly in populations with smaller effective sizes. When internodes are short and effective population sizes are large, ancestral polymorphisms are more likely to be retained in daughter lineages, resulting in extant gene copies with an evolutionary history discordant with the species tree (Edwards 2009; Maddison 1997). More importantly, deep coalescence can affect any taxonomic group for which internodes are short and ancestral effective population sizes are large, making it a potential problem in any phylogenetic analysis.

For bats, deep coalescence has been identified as a problem in resolving intertribal relationships within the subfamily Vespertilioninae (Lack and Van Den Bussche 2010). Vespertilioninae is an extremely specious group (Simmons 2005), and previous molecular phylogenetic analyses have resulted in a near-complete lack of support for basal nodes uniting tribal lineages within the subfamily (Hoofer and Van Den Bussche 2003; Roehrs et al. 2010, 2011). Additionally, all unsupported clades were characterized by extremely short internodes, and a comparative phylogenetic analysis revealed an extremely rapid initial rate of diversification for vespertilionines relative to phyllostomids (Lack and Van Den Bussche 2010), a clade of similar age and generic diversity. These are characteristic signs of incomplete lineage sorting.

For taxonomic groups plagued by deep coalescence, such as the Vespertilioninae, simply increasing the number of concatenated loci will not necessarily result in a correctly resolved phylogeny. When branch lengths are sufficiently short, the majority of loci in the genome will not match the species tree (termed the “anomaly zone”; Degnan and Rosenberg 2006; Liu and Edwards 2009), making the “consensus” phylogenetic signal in conflict with the species’ evolutionary history. As an alternative to concatenation, several “species tree” approaches have been developed [reviewed by Degnan and Rosenberg (2008) and Knowles (2009)]. However, because species tree methods require an adequate sample of gene tree variation and the estimation of ancestral effective population sizes, many loci are required. Furthermore, recent work has shown that species tree methods can be substantially improved when multiple individuals within each species are sampled at each locus (Heled and Drummond 2010; Liu et al. 2008; McCormack et al. 2009). To maximize our ability to accurately produce species trees in the presence of incomplete lineage sorting, as in the case of vespertilionine bats, it will require phylogenomic datasets that include multiple alleles sampled per species. However, this presents a problem with species delimitations. Carstens and Dewey (2010) utilized coalescent models to assess the fit of various taxonomic “scenarios” within the western long-eared *M. lucifugus* clade, and their analysis suggested that most subspecific lineages previously identified for this group likely constitute distinct OTUs, which was in contrast to results of previous phylogenetic analyses and morphological data. This study represents one of the first investigations to combine species tree estimation and species delineation in a single phylogenetic context, displaying the utility and flexibility of coalescent models, and analogous approaches have been developed and implemented for other taxa (Kubatko et al. 2011; Yang and Rannala 2010).

Whereas hybridization and deep coalescence are the result of the evolutionary process (genetic introgression and diversification, respectively), another issue in resolving phylogenies involves systematic errors during the tree construction process (Baurain et al. 2006; Felsenstein 2004; Philippe et al. 2005b). Specifically, for relatively ancient or rapidly evolving lineages, there is a high frequency of substitution saturation producing significant amounts of non-phylogenetic signal or homoplasy (Ho and Jermiin 2004). These issues can sometimes be partially mitigated for protein coding genes by utilizing translated amino acid residues instead of the original nucleotide sequences, but this results in 1/3 of the characters relative to nucleotide sequence data, and amino acid sequences can still be plagued by saturation at deep nodes (Baurain et al. 2006; Rokas et al. 2005). In addition, improved substitution models that more adequately account for saturation, the exclusion of relatively rapidly evolving sites, and/or substituting more slowly evolving taxa for rapidly evolving taxa can similarly reduce systematic error (Baurain et al. 2006).

6.3 Potential Concerns Regarding Phylogenomic Approaches

Some phylogenomic approaches have utilized whole-genome sequence data to infer evolutionary relationships (*Drosophila* 12 Genomes Consortium 2007; Huang et al. 2004; Rokas et al. 2003). However, due to the paucity of taxa for which the entire genome has been sequenced, many phylogenomic studies utilized expressed sequence tags (ESTs; Dunn et al. 2008; Hughes et al. 2006; Philippe et al. 2004). Because of the large number of variable positions available in phylogenomic studies, most of these studies have focused on deep evolutionary relationships. Although fewer phylogenomic studies have been conducted at lower taxonomic levels (i.e., among subfamilies or tribes), a phylogenomic approach has been used with success in resolving relationships within Coleoptera (Hughes et al. 2006). One drawback to using ESTs as phylogenetic markers is the large amount of missing data, resulting in a “gappy” alignment, and the effects of missing data and/or taxa in a large multilocus matrix seem to be variable, ranging from a simple lack of resolution (Sanderson et al. 2010) to positively misleading results (Rokas and Carroll 2005). Recent developments in exome capture allow for targeted sequencing of all coding loci without the issue of missing data (Ng et al. 2009). While this approach relies on known coding sequences, the recent release of draft versions of two bat genomes (*Pteropus vampyrus* and *M. lucifugus*) will allow for targeted enrichment of many coding loci for phylogenetic analyses in bats.

Next-generation sequencing (NGS) technologies offer the promise to revolutionize the field of molecular phylogenetics by providing a means to sequence entire genomes quickly and relatively inexpensively. Most recently, third-generation sequencing platforms have been developed that are capable of sequencing single nucleic acid molecules, rapidly and inexpensively generating massive amounts of data (Eid et al. 2009). As an example of the throughput of this technology, using a third-generation sequencing platform, Pushkarev et al. (2009) were able to sequence a single human genome to 28x average coverage in only a few weeks for less than $10,000, and costs for sequencing entire genomes continue to drop (Metzker 2009). However, even with the rapid advances being made, there still remain logistical difficulties with genome-scale sequencing projects. First and foremost, there are several different NGS technologies that have been developed, and each has a unique set of strengths and weaknesses that must be considered in the design and ultimate goals of the project [i.e., read length vs. the number of reads per run; see Metzker (2009) for a comprehensive review of NGS technology and the various platforms available]. Another difficulty in designing projects involving novel sequencing platforms is the rapidity of technological developments. For example, it is anticipated that early in 2013, DNA sequencing with nanopore technology will become available (Schneider and Dekker 2012), leading to yet another exponential increase in our ability to rapidly generate data. In addition, nanopore technologies allow genome-scale sequencing to be performed on essentially unprocessed organismal material (e.g., direct from blood), making it possible to generate data directly from individuals collected in the field.

Once an NGS platform is chosen, other aspects need to be considered. For example, although the technology to sequence entire mammalian genomes rapidly and efficiently now exists, storage and handling of the large amounts of data remain problematic (Schadt et al. 2010). These challenges can range from the simple task of transferring data among locations to permanent large-scale data storage. The latter of these two obstacles is likely to become even more difficult as data generation continues to decrease in cost but increase in throughput. As examples, the 1,000 Genomes Project (http://www.1000genomes.org/; The 1000 Genomes Project Consortium 2010) and the Genome 10k Project (http://www.genome10k.soe.ecsc.edu) will result in petabytes of data, and data storage is a hurdle to be overcome in these endeavors and any genome-scale analysis undertaken. Moreover, even when storage and handling problems are eliminated, assembling and manipulating entire mammalian genomes from a large number of taxa are similarly problematic. All high-throughput sequencing platforms result in millions or even billions of sequences that must be assembled, annotated, aligned, and analyzed. These are computationally intensive processes. Fortunately, recent advances in multiprocessor, cluster-, and cloud-computing have begun to alleviate the limitations involved in genome assembly and analysis [see Schadt et al. (2010) for a comprehensive review of computational solutions to managing and analyzing genome-scale data]. In addition, there are now available low-coverage draft sequences of a pteropodid (*P. vampyrus*) and a vespertilionid (*M. lucifugus*) genome. As coverage increases and these two genomes near completion, they can serve as reference genomes to speed and improve accuracy in the assembly of other bat genomes (as well as other relatively closely related mammals), reducing the coverage needed for reliable assembly and annotation (Gnerre et al. 2009). Finally, the *Pteropus* and *Myotis* genomes can serve as databases from which to mine nuclear loci and develop degenerate primers for future phylogenetic analyses across all bat species.

6.4 Future Directions

As high-throughput sequencing technologies progress and the ease of generating genome-scale datasets increases, the field of phylogenetics (and now phylogenomics) will expand in the type and breadth of questions that can be addressed. As discussed above, to understand the contribution of processes such as hybridization to the evolutionary history of a species or higher taxonomic group, it is necessary to obtain genome-scale data. The increasing availability of mammalian genomes—especially the two draft bat genomes—will make it possible to identify hundreds of loci appropriate for phylogenetic analysis (i.e., single-copy nuclear genes). Additionally, due to the highly parallel nature of NGS platforms, it is possible to simultaneously enrich many loci, pool samples across many individuals, and through the use of unique genetic barcodes simultaneously sequence essentially an entire project's data in a single sequencing run (Mamanova et al. 2010).

In addition to allowing rapid generation of very large phylogenetic datasets, NGS presents the opportunity to analyze additional "types" and sources of data not previously accessible, such as DNA isolated from ancient or fossil material (Knapp and Hofreiter 2010). For bats, the fossil record is extremely sparse, with very few phylogenetically informative fossils, such as crania or mandibles (Czaplewski et al. 2008; Teeling et al. 2005). As a result, it has been difficult to reliably calibrate molecular clock analyses of bat phylogenies, and many of the currently available chiropteran fossils lead to contradictory results when used independently in dating analyses (i.e., the age of Molossidae; Ammerman et al. 2012). In addition, the molecular clock assumption is violated by many (perhaps even most) datasets, particularly when working at higher taxonomic levels (Bromham and Penny 2003; Drummond et al. 2006), making substitution rate calibrations for dating analyses problematic. A potential solution to the problems in the fossil record and substitution rate calibrations is ancient DNA of known age, which can be utilized by modern phylogenetic analyses to calibrate substitution rates (de Bruyn et al. 2011; Millar et al. 2008; Rambaut 2000). Ancient DNA typically consists of highly degraded and fragmented templates in low copy number relative to microbial and environmental "contaminant" DNA (Millar et al. 2008; Pääbo et al. 2004; Willerslev and Cooper 2007). As a result, standard PCR-based approaches are plagued by contamination, and many studies using direct PCR amplification of short ancient DNA fragments have been called into question (Adcock et al. 2001; Golenberg et al. 1990; Poinar et al. 1993). More recently, NGS platforms have been used to specifically target multiple loci from relatively smaller, dilute ancient DNA samples (Briggs et al. 2009), enrich these loci, and directly sequence essentially all sequences present in a given sample, making it easier to identify and separate contaminating from target DNA while avoiding the inherent bias of PCR-based approaches toward longer fragments (Maricic et al. 2010; Millar et al. 2008).

While phylogenetic analysis of ancient DNA has provided considerable insight into human evolution (Briggs et al. 2009) as well as many other taxa [reviewed by Millar et al. (2008)], we are aware of only one analysis of ancient DNA in bats, and these samples were less than 1,000 years old and used a standard PCR and Sanger-sequencing approach (Bogdanowicz et al. 2009). Nonetheless, high-throughput sequencing of ancient bat DNA could be especially informative for bats due to the depauperate chiropteran fossil record (Czaplewski et al. 2008; Teeling et al. 2005). Phylogenetic analysis of ancient bat DNA could be used to more reliably calibrate phylogenies in molecular dating analyses, provide insight into historical biogeography of both extinct and extant species, directly examine the effects of historical climatic events on bat evolution, and accurately utilize both extinct and extant taxa in comparative phylogenetic analyses and ancestral state reconstructions.

In addition to obtaining molecular samples from fossil materials such as bones or teeth, ancient fecal deposits can also serve as a source of DNA that can be utilized in phylogenetic analyses. Greenwood et al. (2001) and Poinar et al. (2003) were able to extract, sequence, and analyze both mitochondrial and nuclear DNA, respectively, from late Pleistocene (>10 kya) fecal deposits to inform the phylogenetic relationships among extinct ground sloths and their extant relatives. Cave-dwelling bats deposit enormous amounts of guano in a relatively cool environment that is shielded from the harmful effects of UV radiation and has been shown to contain an abundance of bat DNA (Puechmaille et al. 2007). In addition, these guano piles can accumulate and stratify over many years, potentially providing a temporal record of genetic diversity. While traditional PCR and Sanger-sequencing can be applied to these types of ancient samples, without the high cost and time investment of cloning many samples, only a very small proportion of genetic diversity will likely be sampled. As an alternative, utilizing universal primers for multilocus target enrichment followed by direct sequencing on an NGS platform will produce exponentially more data, but without the fragment length and copy number bias of PCR and without cloning. By using high-throughput sequencing platforms to sequence DNA from ancient guano deposits, an entirely new and fruitful source of phylogenetic data can be obtained.

Traditionally, genome-level characters have been limited in their utility for phylogenetic analyses due to the difficulty in their identification (Rokas and Holland 2000). As more and more genomes have been sequenced, the types of data being utilized in phylogenetic analyses have expanded (Boore 2006). In addition to nucleotide and amino acid sequences, genome-level characters include gene order, presence/absence of genes and introns (Krauss et al. 2008), biochemical pathways, insertion of numts (Richly and Leister 2004), and transposable elements [see Boore (2006) for an exhaustive review of genome-level characters]. While these genome-level characters add phylogenetic information, the most attractive aspect of these characters is that homoplasy is very unlikely even over very long timescales. For example, with DNA sequence data, there are only four possible character states (A, C, G, and T) for each site, and convergence upon the same nucleotide can occur to the extent that the level of non-phylogenetic signal can overwhelm the level of phylogenetic signal in the dataset (Baurain et al. 2006; Olsen 1987). Conversely, the insertion of a transposable element can occur at potentially millions of positions (obviously within the constraints of selection on gene function and genomic structure) across the genome, making homoplasy essentially nonexistent (Boore 2006; Rokas and Holland 2000).

Whereas many genome-level characters mentioned above are likely candidates for phylogenomic analyses in bats, recent surveys of a draft sequence of the *M. lucifugus* genome revealed novel possibilities. Transposable elements make up a significant proportion of nearly all eukaryotic genomes (i.e., ~50 % of the human genome; Lander et al. 2001). One type of transposable element, class I transposable elements (DNA transposons), is active in many groups of eukaryotes but is thought to be effectively "extinct" in most mammalian genomes (Lander et al. 2001; Waterston et al. 2002). However, at least nine families of class I transposable elements exhibiting insertion activity over approximately the last 40 million years have been discovered in various groups of vespertilionid bats, and at least one of these families is still active (Pritham and Feschotte 2007; Ray et al. 2007, 2008). The distribution of these elements across various groups within Vespertilionidae, as well as the ongoing insertion activity of at least one of the transposon families, suggests that these may represent excellent sources of non-homoplastic phylogenetic markers. For vespertilionids, this is especially exciting as Lack and Van Den Bussche (2010) found that the mean substitution rate for vespertilionids across both nuclear and mitochondrial loci was approximately 50 % higher than that of phyllostomids. This suggests that systematic error may be responsible for some of the difficulty in resolving many of the basal vespertilionid lineages, and recent transposon insertion events may be able to provide phylogenetic resolution where DNA sequence data have failed.

Phylogenetic analyses have been vital in understanding the origins of echolocation (Eick et al. 2005; Jones and Teeling 2006; Teeling 2009; Teeling et al. 2000) and parallel and convergent evolution of dim-light vision (Shen et al. 2010), and more recent studies combining functional genetics and phylogenetics have identified at least two genes that function in echolocation and were important in the evolution of the echolocating phenotype (Li et al. 2007, 2008). Similarly, evolutionary developmental studies have identified multiple genes and developmental pathways contributing to the evolution of the bat wing from the forelimb of a nonvolant ancestor (Chen et al. 2005; Hockman et al. 2008; Ray and Capecchi 2008; Sears et al. 2006; Wang et al. 2010; Weatherbee et al. 2006). With the new genomic resources available, it should now be possible to further characterize the developmental pathways and evolutionary changes responsible for the evolution of the bat wing and echolocation from a nonvolant ancestor incapable of echolocation. Similarly, phyllostomid bats represent arguably the most phenotypically diverse mammals on the planet (Baker et al. 2000, 2003; Simmons 1998), consisting of a diverse array of feeding strategies including nectarivory, insectivory, frugivory, and sanguinivory. As a result of their behavioral and dietary diversity, they have evolved an amazing array of morphological adaptations. For example, nectarivorous phyllostomid bats have evolved adaptations to hovering flight (Norberg et al. 1993; Winter 1998; Winter et al. 1998), an elongated rostrum, an elongated and extendible tongue with papillae and grooves for obtaining nectar and/or pollen (Winter and von Helversen 2003), and physiological adaptations to maintain a high metabolic rate and efficiently and rapidly digest nectar and pollen (Herrera and del Rio 1998; Schondube et al. 2001; Voigt and Speakman 2007). In a similar fashion, sanguinivorous vampire bats have modified cranial and dental morphology to acquire blood meals (Birney and Timm 1975; Davis et al. 2010; Griffiths and Criley 1989), a highly distensible and vascularized stomach and alimentary tract to accommodate the need for feeding on large quantities of blood and the rapid absorption of water and nutrients (Delpietro and Russo 2002; Mitchell and Tigner 1970), modification of digestive enzymes (Schondube et al. 2001), and renal specialization to rapidly excrete water (Bhatnagar 1988; Wimsatt and Guerriere 1962). These are just two examples of the impressive adaptations to highly diverse feeding strategies illustrating the potential for studies of adaptation and evolution in Phyllostomidae.

The endpoint of any phylogenetic analysis is (hopefully!) a resolved phylogeny reflecting evolutionary relationships of species, functional relationships of genes, paralogs in a gene family, etc. Ultimately a tree topology will be utilized to inform studies of adaptation and evolution. Bats represent an extraordinary group characterized by evolutionary novelty (i.e., echolocation and powered flight) and adaptation (e.g., seven distinct feeding guilds within the family Phyllostomidae) to an extent arguably paralleled by no other group of mammals. As we move into an era of phylogenomics, it is time for bats to move to the forefront of the study of evolutionary novelty and adaptation, which for mammals has been dominated by rodents due to the mouse and rat models being so ubiquitous in all aspects of biology. With the development of massively high-throughput sequencing platforms, the accompanying development of greater computational abilities, and the recognition that there is a need for the development of additional model systems (Abzhanov et al. 2008), bats should be obvious choices for future study in fields such as genomics and evolutionary development.

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Chapter 7
Of Bats and Molecules: Chromosomal Characters for Judging Phylogenetic Relationships

Marianne Volleth

Abstract Traditionally, morphological characters were used for judging phylogenetic relationships. These features, however, are prone to convergent evolution, and therefore, some aspects of intrafamilial and most interfamilial relationships remained unsolved to a large extent. In recent times, genetic features were used to elucidate phylogenetic relationships in Chiroptera. In the following chapter, a short introduction to classical and molecular cytogenetic methods is given. Furthermore, types of chromosomal rearrangements detected by application of such techniques are described. Amongst the large number of characters obtained by comparative karyological analysis, two cytogenetic features, i.e. inv 2 and inv 5, could serve as synapomorphies for the suborder Pteropodiformes. This finding supports recent molecular genetic results which proposed a basal division of Chiroptera into Pteropodiformes and Vespertilioniformes, rejecting the traditional division into Mega- and Microchiroptera. Concerning the phylogenetic relationships within the superfamily Emballonuroidea, however, cytogenetic results are not consistent with traditional views. On the one hand, a common character connecting the genera *Taphozous* and *Emballonura* was not found. On the other hand, similarity in three chromosomal features suggests a closer relationship between *Emballonura* and *Nycteris* than hitherto suspected.

7.1 Introduction

In former times, taxonomists could only use morphological characters for judging phylogenetic relationships in Chiroptera. These traditionally used features were applied successfully to define the 18 extant bat families. Also, the traditional

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_7, © Springer Science+Business Media New York 2013

concept of two chiropteran suborders, i.e. Megachiroptera, comprising only one family, the fruit-eating Pteropodidae, and echolocating Microchiroptera, was strongly supported by several morphological characters (Miller 1907). The 17 microchiropteran families were divided into two infraorders, named Yinochiroptera and Yangochiroptera, based on whether their premaxillaries were moveable/absent or fused relative to their maxillaries (Koopman 1984, 1985). However, the results of DNA sequence analyses (Springer et al. 2001; Teeling et al. 2000, 2002, 2005) changed this view dramatically. With the exception of Nycteridae and Emballonuridae, all former yinochiropteran families are closer related to Pteropodidae than to the remaining former microchiropteran families. Therefore, the newly formed suborder (Pteropodidae and parts of Yinochiroptera) was named Yinpterochiroptera (Springer et al. 2001; Teeling et al. 2002).

The remaining microchiropteran families constitute the suborder Yangochiroptera (sensu Teeling et al. 2002, i.e. Yangochiroptera sensu Koopman plus Nycteridae and Emballonuridae). As the composition of taxa in Koopman's suborders Yino- and Yangochiroptera differs from that in Yinpterochiroptera and Yangochiroptera defined according to the DNA data (Springer et al. 2001; Teeling et al. 2002), Hutcheon and Kirsch (2006) proposed new subordinal names. The Pteropodiformes comprise Craseonycteridae, Hipposideridae, Rhinolophidae, Megadermatidae, Rhinopomatidae and Pteropodidae and equal the term Yinpterochiroptera. The remaining microchiropteran families are subsumed in the suborder Vespertilioniformes (replacing Yangochiroptera sensu Teeling et al. 2002). For the reason of clarity, the terms Pteropodiformes and Vespertilioniformes are used here.

The following chapter provides a short overview of the present knowledge in chiropteran chromosomal evolution.

7.2 Conventional Cytogenetics

7.2.1 Non-differential Staining

Before the introduction of chromosome-banding techniques, the chromosomes could only be non-differentially stained using the Giemsa stain. The information obtained by this technique was the number of chromosomes and the number of chromosomal arms within a cell, resulting in the diploid chromosome number ($2n$) and the fundamental number (FN) for a given species. But even these very basic data are known at present only for about half of the roughly 1,100 bat species (Baker 2006).

The diploid chromosome numbers found within Chiroptera range from the lowest with $2n=14$ in *Vampyressa melissa* (Gardner 1977) to the highest of $2n=62$ in some rhinolophids (*Rhinolophus affinis* and others; see review by Zima et al. 1992). Often, all members of a certain genus possess the same $2n$. For example, all *Miniopterus* species show 46 chromosomes, and the genus *Eptesicus* is characterised by 50 chromosomes (Zima and Horacek 1985). The opposite of such genera

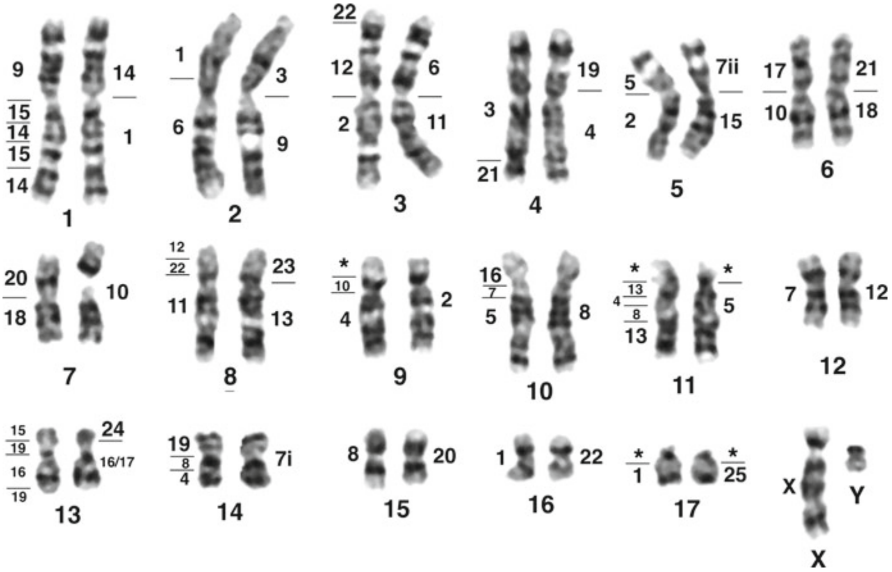

Fig. 7.1 G-banded karyotype of a male *E. spelaea* with indication of homologous chromosomal segments to human (*left*) and *M. myotis* (*right* of each chromosomal pair) revealed by FISH with human and *Myotis* whole chromosome painting probes, respectively. *Asterisks* indicate heterochromatic regions which were not painted by any probe. The right homolog of pair 7 clearly shows a secondary constriction (bearing a NOR)

with a slow rate of cytogenetic changes are genera where nearly every species shows its own characteristic karyotype; examples are the vespertilionid genera *Neoromicia*, *Tylonycteris* and *Rhogeessa* (Kearney et al. 2002; Volleth et al. 2001; Bickham and Baker 1977; Baird et al. 2012).

7.2.2 *Banding Techniques*

For a detailed analysis of a certain karyotype and, of course, for a comparison of two different karyotypes, each single chromosomal pair needs to be individually distinguished. Such discrimination can be obtained by different techniques. Mostly the so-called G-banding technique is applied (see Fig. 7.1). A technical description of cytogenetic methods including banding techniques adapted for use in Chiroptera has been given in Volleth et al. (2009).

By comparing karyotypes of closely related taxa, the prevailing mode of chromosomal evolution was found to be the Robertsonian translocation, i.e. centric fusion (Robertsonian fusion) and centric fission (Robertsonian fission; Baker and Bickham 1980; Bickham and Baker 1979). To enable an easy comparison between karyotypes which evolved mainly by Robertsonian rearrangements and differ therefore in chromosomal arm combinations, Bickham (1979) disregarded in his description of the *Myotis* karyotype the common rule of numbering whole chromosomes using instead a system in which chromosomal arms are numbered. Following his proposal, the autosomal arms in karyotypes of the family Vespertilionidae are numbered according to their size. In bi-armed chromosomes, numbers of both chromosomal arms are connected by a slash. The four bi-armed chromosomal pairs of the genus *Myotis* read, for example 1/2, 3/4, 5/6 and 16/17.

The prevalence of Robertsonian changes, however, resulted in identical arm combinations not only in closely but sometimes also in distantly related taxa. In this case, convergent evolution (homoplasy) has to be taken into consideration. A second concept for explaining the occurrence of identical arm combinations in distantly related taxa has been proposed recently (Robinson et al. 2008). Hemiplasy is the retention of a chromosomal polymorphism through multiple speciation events (Robinson and Ropiquet 2011). In the case of Robertsonian translocations, the initial heterozygous condition, i.e. one fusion chromosome and two non-fused elements, would persist over some time resulting later in a homozygous, fused condition in some descendants and in a homozygous, non-fused situation in others, irrespective of additional features leading to speciation. For example, hemiplasy was proposed as explanation for some of the shared characters in the chromosomal evolution of Bovidae (Robinson and Ropiquet 2011). The persistence time for such polymorphic conditions was estimated as only few (3–5) million years. Therefore, hemiplasy might be taken into consideration only for intrafamilial karyotype evolution in Chiroptera.

The occurrence of only one common Robertsonian translocation chromosome in two taxa should for the reasons discussed above not be taken itself as indication for a close phylogenetic relationship. For statistical reasons, strict rejection of alternate hypothesis would require even three common features (Robinson et al. 2008; Wadell et al. 2001). For example, the presence of three common Robertsonian fusion chromosomes with the arm combinations 7/11, 8/9 and 10/12 in the vespertilionid genus *Neoromicia* (Kearney et al. 2002) is a clear indication for common ancestry. In addition to Robertsonian translocations, other chromosomal rearrangements have also played a role in karyotype evolution of bats. Seven small inversions have been found by comparing G-banded karyotypes of Vespertilionidae. These features have been used to construct a cladogram and to define a “basic” karyotype for this family (Volleth and Heller 1994).

Conventional cytogenetics enabled intrafamilial comparison of bat karyotypes. As a result, Baker and Bickham (1980) recognised three patterns of karyotype evolution in bats: conservatism (e.g. *Myotis*), karyotypic orthoselection (G-band patterns largely conserved) and karyotypic megaevolution (G-band pattern severely altered, e.g. *Tonatia*).

When species are compared which belong to two different bat families, however, identification of homologous chromosomal arms on the basis of G-banded karyotypes is a difficult task. An unequivocal identification and therefore a deep insight into chromosomal evolution in bats were enabled by the application of modern cytogenetic techniques, i.e. fluorescence in situ hybridisation (FISH).

7.3 Molecular Cytogenetics or FISH

7.3.1 Methodology

The development of molecular techniques 20 years ago revolutionised chromosomal analysis. From then on, a certain DNA sequence could be multiplied by polymerase chain reaction (PCR), labelled with fluorescent dyes and used as a so-called probe to detect homologous DNA sequences in target molecules irrespective whether they are bound to a membrane or attached to a microscopic slide as for cytogenetic research. Under physiological conditions, DNA molecules are present as double strands. Melting DNA under certain conditions results in single-stranded DNA. This process is named denaturation. "Renaturation", i.e. annealing of two single strands to a double strand, is one of the most used properties of DNA molecules in genetics. Annealing takes place when the sequence of the first strand is complementary to that of the second strand. This process, hybridisation, enables the detection of chemically labelled probes containing DNA sequences of interest on chromosomes. Most of the DNA probes used in comparative cytogenetics are so-called whole chromosome painting probes (WCP) which contain the genetic information of a complete chromosome from a certain species. Such probes can be obtained by flow cytometry, i.e. using fluorescence-activated cell sorters (FACS). With this highly specialised technique, the chromosomes are separated according to size and base pair composition (GC/AT content ratio; Carter et al. 1990; Yang et al. 1995).

Optimally, each fraction obtained by this technique consists of chromosomes belonging to the same chromosomal pair from the species investigated. Sometimes, however, two different chromosomes displaying similar properties cannot be separated by FACS. In the next step, the DNA of the sorted chromosomes is amplified by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR; Telenius et al. 1992) and labelled with fluorescent dyes which emit light of visible wavelength after radiation with ultraviolet (UV) light. Thus, the location of the probe DNA is detected by the areas of emitted light. In cytogenetics, the target DNA sequences are found at their original location ("in situ"), i.e. embedded in the chromosomes, which have been spread onto glass slides for microscopic investigation. Therefore, this method has been called *f*luorescence *i*n *s*itu *h*ybridisation or FISH. The first FISH probe set was made from the human chromosomal complement. This probe set was used for FISH not only in man but also in different mammalian species, and the technique is then called Zoo-FISH (Scherthan et al. 1994).

7.3.2 Application of Whole Chromosome Probe Sets in Chiroptera

At the beginning, only the human probe set was available for FISH. The hybridisation of all 23 human probes (22 autosomal pairs plus the X; the Y chromosome differs too much between species to enable hybridisation) resulted in 46 different segments on the karyotype of *Myotis myotis*, consisting of 21 autosomal pairs plus X (Volleth et al. 2002). Hybridisation efficiency, however, is better if the phylogenetic distance between the probe species and the target species is lower. Therefore, it is much easier to obtain good results when chiropteran-specific chromosome probes are used. *M. myotis* (MMY) was the first bat species with a flow-sorted probe set (Ao et al. 2006). This probe set enables an easy comparison of the *Myotis* karyotype with chromosomal complements of bats from other families where conventional cytogenetics had failed to detect homologous chromosomes due to extensive changes in the G-banding pattern.

A comparison of the results received by application of human and *Myotis* paints is given in Fig. 7.1 for the flying fox *Eonycteris spelaea*.

Meanwhile, whole chromosome paints have been established by Yang and co-workers for *Aselliscus stoliczkanus*, *Phyllostomus hastatus*, *Carollia brevicauda* and *Macrotus californicus* (Mao et al. 2007; Pieczarka et al. 2005; Sotero-Caio et al. in preparation).

The results obtained with the human and the *Myotis* probe sets showed that during karyotypic evolution in bats, complete chromosomes or at least whole chromosomal arms have been conserved with only few exceptions. A certain chiropteran karyotype can be thought of being composed of a rather small number of chromosomal segments, i.e. 26. As they have remained as entities during chromosomal evolution, these chromosomal segments were called “evolutionary conserved units” (ECUs; Volleth et al. 2002). The numbers given with the ECUs refer to the homology to human chromosomes. ECU 17, for example, contains homologous sequences to human chromosome 17. Chiropteran karyotypes can now be described by species- or genus-specific combinations of these units into chromosomes. A certain ECU, however, although being composed of homologous DNA sequences, might display with different chromosomal shapes, characterised mainly by the position of the centromere. For example, ECU 7a is a small metacentric chromosome in phyllostomids and molossids, a small acrocentric chromosome in vespertilionids and, after centric fission, present as two small acrocentric chromosomes in rhinolophids. By comparing different karyotypes and analysing changes in the appearance of the ECUs, characters of possible phylogenetic importance can be described. FISH enables therefore a deep insight into the process of karyotype evolution in bats. On the level of the G-banding pattern, some ECUs seem to have remained unchanged in many families and are therefore considered as ancestral (plesiomorphic) elements. Other ECUs are found in several differing character states which can be used for phylogenetic analysis.

7.3.3 *Description of Chromosomal Characters Based on FISH*

Altogether, roughly 30 species representing all major bat families have been studied by FISH with human and bat paints in the last years (see review by Volleth and Eick 2012). By comparing these data, chromosomal changes which might be of phylogenetic importance have been deduced. All 12 chromosomal features shown in Table 7.1 have been found in more than one taxon and represent thus no autapomorphies.

Table 7.1 Distribution of shared chromosomal characters revealed by FISH

Character MMY	fus 1/2	fus 3/4	fus 5/6	fus 7i/7ii	fus 9/11	fus 13/23	shape 8	shape 10	shape 12	shape 20	inv 2	inv 5
ECU	14-15 4a-10b	1a-6b 3a-21	13-8b-4c 12a-22a	4b-8c- 19b 5b	6a 2a	11a 11b-22b- 12b	5a-7b-16b	18-20	7a	8a	4a-10b	13-8b- 4c
Pteropodiformes												
Eonycteris	-	-	-	-	-	+	b	b	b	b	+	+
Rousettus	-	-	-	-	-	+	b	b	b	b	nk	nk
Cynopterus	-	-	-	-	-	-	a	fis	fis	a	nk	nk
Rhinolophus	-	-	-	-	-	-	fis	fis	fis	b, fis[7]	+	+
Hipposideros	-	-	-	-	-	+	fis	b	fis	b	+	+
Aselliscus	-	-	+	-	-	+	fis	b	b	a	nk	+
Megaderma	-	-	-	-	-	+ / r	fis	fis	fis	fis	+ / r1	+ / r
Vespertilioniformes												
Nycteris	-	-	+ / r1	-	-	-	fis[5]	fis	fis	b	- / r2	-
Emballonura	-	-	-	-	-	-	fis[6]	fis	fis	fis	-	-
Taphozous[1]	-	-	-	-	-	-	a	fis	fis	b / r1	- / r3	-
Glossophaga	+	-	+ / r2	-	+	-	a	b	b	a	- / r4	-
Phyllostomus[2]	+	-	+ / r2	-	+	-	a	b	b	r2	nk	nk
Diphylla[1,8]	-	-	nk	-	-	-	a	b	b	a	nk	nk
Myzopoda[1]	-	+	-	-	+	-	fis[6]	a / r?	b / r?	r3	nk	nk
Myotis[3]	+	+	+	+	-	-	a	a / r1	a / r1	a	-	-
Miniopterus	+	-	+	+	-	-	a	a / r2	b	a	-	-
Molossus[4]	+	-	-	+	-	-	a	b	b	a	-	-
Natalus	+	-	+	+	-	-	a	b	a / r2	a	-	-

(continued)

Table 7.1 (continued)

The characters are described in homology to *M. myotis* chromosomal arms (MMY) or to human homology (ECU)

MMY, *Myotis myotis*; ECU, evolutionary conserved unit (numbers refer to human chromosomes); inv, inversion; fus, centric fusion; nk, not known; r, rearranged (r1, r2, r3, r4 differing rearrangements shaped the respective chromosomes; independent numbering in each column)

A + sign indicates that the character is present, a – sign that the character is missing

shape: a, acrocentric, either as a single chromosome or as one arm of a bi-armed chromosome; b, bi-armed; fis, fissioned

Grey-shaded columns: analysis requires FISH with human (inv 5) or lemur (inv 2) probes

[1] Data from literature (Mao et al. 2008; Sotero-Caio et al. 2011; Richards et al. 2010)

[2] F. Yang, personal communication

[3] Representative of the vespertilionid "basic" karyotype

[4] Similar condition found in *Mormopterus* and *Mops*

[5] Breakpoint differs from that in Rhinolophoidea

[6] Breakpoint not known

[7] Chromosome either present as bi-armed element or, after centric fission, as two acrocentric chromosomes

[8] Similar condition found in *Diaemus*

Six of these characters are *Robertsonian fusions* where two acrocentric chromosomes fuse at the centromere to constitute one bi-armed chromosome. In all but two of the genera studied from the suborder Pteropodiformes, a fusion chromosome composed of the ECUs 11a and 11b-22b-12b (MMY 11 and MMY 23) has been found. In *Cynopterus sphinx* and the genus *Rhinolophus*, centric fission events are assumed to have occurred (see column "reversals" in Table 2). In none of the taxa studied belonging to the Vespertilioniformes, a fusion chromosome MMY13/23 has been reported up to now. This fusion chromosome could therefore represent a common character for Pteropodiformes. However, whether this feature is a derived or an ancestral character remains unclear. One indication for the ancestral condition might be the fact that this fusion chromosome comprises both chromosomal segments which harbour sequences homologous to human chromosome 11 in bats. Synteny conservation of *Homo sapiens* (HSA) chromosome 11 has been found in many other mammalian taxa and therefore been proposed as character of the putative mammalian ancestor (Robinson and Ruiz-Herrera 2008).

Three fusion chromosomes could be important for the phylogenetic relationships in the suborder Vespertilioniformes. First, the fusion product of ECU 4b-8c-19b (i.e. the proximal part of Myotis MMY 7, called from here on 7i) and ECU 5b (the distal part of MMY 7, called here 7ii) was proposed as a synapomorphic character for Vespertilionoidea (as 4b-19b in Volleth et al. 2002 because the tiny HSA 8 homologous segment was detected later; see Volleth et al. 2011). This statement remains valid as such a combination has not been found in any non-vespertilionoid taxon up to now. The fusion product of ECU 14–15 with ECU 4a-10b (resulting in MMY 1/2) was also found in all Vespertilionoidea but, in addition, in the phyllostomid genera *Glossophaga* and *Phyllostomus* (Volleth et al. 1999, F. Yang personal communication). Whether the occurrence of this fusion product in Phyllostomidae is the result of convergent evolution or can be regarded as a common feature for Noctilionoidea and Vespertilionoidea remains open at the moment.

Even more complex is the situation for the third case, the centric fusion between ECU 13-8b-4c and ECU 12a-22a, resulting in MMY 5/6. This combination was found in vespertilionids, natalids and miniopterids, but not in molossids. In nycterids, a rearranged chromosome is present, containing two-thirds of MMY 5 and two-thirds of MMY 6, resulting in a clearly different G-banding pattern compared to MMY 5/6 (own unpublished results). In addition to sequences homologous to MMY 6, *Glossophaga* chromosome 12/10-11 harbours a very small segment homologous to human chromosome 13 and therefore to MMY 5 (own unpublished results). For both families, Nycteridae and Phyllostomidae, the presence of the complete MMY 5/6 homolog in their ancestors could be taken into consideration. Under this assumption, a MMY 5/6 homologous chromosome could have appeared in the karyotype of the vespertilioniform ancestor. The presence of a MMY 5/6 homolog in the hipposiderid *Aselliscus* (Mao et al. 2007) points to the possibility that such combination might have been present in the chiropteran ancestor. At the moment, however, the assumption of convergent evolution in *Aselliscus* and Vespertilioniformes seems to be more likely.

Some of the evolutionary conserved units changed their shapes during chromosomal evolution in bats. Originally bi-armed chromosomes can be found as two acrocentric chromosomes after *centric (Robertsonian) fission* or, after an inversion or centromere shift, as a single acrocentric chromosome. Such a scenario is assumed for ECUs 18–20 and 7a, i.e. homologs to MMY 10 and 12, respectively. The bi-armed shape of both ECUs has been found in approximately half of the taxa studied throughout the order (Table 7.1). This condition is therefore very likely the ancestral one. The result of a centric fission of a bi-armed element, i.e. two acrocentric chromosomes, would then be regarded as derived. However, independently occurring centric fissions of the same ancestral bi-armed element lead to identical elements on the level of microscopic resolution. Therefore, the possibility of convergent events must be considered not only in the case of centric fusions but also in the case of centric fissions. For example, the above-mentioned bi-armed chromosomes ECU 18–20 (MMY 10) and ECU 7a (MMY 12) underwent fissions in the ancestor of Emballonuroidea, in *Megaderma*, in *Rhinolophus* and in *Cynopterus* (Volleth et al. 2002; Ao et al. 2007; Mao et al. 2007, 2008). Amongst Hipposideridae, while conserving ECU18-20 in the ancestral state in the whole family, ECU 7a has also been split in the genus *Hipposideros*, but not in *Aselliscus* (Volleth et al. 2002; Mao et al. 2007). As a consequence of the observation of multiple fission events in these two ECUs, they have not been treated as cytogenetic signatures in Fig. 7.2.

In contrast, centric fission of another bi-armed element is very likely a valuable cytogenetic character. In the case of ECU 5a-7b-16b, homologous to MMY 8, the character state found in the flying fox *Eonycteris*, with 16b in the short arm and 7a-5a in the long arm of a bi-armed chromosome (Volleth et al. 2002), is assumed to represent the ancestral condition at least for the suborder Pteropodiformes. As all members of the Rhinolophoidea show this ECU separated into two elements, 16b and 7a-5a, a single centric fission in the ancestor of this superfamily can be assumed as apomorphic event. The breakpoint of the MMY 8 homolog in *Nycteris* and *Emballonura* is clearly different from that in Rhinolophoidea (own unpublished results). In *Myzopoda*, presenting also with two MMY 8 homologous elements (Richards et al. 2010), the breakpoints are not known yet.

Nucleolus organising regions (NORs), harbouring the genes coding for ribosomal RNA, have been found at many different locations in mammalian genomes. NORs are found at two basically different locations (1) close to a telomere or on a minute short arm, i.e. in a terminal location and (2) within a chromosome, often close to the centromere. In the latter case, the NOR is often visible in the microscope as a “gap” and has therefore been called “secondary constriction”, considering the centromere as primary constriction. For detection of these secondary constrictions, mostly no specialised banding technique is necessary in contrast to the terminally located NORs which require silver staining or FISH to be verified. Species displaying terminally located NORs often posses multiple sites which change their location easily during karyotype evolution even amongst species of the same genus (Volleth 1987). NORs visible as secondary constrictions, however, are much more suited as phylogenetic characters because in many cases, there is only one pair of NORs per karyotype showing a higher evolutionary stability.

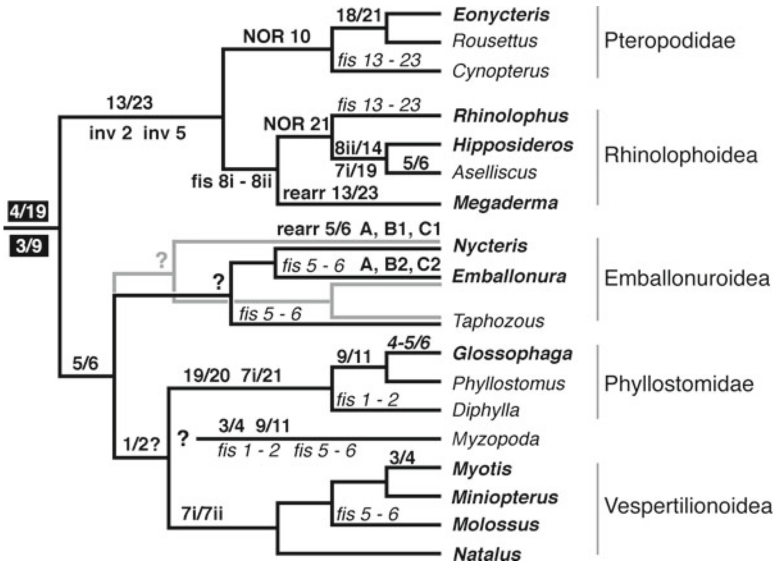

Fig. 7.2 Shared chromosomal characters mapped onto a DNA-based phylogenetic tree. Taxa without FISH data have been omitted. For each character, the proposed first occurrence and further development in succeeding taxonomic levels is indicated. Reversals are shown in *italics*. Chromosomal arm numbers are given according to *Myotis* homology. Two numbers connected by a slash indicate a Robertsonian (centric) fusion between both elements. Abbreviations: fis, fission; NOR, nucleolus organising region; inv, inversion; rearr, rearranged. For genera indicated in **bold**, the complete dataset is available (see Table 7.1). Data are included from literature as indicated in Table 7.1. The unsolved position of *Myzopoda* is indicated by a *question mark*. The Robertsonian fusion chromosomes which *Myzopoda* shares with Phyllostomidae and Vespertilionidae, respectively, represent presumably homoplasies. The relationships within Emballonuroidea based on DNA sequence data are shown in *grey*; those proposed by cytogenetic signatures are shown in *black*. Character A means NOR at 12i, B1 is the combination of a part of 15 with 25, B2 is the combination of 15 with 25, C1 is the combination of 16/17 and 2 in one chromosome and C2 is the combination of 16/17 and 2 in one arm of a chromosome. For the chiropteran ancestor, two Robertsonian fusion chromosomes are proposed (*white* numbers on *black* background) which have been preserved only in the Pteropodidae genera *Eonycteris* and *Rousettus*

The probability that this single location is the same just by chance is not very likely. Two shared characters within Pteropodiformes are locations of NORs. All Pteropodidae shown in Fig. 7.2 have a single NOR on the MMY 10 homolog. Similarly, all members of Rhinolophidae and Hipposideridae studied up to date possess a NOR on the MMY 21 homolog.

By comparison of the *G-banding patterns* of homologous chromosomal segments (i.e. ECUs) between different taxa, it became obvious that there was apparently no change in the banding pattern in many cases. For example, ECU 1a-6b (MMY 3) can be identified in nearly every chiropteran karyotype without applying FISH due to the characteristic G-banding pattern. In contrast, some ECUs show such large differences in the G-banding pattern that homology could have never been proven without application of FISH. In these cases, the intrachromosomal order has obviously been changed during karyotype evolution. One example is ECU 13-8b-4c (MMY 5), where the rearrangement was detected by using the human whole chromosome painting (WCP) set; see column “inv 5” in Table 7.1. In all Pteropodiformes studied up to now, the small segment homologous to human chromosomes 4 and 8 is situated within the large HSA 13 homologous segment: 13-4c-8b-13 (Volleth et al. 2002; Mao et al. 2007). This is thought to be an apomorphic condition because the prevailing pattern of the Vespertilioniformes shows the HSA 4–8 segment in the distal and the HSA 13 part in the proximal position (13-8b-4c). The distally located HSA 4–8 segment in chromosome 12 of *Megaderma spasma* described by Mao et al. (2008) could meanwhile be shown to be capped with a small HSA 13 homologous segment. In addition, a second segment homologous to HSA 13 was detected, suggesting that a chromosome with the apomorphic condition, 13-8b-4c-13, has been split into two parts in *Megaderma* (own unpublished results). Therefore, the condition in *Megaderma* could be derived by fission from the state found in other Pteropodiformes and is not similar to that in Vespertilioniformes.

Another possibility for detecting intrachromosomal cryptic rearrangements is the use of FISH probes from species where chromosomes of interest have been fragmented into smaller parts compared to the human genome. This is for instance the case for the black lemur, *Eulemur macaco* (EMA), where homologous sequences to HSA 4 are found on five different chromosomes (Müller et al. 1997). The largest HSA 4 homologous segment in bats (MMY 2) shows homology to the following four EMA chromosomes: 6, 12, 19 and 20. MMY 2 and its counterparts in other Chiroptera are composed of layers of thin G-dark and G-light bands like a sandwich. Therefore, G-band analysis failed to detect any differences between the MMY 2 homologs. With the application of HSA 4 homologous EMA probes, however, the signal pattern present in Pteropodiformes differed by inversion of a small segment from the pattern seen in Vespertilioniformes. Comparisons with the patterns found in human and the European mole (serving as out-groups) suggest the vespertilioniform pattern as the more ancestral one. The inverted state found in Pteropodiformes could therefore be regarded as a synapomorphy for this suborder (Volleth et al. 2011).

7.4 Phylogenetic Considerations

In order to enable a comparison of cytogenetic and molecular genetic results, those shared cytogenetic characters which probably could serve as synapomorphies for the different taxonomic levels were plotted onto the most recent molecular tree in Fig. 7.2 (Teeling et al. 2012; Meredith et al. 2011). Additionally, common chromosomal features proposed as cytogenetic signatures for the different taxonomic levels have been summarised in Table 7.2.

A comparison of all currently available data resulted in the assessment of only few characters which could be proposed as plesiomorphic features for the karyotype of the chiropteran ancestor. Amongst them are the fusion chromosomes MMY 4/19 and

Table 7.2 Common chromosomal characters proposed as cytogenetic signatures

Taxonomic level	Character	Homoplasies	Reversals	Remarks
Chiropteran ancestor	4/19			Synteny conservation HSA 3
	3/9			Synteny conservation HSA 6
	Bi-armed 10			
	Bi-armed 12			
Pteropodiformes	inv 2		fis in *Cynopterus*	Synteny conservation HSA 11
	inv 5		fis in Rhinolophidae	
	13/23			
Pteropodidae	10 with NOR			
Eonycteris+Rousettus	18/21			
Rhinolophoidea	fis 8i/8ii	*Myzopoda?*		Breakpoint not known for *Myzopoda*
Rhinolophidae+Hipposideridae	21 with NOR			
Hipposideridae	7i/19			
	8ii/14			
Vespertilioniformes	5/6	*Aselliscus*	fis in Emballonuridae fis in *Myzopoda* fis in Molossidae	Rearranged in Nycteridae, rearranged in Phyllostomidae
Nycteridae+Emballonuridae	None			
Nycteris+Emballonura	12i with NOR			
	comb 15 with 25			
	comb 2 with 16-17			
Vespertilionoidea+Phyllostomidae	1/2?		fis in *Myzopoda* fis in Desmodontinae	1/2 present in Phyllostominae and Glossophaginae; ancestral condition for Noctilionoidea not known
Phyllostomidae	19/20			
	7i/21			
Vespertilionoidea	7i/7ii			
Molossidae+Miniopteridae+Vespidae	None			
Vespertilionidae+Miniopteridae	None			

Fusion chromosomes are indicated by two numbers connected by a slash, e.g. 13/23. Numbers in the column "Character" refer to *Myotis* chromosomal arms fis, fission; comb, combination of chromosomal arms (or parts of it) in the same chromosome; NOR, nucleolus organising region; HSA, *homo sapiens*

MMY 3/9, because they are a combination of both chiropteran segments with HSA 3 and HSA 6 homologous sequences, respectively. Synteny conservation of homologous sequences to human chromosomes 3 and 6 has been found in many mammalian species and therefore proposed as plesiomorphic trait for Eutheria (Biltueva et al. 2004; Richard et al. 2003; Frönicke 2005). Fusion chromosome MMY 13/23, displaying conserved synteny of HSA 11, is a common feature for Pteropodiformes but could probably also represent an element of the chiropteran ancestor.

Homologous chromosomes to ECU 7a and 8a, i.e. the major part of HSA 7 and the long arm of HSA 8, have been proposed as ancestral entities for Eutheria (Robinson and Ruiz-Herrera 2008). Therefore, it seems likely that these ECUs also consisted of one single chromosome each (homologs to MMY 12 and MMY 20) in the chiropteran ancestor. Centric fission of both elements must then have occurred independently in several lineages of both suborders (see Table 7.1). The same consideration could apply for ECU 18–20 (MMY 10), although the sequence combination in this ECU is chiropteran specific and has not been proposed for the eutherian ancestor.

All hitherto examined members of the suborder Pteropodiformes share two characters, inversions in the MMY 2 and MMY 5 homologous chromosomes, which can be regarded as synapomorphies for this suborder (Volleth et al. 2011). Further, phylogenetic relationships within this suborder proposed by DNA data are fully supported by cytogenetic results.

A definition of synapomorphic characters for the taxonomic levels within Vespertilioniformes, however, is a difficult task. The first occurrence of three fusion chromosomes in question, i.e. 5/6, 1/2 and 7i/7ii, during chromosomal evolution of this suborder has provisionally been assessed (see Fig. 7.2). Analyses of additional genera, especially from the superfamily Noctilionoidea, could provide further helpful information.

Concerning interfamilial relationships of this suborder, there exist two challenging points. One concerns the position of the endemic Malagasy family Myzopodidae. Results of recent DNA studies place Myzopodidae either as the basal branch of the Noctilionoidea (Miller-Butterworth et al. 2007; Teeling et al. 2012) or as early offshoot of the Vespertilionoidea (Eick et al. 2005; Meredith et al. 2011; together with *Taphozous*: Agnarsson et al. 2011). The cytogenetic investigation of *Myzopoda aurita* (Richards et al. 2010) revealed two interesting Robertsonian fusion chromosomes beneath many autapomorphic characters. One is the combination MMY 3/4 which is found only in Vespertilionidae and *Myzopoda*. Another fusion product found in *Myzopoda* is the combination MMY 9/11 which has also been reported for the phyllostomid genera *Glossophaga and Phyllostomus*, but not for Desmodontidae (Sotero-Caio et al. 2011). At present, it remains unclear whether these cytogenetic characters are the result of convergent evolution or indicate common ancestry. Surprisingly, own unpublished results from Emballonuroidea revealed some curious chromosomal similarities to the published karyotype of *Myzopoda*. Application of human WCPs could help to further characterise the rearrangements as being species specific or shared with other taxa. Interestingly, examination of the placentation in *Myzopoda* and comparison with the vespertilioniform superfamilies yielded no support for inclusion of *Myzopoda* in Noctilionoidea or Vespertilionoidea but some indications for a possible association of Myzopodidae with Emballonuridae (Carter et al. 2008). Altogether, the phylogenetic position of *Myzopoda* seems to be quite unsolved at the moment (see question mark in Fig. 7.2).

The second unsolved problem concerns the relations within the superfamily Emballonuroidea. The families Nycteridae and Emballonuridae have been shown to be close relatives in all molecular phylogenies (Eick et al. 2005; Miller-Butterworth et al. 2007; Meredith et al. 2011; Teeling et al. 2012). In addition, monophyly of Emballonuridae was shown in several analyses (Lim et al. 2008; Meredith et al. 2011; Miller-Butterworth et al. 2007; Ruedi et al. 2012; Teeling et al. 2012) with exception of Agnarsson et al. (2011). However, for the reason of the clear separation and age of diversification of Emballonurinae and Taphozoinae (about 50 million years ago), Ruedi et al. (2012) proposed to elevate both subfamilies to family status. In contrast to the above-mentioned results, cytogenetic analyses revealed no character which could serve as a synapomorphy for Emballonuroidea. In addition, we did not find any common chromosomal feature connecting *Taphozous* (Mao et al. 2008) and *Emballonura* (own unpublished results). Surprisingly, in the karyotypes of *Emballonura* and *Nycteris*, three similar features have been found which could hardly be interpreted as convergences (own unpublished results). Due to the highly rearranged karyotypes in both genera, however, the chromosomes mentioned are not completely identical, but show some differences. To demonstrate the discrepancy regarding the suggested relationships, the molecular-based phylogeny is shown in grey, the chromosomally deduced relationships in black in Fig. 7.2. Further studies with extended species coverage are certainly necessary to clarify these ambiguities.

7.5 Conclusions

Molecular and cytogenetic studies resolved interfamilial relationships in bats to a high extent. In contrast, the intrafamilial relations still need further clarification. Sampling of genetic data from hitherto not represented small bat families, and addition of further members of the large families should result in more robust phylogenies (Zwickl and Hillis 2002). Further, the description of new bat species should not only include morphological characters but also genetic features.

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Part II
Ecology and Behaviour

Chapter 8
Aeroecology

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Eli S. Bridge, and Thomas H. Kunz**

Abstract Aeroecology is an emerging scientific discipline that seeks to broaden understanding about the ecological function and biological importance of the aerosphere. The unifying concept of this interdisciplinary field is a focus on the aerosphere itself and the myriad airborne organisms that inhabit and depend upon this environment for their existence. In this chapter, we discuss the conceptual framework of aeroecology and underscore the technological advances that support for an interdisciplinary approach to studying the aerosphere.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_8, © Springer Science+Business Media New York 2013

8.1 Introduction

When we think about habitat for bats or other creatures, we often think about the vegetation communities associated with where an animal forages or the physical landscape features that an animal uses for periods of rest (e.g., caves). For volant animals like bats, the air through which they fly is also arguably a habitat. Air is a fluid medium utilized by bats and birds along with their prey for critical activities such as foraging, dispersal, and migration. The aerosphere—the relatively thin substratum of the troposphere closest to the Earth's surface that supports life—has long been studied in the context of meteorological conditions and functional ecosystem relationships, such as nutrient cycling and gas exchanges, but it has not been recognized as a separate ecosystem until relatively recently (Kunz et al. 2008).

Aeroecology is an emerging scientific discipline that seeks to broaden understanding about the ecological function and biological importance of the aerosphere (Kunz et al. 2008). The unifying concept underlying this new interdisciplinary field of study is a focus on the aerosphere itself and the myriad airborne organisms that inhabit and depend upon this environment for their existence. Biologists that study animals that use the aerosphere have typically focused on behavior, ecology, and evolution of specific taxonomic groups (e.g., vertebrates, arthropods) or specific physiological or behavioral functions, such as thermoregulation, water balance, respiration, or flight. The aerosphere has been studied extensively by atmospheric scientists and meteorologists with the goal of expanding our understanding of meteorology and ability to predict weather. Aeroecology provides a unifying framework for investigating how dynamic properties of meteorological conditions at local, regional, and global scales affect organisms that depend on air for foraging and movement (Fig. 8.1).

Marine biology has long been recognized as a stand-alone discipline that unites a diversity of scientists, including oceanographers, ecologists, and organismal biologists. The unifying theme in marine biology is the aqueous fluid of the oceans and the interactions of animals with that habitat. Similarly, aeroecology unites scientists that study the physical aerosphere with ecologists and organismal biologists that study species that use this medium as habitat. By connecting scientists across existing disciplines into a common framework, aeroecology advances collaborative science and addresses both fundamental scientific questions as well as applied research topics important for conservation.

Unlike aquatic ecosystems, no animal spends its entire life in the aerosphere. Yet many species spend a significant portion of their lives in this environment (Wilcove 2008). The aerosphere is a critical connective habitat for species that spend time in terrestrial or semiaquatic ecosystems but depend on the aerosphere for daily or seasonal movements. Because of their ability to move over large spatial extents, volant organisms such as birds, bats, and insects contribute to the ecological integrity of multiple ecosystems that span geopolitical boundaries linked by migration or dispersal through the aerosphere. Aeroecology provides a framework for understanding the effects of global phenomena such as climate change and anthropogenic alteration of diverse landscapes on biodiversity, global health, and ecological integrity

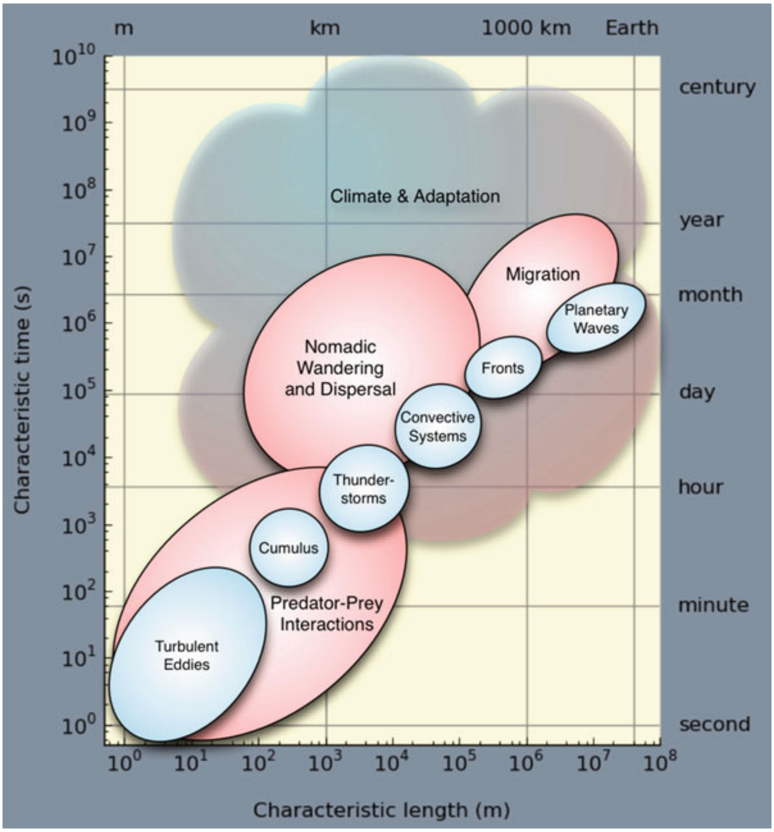

Fig. 8.1 Conceptual diagram of the spatial and temporal scales of common meteorological phenomena and movement behaviors of bats in the aerosphere. Areas of *overlap* indicate the scales at which movements of bats in the aerosphere can be influenced by prevailing meteorological conditions (copied with permission from BAMS)

(Kunz et al. 2008). Many of the questions addressed by aeroecology require the use of advanced technological tools because of the logistical challenges of studying animals in the aerosphere.

8.2 Important Questions in Aeroecology

Kunz et al. (2008) laid out a vision for aeroecology that incorporated both technological solutions for studying animals that use the aerosphere as well as outlining some of the key questions that unite aeroecology. Here we outline some of the cutting-edge work currently being conducted in aeroecology and discuss current advances in integrative approaches that hold promise for future endeavors in this arena.

8.2.1 Climate/Weather and Aerial Behavior of Bats

Predicting impacts of climate change on animal populations requires understanding how animals respond to variation in climate and weather patterns. Recent studies have shown that changes in seasonal climate, specifically drought, can have negative impacts on fitness in some bat species, including reproductive rates (Adams 2010) and annual survival (Frick et al. 2010b). Studies that address how both climate and weather conditions influence movement and foraging behavior of bats in the aerosphere will add to understanding how long-term shifts in climate may influence bat populations.

Anthropogenic climate change has caused shifts in phenology, such as spring arrival, emergence, and reproduction of many temperate species (Both and Visser 2005). Aeroecological investigations in phenology and migratory behavior should provide new information about phenology and whether bats are at risk from decoupling of resource–consumer interactions, as has been shown for some migratory passerines (Visser et al. 2005). Long-term monitoring of phenological patterns, such as arrival and departure from colonial roosts, can determine how bat populations respond to shifts in climate. Emergence counts and arrival/departure times from colonies can be done with traditional approaches of visual inspection on the ground or through remote-sensing technologies, such as radar, for species that fly at sufficient altitudes to be detectable (e.g., *Tadarida brasiliensis*) (Frick et al. 2012). Following bats during migration has thus far not been logistically feasible, except for very large-bodied bats outfitted with global positioning system (GPS) or satellite telemetry (Smith et al. 2011; Tsoar et al. 2011). Answering questions about long-distance migratory patterns will require technological advances in individual tracking devices that are currently in development coupled with integration of various remote-sensing instruments such as networked radars.

We still know relatively little about the scale of movements in most bat species. Numerous studies over the past several decades using small radio-tracking transmitters on bats have advanced knowledge considerably about selection of roost types and local foraging movements (Amelon et al. 2009). Given the relatively short life span of these types of devices (usually 1–2 weeks) and the labor necessary for tracking nightly foraging movements, there is still a considerable gap in knowledge about both seasonal movements of bats and how factors such as weather and prey availability influence foraging behavior. Technological advances in both individual tracking devices and remote-sensing systems such as radar offer promising opportunities toward breaking some of the logistical barriers to empirically investigating these questions.

8.2.2 Population Monitoring

Although estimating population sizes and determining population trends is not exclusive to aeroecology, there is some exciting work being done on censusing bats at large colonies and tracking changes in colony size using thermal infrared imaging, near-infrared video, and radar technologies under the umbrella of aeroecology. Monitoring bat populations is important for determining impacts of natural and anthropogenic stressors on populations and developing effective conservation strategies (Hayes et al. 2009; O'Shea et al. 2003). Counting bats at hibernacula has been a traditional method of monitoring bat populations for species that hibernate in known hibernacula (Kurta and Kennedy 2002). This technique is highly valuable and has been pivotal in estimating impacts of white-nose syndrome (WNS) on bats in eastern North America (Frick et al. 2010a; Langwig et al. 2012). For species that do not hibernate, however, estimating bat population sizes and trends through time remains challenging (O'Shea and Bogan 2003).

In particular, accurately estimating colony sizes for large colonies of bats, such as Brazilian free-tailed bats, has been a long-standing challenge. The development of thermal infrared video provides a reliable method for censusing bat colonies and has been used successfully for estimating colony sizes (Kunz et al. 2009), including very large aggregations of Brazilian free-tailed bats (Betke et al. 2008; Hristov et al. 2010). Betke et al. (2008) developed computer vision algorithms for counting individual bats in a field of view with thermal infrared imagery. These methods have led to insights about population trends (Betke et al. 2008) and daily and seasonal fluctuations in colony size (Hristov et al. 2010).

Radar provides a potential alternative means of estimating bat populations for certain species. There are two basic approaches to using radar for estimating colony sizes of bats. Small, portable radars capable of fine spatial resolution can be positioned near a bat colony and used to observe and count individuals as they depart. This method is akin to the thermal infrared imagery technique. Conversely, larger and more powerful radars that exist as part of weather radar networks can detect bats and other aerial organisms as they scan the aerosphere in the vicinity of a particular colony site. In this case, the number of individuals is inferred from the strength of the received radio wave signal scattered by a bat or collection of bats. Using radar for estimating numbers of bats is a developing method and needs further validation, for example, through the use of complementary observations from thermal infrared video. As we discuss in more detail below, both of these radar approaches are associated with various strengths and limitations.

8.2.3 Aeroecology for Conservation

The aerosphere is affected by human activities and conditions such as air pollution, artificial light sources, and anthropogenic structures (e.g., skyscrapers, communication towers, and wind turbines) that may cause direct mortality or disruption of activities such as feeding, dispersal, migration, and courtship (Kunz et al. 2008). Habitat destruction and modification can also alter migratory or foraging activities in the aerosphere. Effective conservation policies will depend on understanding how bats are affected by these myriad threats in the aerosphere.

Mortality of bats from collisions with wind turbines at wind energy facilities has caused considerable concern for bat conservation (Kunz et al. 2007a, b; Arnett et al. 2008). Studies have shown that fatalities at wind turbine facilities are predictable based on seasonal periods (fall migration) as well as daily meteorological conditions, such as wind conditions (Arnett et al. 2011, Chap. 20). Determining migratory patterns of bats and how migratory movements are influenced by meteorological conditions will help identify high-risk areas for siting wind facilities. Remote-sensing technologies such as radar and thermal infrared video, especially when coupled with tracking devices, are valuable resources for studies on fatalities associated with wind energy facilities. A better understanding of migratory routes and relationships between weather and bat movements will be key for developing effective conservation for species affected by wind energy development.

8.3 Tools Used in Aeroecology

Investigating the behavior and movements of bats in the aerosphere has always been and will remain logistically challenging. Aeroecology addresses this challenge head on through an approach that focuses on technological advances and integration across multiple technologies and scientific disciplines. We discuss some of the key technological tools in an aeroecologists' toolbox with attention to how they can be used now or in the near future for advancing aeroecology. This is not meant to be an exhaustive list, but rather highlights certain tools we feel are particularly helpful or promising. Although acoustic recording devices are an important and rapidly evolving technology for aeroecological studies, we reference readers to chapters in this volume and elsewhere (Parsons and Szewczak 2009) dedicated to this topic.

8.3.1 Radar Aeroecology

Remote-sensing tools offer opportunities for investigating ecological processes at spatial and temporal scales that have traditionally thwarted authoritative understanding of ecological dynamics in the aerosphere. The capacity of radars to detect biological scatterers in the aerosphere has been known for over 60 years (Lack and Varley 1945), and much has been learned about the aerial behavior of birds, bats, and flying arthropods through the assistance of this technology. For the most part, these studies have been conducted using small radars that were specifically adapted for observations of volant species (e.g., Bruderer et al. 1999; Chapman et al. 2011; Harmata et al. 2003). Since these studies have largely been taxa dependent, some investigators have self-classified their work as belonging to either radar ornithology or radar entomology. Here, we adopt a taxonomically broader and more integrative approach under the name of radar aeroecology (Chilson et al. 2012b). Several overview papers have been written that discuss the utility of radar for biological studies (Diehl and Larkin 2002; Chilson et al. 2012b; Gauthreaux and Belser 2003).

A considerable boon to biological studies using radar and one that has helped lead to the advancement of radar aeroecology as an integrative discipline has been the development of operational networks of radars for the observation of weather. Gauthreaux began using weather radars in the USA for biological research not long after these facilities were established in 1959 (Gauthreaux and Livingston 2006). In the interim, many others have followed suit using networked weather radars across several countries (Buler and Moore 2011; Diehl et al. 2003; Dokter et al. 2011; Horn and Kunz 2008; Kelly et al. 2012; van Gasteren et al. 2008). As one example, we consider how the network of weather radars within the USA are being exploited for aeroecological studies.

The NOAA National Weather Service (NWS) maintains and operates 159 Weather Surveillance Doppler Radar (WSR-88D) installations collectively known as NEXRAD. This network provides near-continuous coverage of the airspace corresponding to roughly the lowest 10 km for the conterminous continental USA. NEXRAD stations regularly detect scatter from airborne animals, including bats, birds, and insects (bioscatter) (Fig. 8.2). Data are updated every 5–10 min and the entire data archive, which goes back to the early 1990s, is now publicly available through the National Climatic Data Center (NCDC). The benefits and uses of NEXRAD for weather monitoring and forecasting are well demonstrated; however, the use of this radar network for aeroecological studies focused on bats remains limited (Frick et al. 2012; Horn and Kunz 2008).

Weather radars probe the surrounding airspace using a particular volume coverage pattern (VCP) suitable for prevailing meteorological conditions. Data are reported and stored in spherical coordinates with observations binned into discrete units of azimuth angle, elevation angle, and range with the origin of the coordinate system located at the radar installation. For each bin, three conventional radar products are reported: radar reflectivity factor (Z), radial velocity (v_r), and spectrum width (σ_w). The measure of backscattered intensity, radar reflectivity factor (Z), can be directly related to the number of aerial organisms occupying the aerosphere (Chilson et al. 2012c) and therefore is the appropriate measure for identifying aggregations of animals for phenological studies (Kelly et al. 2012) or estimating aerial densities (Chilson et al. 2012c). Radial velocity (v_r) can be used to detect patterns in migration by looking at flight directions and speeds of bioscatterers (Gauthreaux and Belser 1998).

A recent and key innovation for increasing the utility of NEXRAD data for ecological studies is the visualization of NEXRAD data in real time in a Cartesian grid that mosaics across different radar installations (Fig. 8.2). Radar data from NEXRAD are fused with observations from other meteorological instruments into one collective data product provided by the National Atmospheric and Oceanic Administration (NOAA) National Severe Storms Laboratory (NSSL). This database is known as the National Mosaic and Multi-Sensor Quantitative Precipitation Estimation (NMQ) system (nmq.ou.edu). Within this framework, NEXRAD data are ingested, controlled for quality, and combined to form a three-dimensional map of Z, which is projected onto a Cartesian grid (Zhang et al. 2004, 2011). The horizontal resolution of the NMQ output is 1 km with 31 vertical levels and a temporal resolution of

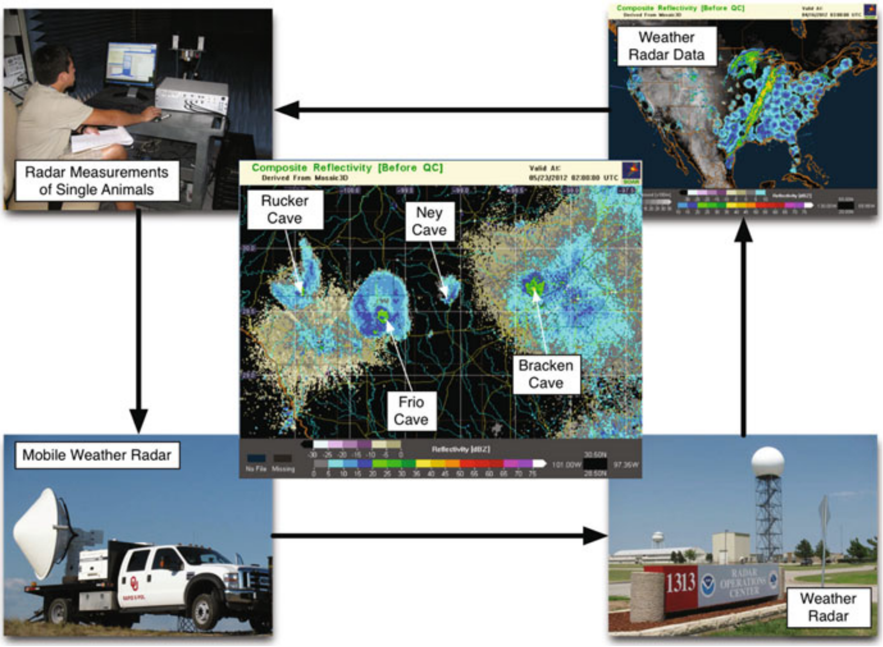

Fig. 8.2 Diagram showing how multiple-instrument sampling is being used to develop quantitative tools for aeroecology. Radar measurements of single animals are conducted in an anechoic chamber (*top right*) and are used in conjunction with both portable radars (*bottom left*) and individual fixed radar installations (*bottom right*) to calibrate and interpret received radar signals from bioscatter. Radar data from networked fixed stations are mosaicked and mapped into a Cartesian grid system (*top right*) that shows continental-scale patterns of bioscatter activity. These maps can be viewed in near real time to watch biological phenomena such as emergences of Brazilian free-tailed bats (*Tadarida brasiliensis*) in south-central Texas (*center*). Emergences are identifiable as distinct clouds of radar reflectivity associated with known cave locations

5 min. Data are displayed as two-dimensional map of composite maximum reflectivity values across the conterminous continental USA. Currently, there is a biologically oriented companion website supported by NSSL called SOAR—Surveillance of the Aerosphere—using Weather Radar that displays the composite reflectivity (CREF) before bioscatter signal has been filtered out for improving meteorological predictions (http://soar.ou.edu).

The advantage of the NMQ/SOAR system is that it allows ecologists to view in real time (and from their desk chair) the behavior of organisms in the aerosphere at unprecedented spatial scales (Fig. 8.2). For example, it is possible to view emergences of Brazilian free-tailed bats from cave and bridge roosts in south-central Texas (Fig. 8.2) and determine patterns of daily timing of emergences as well as seasonal arrival/departures. Novel foraging behaviors of bats in the aerosphere are also being revealed. For example, Fig. 8.3 shows radar reflectivity patterns that suggest that Brazilian free-tailed bats emerged from Frio Cave and foraged for insects aggregated by outflowing winds produced by storm systems. These types of

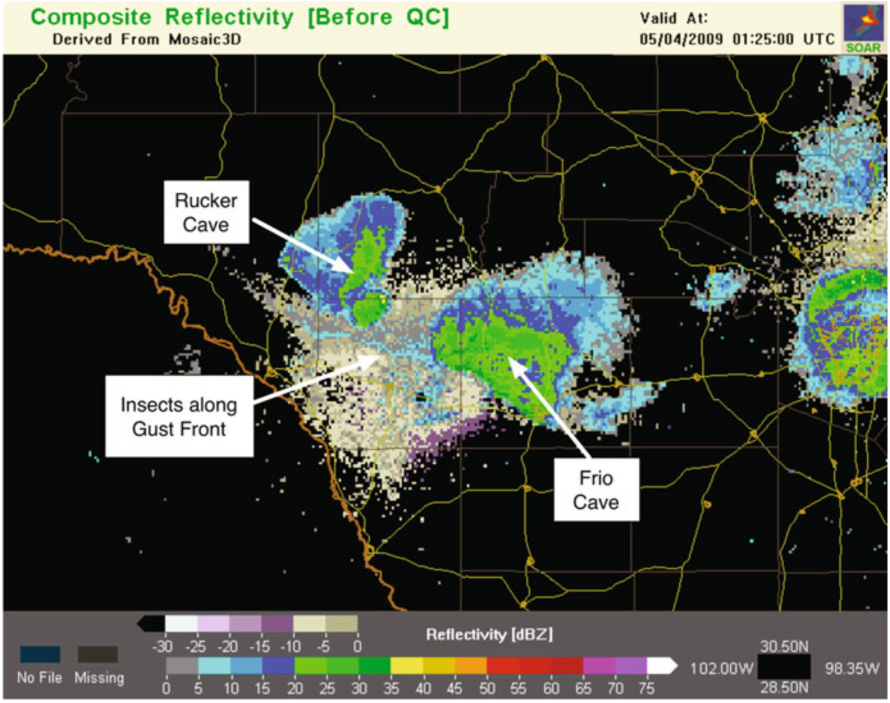

Fig. 8.3 Radar visualization from the SOAR website (soar.ou.edu) showing insects aggregated along a storm-generated gust front and Brazilian free-tailed bats (*Tadarida brasiliensis*) emerging from cave roosts and foraging along the insect buffet

visualizations and observations can help generate hypotheses about behavior of bats in the aerosphere and how meteorological conditions can drive predator–prey interactions in the aerosphere.

Although many insights regarding behavior and distributions of animals in the aerosphere can be made by investigating visualizations of NEXRAD radar imagery, more quantitative interpretations of radar data are needed. Persistent and significant limitations remain in using existing operational systems such as NEXRAD for biological monitoring and aeroecological research. In part, these problems stem from (1) lack of a robust biological nexus within the radar community and conversely a lack of familiarity of radar products among ecologists, (2) absence of radar product outputs focused on bioscatter, and (3) the manner in which radar output data are filtered prior to release to the public (Chilson et al. 2012b). A workshop on radar aeroecology was convened in 2012 at the National Weather Center in Norman, Oklahoma, to address these and other issues (Chilson et al. 2012a). Realizing the full potential of radar aeroecology will require advances in (1) validation studies based on theory and experiments in the laboratory to evaluate reflectivity measurements provided by radars, (2) multi-instrument sampling in the field that can validate approaches across different radar platforms, and (3) development of tools and techniques for mining radar data in concert with field observations and other remotely sensed data.

Meteorologists have long used multiple-instrument sampling techniques for ground-truthing observations and integrating information across sampling domains to quantify characteristics and behavior of weather phenomena (Chilson et al. 2012b; National Resource Council 2009). This same technique of using mobile radars in conjunction with NEXRAD installations is also being employed in the field of radar aeroecology with the aim of using radar data for quantifying densities of animals in the aerosphere. Figure 8.2 depicts a multi-instrument sampling approach that aims to use measurements of individual animals in the laboratory to permit quantitatively interpretations of large-scale observations of bioscatter over continental scales. Reflectivity of individual animals can be measured in the laboratory to determine the backscattering radar cross section (RCS) of an individual animal (e.g., bat). These RCS measurements are necessary for estimating aerial densities of a species from radar reflectivity values (Chilson et al. 2012c). Some smaller portable radars can count discrete echoes which can be used to calibrate reflectivity values from NEXRAD and other large radars, and this method has been successful in estimating bird densities aloft (e.g., Diehl et al. 2003; Dokter et al. 2011). Small portable radars are also useful for helping scale from individuals to groups of bioscatterers (e.g., bats) by having finer spatial resolution than large radars typical of NEXRAD. These portable radars can also be oriented toward biological aggregations of interest (e.g., a bat roost). Radars in the NEXRAD network are at fixed locations, but the data are continuously collected and archived, which offers considerable advantages for long-term monitoring. The ultimate goal is to enable use of NEXRAD data for estimating population sizes so that long-term monitoring of bat species such as Brazilian free-tailed bats can be accomplished through current remote-sensing efforts. Use of the NEXRAD data network for population monitoring would permit retrospective analyses going back through the life of the archive (20 years) as well as continuous monitoring into the future.

Estimating population densities at ground-truthed point localities such as known bat roosts appears quite promising. However, use of this technology for estimating densities during migration is currently limited by the inability to discriminate taxonomic origin of received signals. However, many weather radar installations are now capable of supporting polarimetric operation, which should greatly enhance abilities to discriminate among types of biological scatterers. Whereas conventional weather radars transmit and receive radio waves with a single polarization, polarimetric weather radars use two orthogonally aligned radio waves (Fig. 8.3). Information related to size, shape, and orientation of a scatterer can be extracted by comparing the amplitudes and phases recorded for the different polarizations (Chilson et al. 2012b; Mueller and Larkin 1985; Zrnić and Ryzhkov 1998). Consider, for example, a collection of bioscatterers sampled by a weather radar consisting of both moths and small bats (Fig. 8.4). If the sampled bioscatter predominantly results from one of the two species (either bats or insects), then the resulting polarimetric signature, which is unique to the type of animal in this case, can be used for taxonomic discrimination.

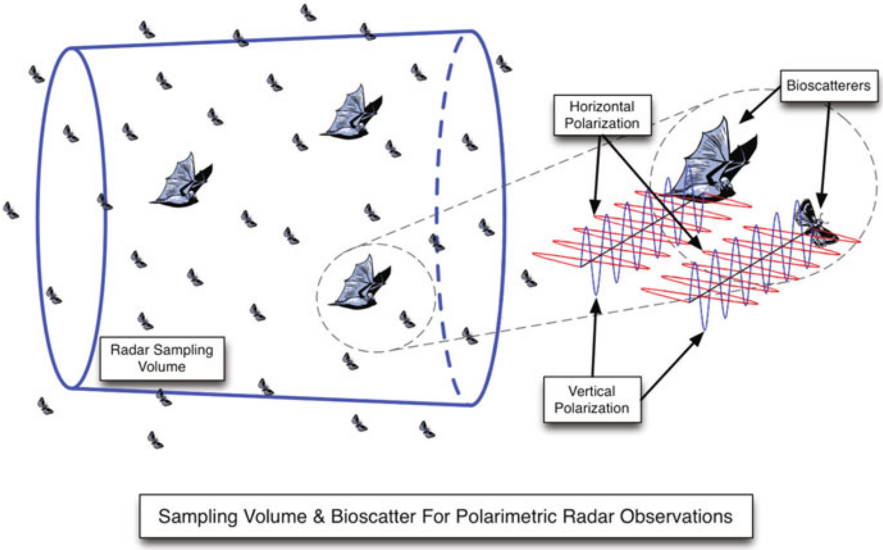

Fig. 8.4 Depiction of a radar sampling volume containing moths and bats. Resulting bioscatter signal contains contributions from both species. Depending on the relative numbers of each species, the backscattered signal may be dominated by one species over the other. Also shown is an illustration of polarimetric radio waves propagating through the aerosphere before interacting with the bioscatterers contained within the sampling volume

Overall, radar aeroecology holds much potential for contributing to understanding of many aspects of bat ecology and conservation, including phenology, foraging behavior of bats in the aerosphere, and long-term population monitoring of targeted species. Certain bat species cannot be studied effectively with radar, particularly those that fly under forest canopies. The Brazilian free-tailed bat has been the classic model organism for radar aeroecology in the USA. Species that aggregate in large groups and fly in open habitats will be good candidates for studies in radar aeroecology that focus on phenology and population monitoring. The ability for dual polarization to help discriminate taxonomic groups in the aerosphere will help increase the utility of radar for migratory studies of bats.

8.3.2 Thermal Imaging

Since the initial development of commercial handheld thermal infrared cameras in the 1980s, ecologists have used thermal cameras as an approach to understanding ecophysiology (e.g., Reichard et al. 2010). Many studies have shown that thermal infrared cameras can provide information about metabolic rates, thermal regulation, and water loss. While the studies are not usually considered aeroecology studies per se, when applied to volant animals, they can be quite revealing. There are very few techniques available that can provide reliable information about the energetics of flight collected through noninvasive means.

Recent advancements in thermal infrared technology have led to wider applications for ecological studies (Hristov et al. 2008). Even the most basic models of thermal infrared cameras have the single most important advantage over visual light camera systems: the ability to see in the dark. This capability allows for imaging bats roosting or moving inside caves, emerging from roosts, commuting across the landscape, and foraging. These behaviors have typically been difficult to observe with other technologies. Thermal infrared video overcomes limitations of normal photography or videography in low light situations that cause blurring and narrow depth of field. There are also benefits to using thermal infrared cameras in daylight, such as obtaining high-contrast images that are valuable for computer vision analysis.

Thermal infrared videography has played an integral role developing aeroecology as an integrative discipline across fields. This collaborative integration has raised interest in how a single data stream can be used for multiple scientific lines of inquiry. For example, while biologists may be interested in understanding movements of individual bats and observing behaviors such as insect capture and obstacle avoidance, collaborators in more technical fields, such as computer science and engineering, may be interested in understanding the movements of large groups of entities in 3D space. Data acquisition and analysis for multiple purposes requires cross-disciplinary communication and understanding.

A very recent and successful contribution of thermal infrared cameras to aeroecology has been their use in censusing large colonies of bats, specifically Brazilian free-tailed bats (Betke et al. 2008; Hristov et al. 2010). It has long been the goal of many ecologists to estimate abundances and understand the ecological role of bats (Kunz et al. 2009). In particular, the large colonies of Brazilian free-tailed bats that form in caves and under overpass bridges in Texas have long fascinated ecologists as they constitute some of the largest aggregations of mammals in the world. Earlier estimates of numbers of bats were based on error-prone methods of counting and led to unreliable estimates of colony size (Betke et al. 2008). A reliable method of estimating colony size was necessary for tracking population trends through time and determining the ecosystem services of these bats to the agricultural industry in Texas (Cleveland et al. 2006).

By taking advantage of a strong collaborative effort between computer science and biology, Betke et al. (2008) developed several tracking and counting algorithms to be used with thermal infrared video of emerging Brazilian free-tailed bats. To collect these videos, a FLIR Merlin system (including a camera and associated computer station) was deployed perpendicular to the flow of bats, usually with a backdrop of sky, which allowed for the highest degree of contrast, and video was recorded throughout emergence (Fig. 8.5). These videos were then processed using standard computer vision techniques, such as background subtraction, to locate moving heat signatures that were identified as likely to be bats. These bats were then tracked from frame to frame using recursive Bayesian filtering and counted as they left the

Fig. 8.5 Thermal imaging can be used to produce high-contrast, high-resolution imagery that can be used by a variety of researchers. (**a**) A visual light image of *Tadarida brasiliensis* emerging from Frio Cave. Thermal cameras are arranged such that their field of view includes the bats with a backdrop of the sky. (**b**) The resulting images provide unprecedented detail of bat flight and group behavior (photo credit N.W. Fuller)

frame of view. When compared to hand-annotated videos of emerging bats, the automated census system was extremely effective and reliable in its final counts (Betke et al. 2008).

Thermal infrared imaging is also being used to describe the movements of organisms in three-dimensional space as they fly through the aerosphere and interact with various types of clutter, including self-clutter (i.e., other bats), stationary obstacles (e.g., trees), and moving obstacles (e.g., predators). Information such as angle of approach, turning radius, 3D wing position, angular velocity, proximity of approach, 3D group structure, and group behavior can be determined using multiple calibrated camera views, each focused on an overlapping frame of view. Understanding the mechanics of three-dimensional flight of animal groups can provide general insights about group structure and individual behavior (Parrish and Hamner 1997). Three-dimensional data from thermal imaging can also be combined with other technologies to better understand the way forest structure influences movement and flight of bats. Research has shown that forest structure plays an important role in bat flight (Lacki et al. 2007). Recent advances in under-canopy LiDAR systems have allowed for even greater resolution of animal flight within forests. For example, data gathered through these analyses has the potential to provide novel research directions and valuable insight into the biomechanics and mastery of flight.

8.3.3 Tracking Individuals

A full understanding of the aggregate phenomena observable by radar and thermal imaging must take into account the motivation and constraints that influence the behavior of individual animals. We must, therefore, give some attention to the activities of the individual to understand the mechanisms that give rise to the emergent properties of groups and populations. Tracking the movements of individual bats is particularly challenging due to the fact that bats are small yet move rapidly over great distances. The majority of bat species weigh less than 20 g (Smith et al. 2003), which severely limits the types of tracking devices that can be attached to bats without unduly affecting their behavior (Bridge et al. 2011). For large bat species, the possibilities for tracking individuals are more expansive. There have been numerous successful efforts to employ satellite tracking to studies of bats, and there are even published guidelines for what equipment is most appropriate for different species (Smith et al. 2011). The smallest signal-transmitting devices capable of functioning on a global scale are satellite transmitters that weigh on the order of 5 g, which is far too large for most bats. Although we can expect increasing miniaturization of complex electronics, a significant decrease in the mass of traditional satellite transmitters is unlikely due to fundamental constraints on the energy requirements of long-distance transmission and the chemical nature of batteries. Unless there is a breakthrough in battery technology, traditional satellite tracking will not be possible for small bats.

Geolocation dataloggers weighing less than 1 g have provided some of the first renderings of migrations by small birds (Bächler et al. 2010; Stutchbury et al. 2009), but these devices rely on determining the precise times of sunrise and sunset, which limits their use on many bat species due to their roosting habits. Moreover, these tracking devices rely on recapturing tagged individuals to obtain data, and such a scenario may be unlikely for many bat species. Nevertheless, there may be some potential for geolocation dataloggers to provide insights into the long-distance movements of some species, particularly tree-roosting bats (Holland and Wikelski 2009).

The future is much more promising with regard to short-range tracking. The requirements for battery power limit the degree of miniaturization possible for radio transmitters. Nevertheless, one can now find transmitters as small as 0.11 g (MacCurdy et al. 2011). The range and longevity of such small transmitters are quite limited (on the order of 2 weeks and 4 km depending on the design), but this high degree of miniaturization enables tracking individuals of even very small bat species. Radio transmitters are not only getting smaller, but radio telemetry systems are becoming more sophisticated. Traditionally, tracking movements of individuals with radio transmitters relied on labor-intensive methods, such as following individuals across the landscape with a portable receiver and antenna, or crude location estimates based on triangulation from multiple directional antennas. In addition, the number of tags that could be monitored at once was usually limited because each tag had to operate at its own frequency. Emerging technologies have automated tracking endeavors and increase the accuracy of location estimates. Moreover, signals from radio tags can be coded such that multiple tags can operate on the same frequency. For example, MacCurdy et al. (2009) have demonstrated a tag location system, in which specialized receivers can discern differences in the arrival time of transmitter signals such that the system can determine the relative distances between an individual tag and several known locations. Once implemented, this sort of “reverse GPS” system can automatically generate locations for an individual every few seconds.

The holy grail of radio tagging—global coverage via a unified set of space-based receivers—is the goal of the International Cooperation for Animal Research Using Space or ICARUS. This system proposes to use devices very similar to traditional radio transmitters in conjunction with highly sophisticated receivers in space to track individual animals. Tests of the system have been carried out using airplanes, and plans have begun for deployment of the first space-based receivers. If the system works as anticipated, ICARUS could provide global coverage for transmitters weighing on the order of 1 g.

Radio-frequency identification (RFID) is another promising technological field that has great potential for tracking individual bats. RFID generally refers to short-distance wireless communication on the order of a few meters to a few centimeters between a small, passive tag and a powered interrogator or reader. The reader emits a carrier wave that powers a transmission from the tag such that the tag does not need a battery. There are forms of RFID communication that use powered tags and that can function over relatively large distances (tens of meters), but most RFID systems have passive tags and limited read range. Passive Integrated Transponders or PIT tags are now familiar to most biologists. These RFID tags are commonly used in animal studies and can weigh less the 0.1 g. Because they do not require a battery, their service life is typically longer than the life span of a bat, and they have already been used to monitor the behaviors of individual bats (e.g., Kerth et al. 2011).

The primary limitation to RFID systems is that their extremely limited read ranges generally restrict their use to situations in which one is monitoring a very localized resource or area (e.g., a small cave entrance). Typical low power systems, such as those that use PIT tags, cannot really track individual movement paths. However, high-frequency systems are in development that can locate individual tags by employing a network of power nodes connected to reading units. Systems of this sort would be able to track individual movements among the network of power nodes, which would typically be an area equivalent to a large room or gymnasium.

In some situations, geographic information can be inferred from intrinsic biomarkers, such as stable isotopes and DNA, which are, of course, free of any size constraints (Cryan et al. 2004; Robinson et al. 2010). Detailed movement paths are beyond the reach of these tools, but if adequate background data are available, they can provide coarse estimates of breeding or wintering locations and/or link groups of individuals to regional populations (Hobson and Wassenaar 2008). Combining the inference from these techniques to generate probability landscapes has led to surprisingly precise geographic assignments in a limited number of bird species (T. Smith and M. Wunder, personal communication), and there is potential for this technique to yield insights into connectivity and movement of bat populations as well.

Not only does tracking individuals have scientific value, but it also provides a means of educating and engaging the public. People identify with individuals, and describing a life cycle from the perspective of an individual bat is appealing to communicate about bats to the general public. Tracking individuals will undoubtedly provide key insights into important issues affecting bats and humans today, including disease transmission, mortality at wind turbines, and consumption of insect pests, and perhaps the narratives revealed by tracking technology will help raise public awareness to these important issues.

8.4 Conclusions

By focusing on integrating across a diversity of disciplines and technological innovations, aeroecology is furthering scientific investigations in a diversity of research arenas. For example, aeroecology is at the forefront of research on topics such as daily and nightly dispersal, migratory patterns, foraging behavior, distribution and quantification of aerial biomass, aerial biodiversity, phenological patterns related to climate change, and impacts of land-use policy. In addition to basic scientific research, aeroecology has the potential to make significant contributions to human society by providing information on issues ranging from aviation safety, agricultural productivity, and siting of wind energy facilities.

A pressing need exists for scientists to identify and use creative technological and analytical solutions for understanding biological phenomena at broad spatial and temporal scales in the aerosphere, given global threats to biodiversity, emerging infectious diseases, and the need to sustain ecological integrity (Kunz et al. 2008). By developing new analytical and interpretive tools from existing and emerging technologies, aeroecology encourages scientists and citizens from a diverse set of disciplines to tackle contemporary conservation and ecological questions in unprecedented ways.

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Chapter 9
Response of Bat Diversity to Forest Disturbance in Southeast Asia: Insights from Long-Term Research in Malaysia

Tigga Kingston

Abstract Southeast Asia is home to over 25 % of the world's bat fauna, but rapid rates of deforestation and habitat degradation threaten species across the region. Ecological bat research in Southeast Asia, which has hitherto lagged far behind that of Neotropical countries, is now gaining momentum with burgeoning efforts to assess the response of bat diversity to anthropogenic habitat modification. Central to interpretation of diversity patterns in disturbed landscapes is an understanding of assemblage composition and dynamics in relatively unmodified habitats. Fifteen years of research in a Malaysian primary rainforest has provided some understanding of bat assemblage structure and spatiotemporal variability in old-growth forests and has generated predictions of ensemble vulnerability that have largely been supported by empirical studies in the surrounding fragmented landscape and other parts of Southeast Asia. The forest-interior insectivorous ensemble, members of the Rhinolophidae, Hipposideridae, Kerivoulinae, and Murininae, is particularly vulnerable to forest loss and degradation, and this group can be further subdivided by their roosting ecology. By virtue of their greater vagility, cave-roosting forest-interior species are somewhat more resilient to fragmentation and degradation than species that depend on forest structures for roosts (cavities, foliage). However, as disturbance intensifies, cave-roosting species are also lost from assemblages and are further imperiled by cave disturbance. I advocate that future research focuses on testing the generality of the findings and predictions from the Malaysian landscape in other parts of Southeast Asia and on determining differential vulnerability within the other Southeast Asian bat ensembles.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_9,

9.1 Southeast Asian Bat Diversity, Threats, and Research Effort

By far the greatest proportion of the world's bat diversity resides in tropical ecosystems (Findley 1993; Willig et al. 2003), but habitats in these ecosystems are some of the most imperiled in the world, subject to rapid degradation, extensive conversion to anthropogenic uses (FAO/JRC 2011), and uncertain futures under synergistic interactions with climate change (Brodie et al. 2012). Our knowledge of bat ecology remains limited in much of the tropics, but in Southeast Asia the need for greater understanding that can underpin conservation action is particularly urgent as only 15 % of primary forest remains (FAO 2010) and less than 10 % of forests are protected (Sodhi et al. 2010a). Deforestation alone may precipitate the loss of as many as 40 % of the region's bat species by the end of this century (Lane et al. 2006).

Southeast Asia comprises 11 countries (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Timor-Leste, Vietnam) grouped into 4 biogeographic subregions: Indochina, Sundaic, Philippines, and Wallacea. The region is subject to rapid rates of land-use conversion yet is characterized by high biodiversity and endemism and is of critical importance for global conservation efforts (Brooks et al. 2006; Sodhi et al. 2010a; Miettenin et al. 2011). It encompasses 34 of the Global 200 Ecoregions (17 %) prioritized by WWF for conservation (Olson and Dinerstein 2002), three (Indonesia, Malaysia, Philippines) of the 17 Megadiversity countries identified by Mittermeier et al. (1997), and 7 of the 20 hotspots of latent extinction risk for mammals (Cardillo et al. 2006), and each of the 4 biogeographic subregions has been identified by Conservation International as Biodiversity Hotspot (Mittermeier et al. 2005; Myers et al. 2000). Not surprisingly, the density of threatened vertebrates in Southeast Asia is among the highest in the world, and the region is the global center of vertebrate extinction risk increases of the past three decades (Hoffman et al. 2010).

Southeast Asia is home to over 25 % of the world's bat diversity. Kingston (2010) reported 330 species for the region, 320 from Simmons (2005), and 10 newly described species to 2007. There have since been a further 12 species described: *Desmalopex microleucopterus* (Esselstyn et al. 2008), *Thoopterus suhaniahae* (Maryanto et al. 2012), *Rhinolophus thailandensis* (Wu et al. 2009), *Hipposideros einnaythu* (Douangboubpha et al. 2011), *H. griffini* (Thong et al. 2012), *Murina cinerea*, *M. beelzebub*, *M. walstoni* (Csorba et al. 2011), *M. eleryi* (Furey et al. 2009), *M. harpioloides* (Kruskop and Eger 2008), *Glischropus bucephalus* (Csorba 2011), and *Myotis phanluongi* (Borisenko et al. 2008). Over 7 % of Southeast Asia's 342 species have been described since 2000, and species discovery rates are likely to remain high, particularly with greater use of molecular techniques (Francis et al. 2010; Khan et al. 2010) and collaborative efforts across species' ranges (Kingston 2010).

Unfortunately, much of the region's bat diversity is at risk. About 20 % of the 323 known Southeast Asian species assessed by the IUCN are currently regarded as threatened or near threatened (assessed as Near-Threatened, Vulnerable, Endangered, or Critically Endangered), with another 20 % Data Deficient (IUCN 2010). This masks the true picture as population trends, quantifications of which underpin the Red List categorizations, are unknown in 57 % of species, with populations decreasing in 24 % of species, and stable in only 18 % of species. Populations of just one species, *Cynopterus sphinx*, are believed to be increasing. Moreover, explicit causes of decline (i.e., threats) are listed for over a third of species and are likely to precipitate further measurable declines should they persist, particularly as many bat species are subject to multiple threats. As might be expected, forest loss and logging are the most prevalent threats to bats, but hunting of bats (for consumption, traditional medicine, and as a control of orchard pests), quarrying (of caves), and urbanization are also major drivers of population declines (Fig. 9.1; Kingston 2010).

Despite the great diversity of species and the intensity of threats, bat research in Southeast Asia is only now gaining momentum and lags Neotropical efforts by at least 20 years. As a crude measure of the differential research effort between Southeast Asia and the Neotropics, a Google Scholar advanced search of articles with the words Chiroptera and any of the 11 Southeast Asian countries reported 4,270 publications (as of March 2012), whereas that of Chiroptera and the 21 mainland Neotropical countries from Mexico to Argentina and the nations of the Greater Antilles reported over 15,100, despite roughly equivalent species richness (~340 species). Moreover, of the 823 Mammalian Species accounts published to date, 215 are of bats, of which 134 are of Neotropical compared to 48 of Palaeotropical species, with only 8 species from Southeast Asia. Although this may in part be a reflection of the lack of familiarity with Mammalian Species outside the Americas (as it is a publication of the American Society of Mammalogists), it gives some indication of the state and distribution of bat knowledge, as each account "summarizes the current understanding of the biology of an individual species including systematics, distribution, fossil history, genetics, anatomy, physiology, behavior, ecology, and conservation" (ASM 2012).

Nonetheless, the last 10–15 years has seen a substantial increase in research effort, with just fewer than 3,000 publications since 2001, more than two-thirds of the total reported by the Google Scholar search. However, despite the intensity and immediacy of threats to Southeast Asian bat diversity, relatively few publications have focused on the response of bats to the rapid land-use changes underway in the region (but see Fukuda et al. 2009; Furey et al. 2010; Hughes et al. 2012; Phommexay et al. 2011; Turner 2011, and prior work summarized in Kingston 2010), yet the long-term persistence of much of Southeast Asia's diversity will ultimately hinge on species' ability to persist in landscapes that have been modified by man (Sodhi et al. 2010b). Comparative studies of diversity under different land uses and configurations of land-use elements will play a critical role in elucidating management strategies that maximize biodiversity, as well as the development of certification schemes that incentivize management changes [e.g., the UN's Reducing Emissions from Deforestation and Forest Degradation (REDD) program and High Conservation Value Forests of the Forest Stewardship Council]. Meaningful interpretation of such studies, however, depends in large part on understanding assemblage diversity and dynamics in relatively unmodified habitats and landscapes, as this provides the baseline for assessing the value of modified landscapes.

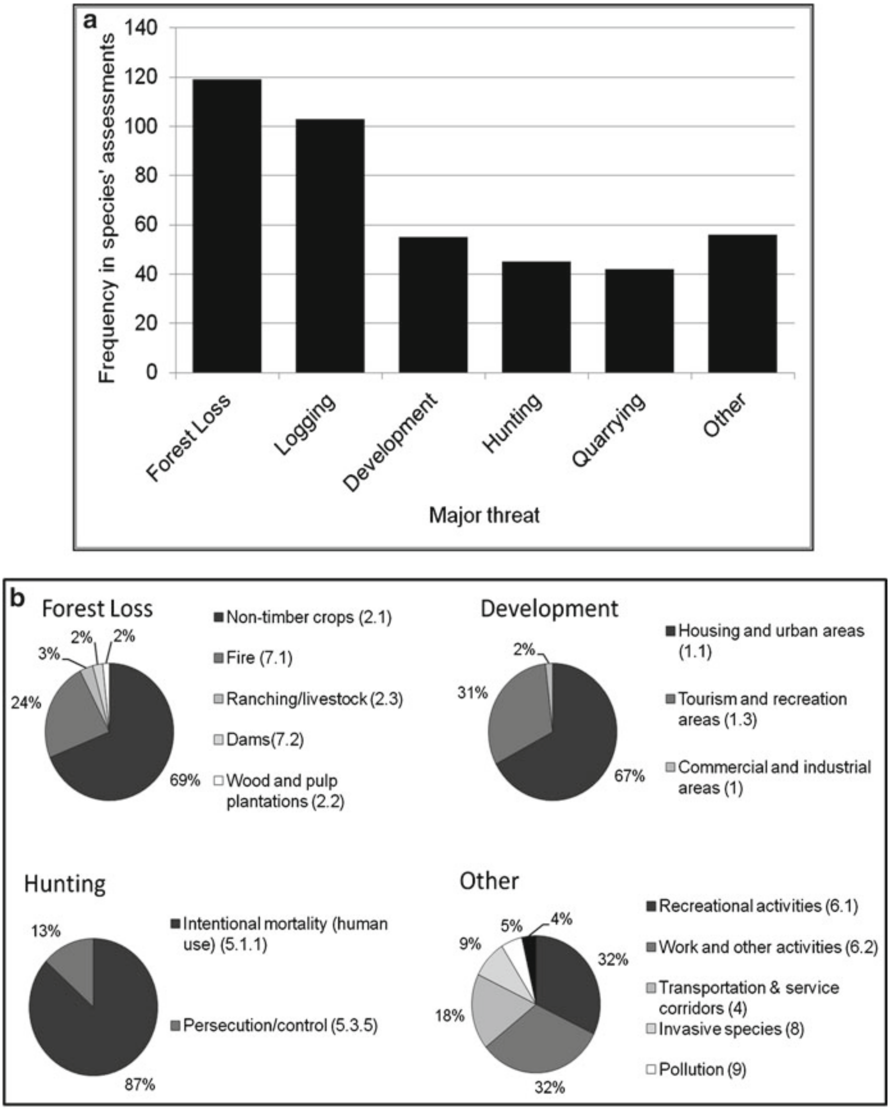

Fig. 9.1 Frequency of specific threats listed in IUCN assessments of Southeast Asian bat species. (**a**) Distribution of major threats across species assessments. The listed categories are derived from the IUCN hierarchical classification, although Forest Loss includes elements of two IUCN categories (Agriculture and Natural System Modifications). (**b**) Breakdown of categories in major threats, by finest level of IUCN threat classification (given in *parentheses*). Quarrying (3.2) in (**a**) was already at the finest resolution, and the primary subcategory of logging (5.3) was 5.3.5—motivation unknown/unrecorded

9.2 Assemblage Structure and Spatiotemporal Dynamics in Unmodified Habitat

Progress in establishing baseline measures of the distribution and composition of Southeast Asian bat diversity has been slow. Historically, and still today in some cases, access to some of the more remote, unmodified areas has been restricted by civil and international conflicts or simply by the expense and logistical difficulty of gaining access. In addition, although there has been a growing interest in biodiversity studies over the last couple of decades, bats have rarely been considered a priority group, and expertise in several countries is restricted to a few individuals. Not only is the availability of inventories from protected areas and other unmodified systems consequently very patchy, but generating comprehensive inventories is complicated by the differences in efficacy of particular capture methods for different ensembles (Table 9.1) and the influence of habitat structure on capture success. Multifaceted studies that combine capture methods are essential for the generation of complete/near-complete inventories (Furey et al. 2010; Kingston et al. 2003; Phommexay et al. 2011; Sedlock et al. 2008), but have been difficult to standardize to enable quantitative comparisons among studies, localities, and habitats.

In the face of limited and incomplete inventories, species composition at a given locality can be inferred by overlaying species' distributions delineated by inter- and extrapolation of locality records by regional experts (Boitani et al. 2006; Struebig et al. 2010) or predictively modeled from niche or climate envelopes (Hughes et al. 2012). While this can be an effective approach for macroecological analyses, for example, gap analysis of protected area coverage in Borneo (e.g., Struebig et al. 2010) or changes in species richness in response to climate change across mainland Southeast Asia (Hughes et al. 2012), studies of diversity responses to habitat alteration at the landscape scale generally require finer-scale inventories that can establish the pre-disturbance or baseline assemblage composition, particularly as natural spatiotemporal variability in these baselines could affect the interpretation of diversity patterns in disturbed habitats.

Variation in baseline assemblage composition in space and time within continuous rainforest habitat has been the focus of long-term research in Krau Wildlife Reserve (KWR), Peninsular Malaysia. KWR encompasses approximately 620 km^2 of unmodified, dipterocarp forest which supports the greatest diversity of bats, with over 70 species, reported for any site in the Palaeotropics to date (Kingston et al. 2006). Following initial research on assemblage composition and structure at a single study site (Kuala Lompat) within the forest (Kingston et al. 2000, 2003), a larger study was initiated to capture spatiotemporal variability at the landscape scale (see Kingston et al. 2006; Rossiter et al. 2012). The study was restricted to insectivorous bats of the rainforest interior as this ensemble can be consistently sampled with four-bank harp traps (Francis 1989; Table 9.1), and is predicted to be most vulnerable to forest loss and degradation (Kingston 2010; Kingston et al. 2003). Five spatially independent sites were established within the lowland rainforest and each was surveyed 4–6 times. Analyses of over 16,000 captures of 31 species (predominantly members of the Hipposideridae, Rhinolophidae, Kerivoulinae, and

Table 9.1 Efficacy of survey methods for the five main bat ensembles of Southeast Asia. Methods highlighted in gray provide the most effective coverage for the particular ensemble. Details of the methods can be found in Kunz et al. (2009)

		Ensemble				
		Forest interior insectivores	**Edge and gap insectivores**	**Open space insectivores**	**Below canopy plant-visiting bats**	**Canopy plant-visiting bats**
Other ensemble definitions		Narrow or highly cluttered space[1], Strategy I[2]	Background clutter/edge space[1], Strategy II[2]	Uncluttered space/open space[1], Strategy III[2]	Understory and below canopy[3], Strategy IV[2]	Above canopy[3], Strategy V[2]
Dominant taxa		Rhinolophidae, Hipposideridae, vespertilionid subfamilies Kerivoulinae, Murininae	Vespertilionids, and small emballonurids	Molossidae, large Emballonuridae, Miniopteridae, larger vespertilionids	Pteropodidae - mainly smaller species that use "steady state" resources e.g. *Cynopterus* spp., *Balionycteris maculata*[4]	Pteropodidae that depend on "big bang" resources e.g., *Pteropus* spp., *Dyacopterus* spp*., Eonycteris* spp.[4]
Success of survey/capture method	**Harp traps (four-bank)**	Effective - provided habitat has relatively closed understory so bats can be funneled into traps. Harder in plantations or open habitats, and may be difficult to bring many to remote areas (although most are collapsible)	Typically do not forage at harp trap level in primary forest, but if funneled by vegetation or nets (e.g., over streams, or just before a gap), get occasional captures	Typically do not forage in understory clutter where harp traps effective	Can occasionally be captured in harp traps set in understory, but not in numbers representative of abundance	Typically do not forage in clutter where harp traps effective
	Mist nets	Many species detect and avoid. Can be effective for larger species with lower frequency echolocation calls (e.g., large *Rhinolophus* or *Hipposideros*) and the passive-listening *Nycteris* spp. or *Megaderma* spp.		Effective over rivers or near roosts where animals foraging low, otherwise often flying above ground-net height. Stacked systems (e.g., over rivers) increase capture rate	Nets set near fruiting/flowering trees, over rivers, as stacked or vertical or canopy systems most effective in primary forest. Ground nets in village fruit plantations effective for common species e.g. *Cynopterus* spp., *Eonycteris* spp., *Dobsonia* spp. Ineffective in light conditions (after moonrise, street lamps) or high winds as the nets become visible to the bats.	
	Acoustic surveys	Many species use low intensity and/or high frequency calls that attenuate too rapidly for effective monitoring. Poor discrimination of call characteristics among species of Kerivoulinae and Murininae. Surveying for species of large hipposiderids or rhinolophids with low frequency (<60 kHz) calls could be effective.	Sufficiently intense for acoustic monitoring, but difficulties arising in developing call libraries because capture rates are so low (unless cave-dwelling)		Do not use laryngeal echolocation	
	Roost surveys	Cave surveys (using harp traps, mist nets, and acoustic techniques) are effective for those species that roost in caves (~50%). Searches of tree hollows (live and fallen), culverts, houses, bamboo culms, furled leaves (e.g.,banana) can generate presence data, but are hard to standardize unless based on transects. Misses species that roost in foliage.				
	Other methods		Flick-netting, funnel traps, but low capture rate and hard to standardize			

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1 = *Sensu* Schnitzler & Kalko 1998, based primarily on acoustic challenge to bats
2 = *Sensu* McKenzie et al. 1995, based primarily on wing morphology and foraging space
3 = *Sensu* Hodgkison et al. 2004a, based on wing morphology and foraging space
4 = Hodgkison et al. 2004

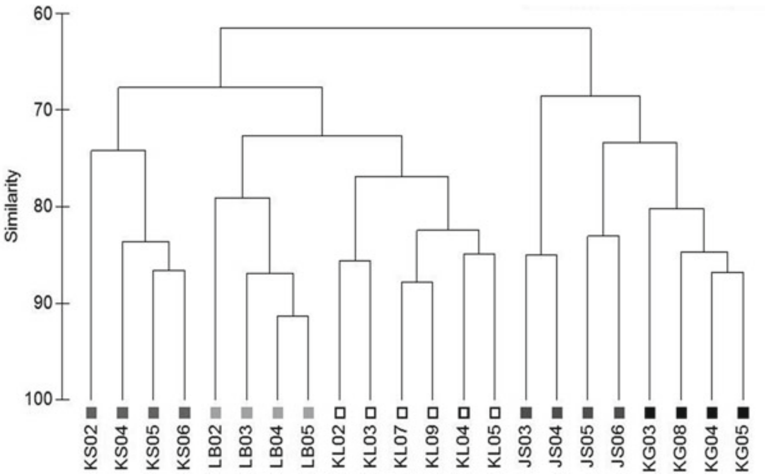

Fig. 9.2 Cluster analysis of Bray–Curtis similarity resemblance matrix derived from log(x + 1) capture index (number of individuals/number of complete harp trap nights) for each species for each survey. Surveys cluster primarily by location. Group average used, analysis conducted in Primer v 6. *KL* Kuala Lompat, *LB* Lubuk Baung, *KS* Kuala Serloh, *KG* Kuala Gandah, *JS* Jenderak Selatan. Number following locality indicates year of survey, e.g., *KL02* survey of Kuala Lompat in 2002

Murininae) indicate that although species richness of the study grids shows some spatiotemporal variability, there were no consistent effects of time and limited effects of space (T Kingston, unpublished data). However, composition of the assemblages (which includes the abundance of species) varied and clustered primarily by study grid (Fig. 9.2), indicating landscape effects on composition. Absolute and rank abundance of some species (e.g., *Kerivoula intermedia*) were relatively consistent across the landscape (the five study grids), but other species, primarily those dependent upon caves (e.g., *Hipposideros cervinus*, *Rhinolophus lepidus*), varied substantially (Fig. 9.3).

This natural variability in species abundance across a continuous unmodified landscape has several implications: (1) baseline surveys of large unmodified areas that plan to generate composition data rather than simple presence lists (as may be key when assessing the impacts of disturbance on diversity) will maximize return on effort if they can survey multiple spatially independent sites within the area; (2) the presence of key landscape features, such as caves, can substantially influence the baseline assemblage composition; and (3) there is potential for passive sampling effects to influence both interpretation of the baseline assemblage and the actual composition of the remnant assemblage in fragmented or disturbed habitats.

Temporal variability of relative abundance, though less pronounced than the landscape variability, can also be substantial (Fig. 9.3), with abundance increasing, decreasing, or remaining relatively stable across time and with directions of change within a species varying across the landscape. This variability in individual species abundance in space and time compounds to substantial spatiotemporal complexity in assemblage structure, further complicating efforts to establish baselines for disturbance studies and perhaps calling into question the very nature of "an assemblage."

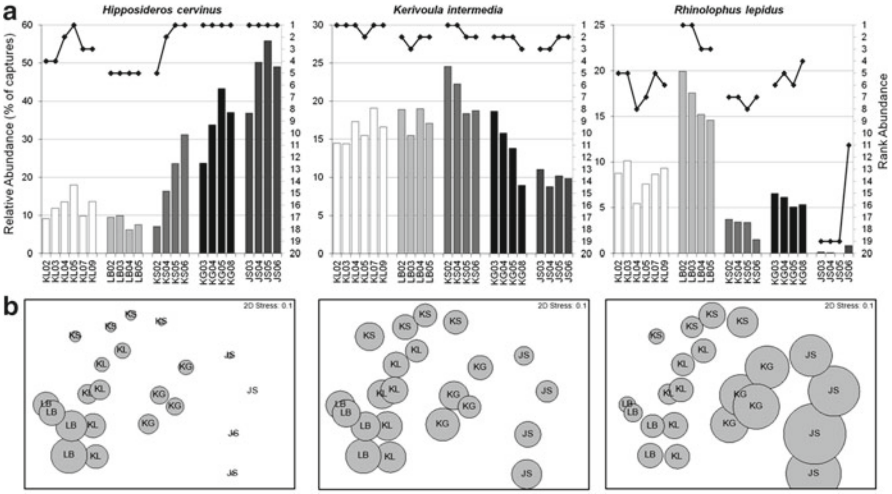

Fig. 9.3 Spatiotemporal variability in species abundance and assemblage composition at Krau Wildlife Reserve, Peninsular Malaysia. Datasets exclude juveniles and within-year recaptures. (**a**) Spatiotemporal variability in relative abundance (*bars*—primary vertical axis) and rank abundance (*lines*—secondary vertical axis) for three of the most common species captured in harp trap surveys of the five study grids in Krau Wildlife Reserve (KWR). (**b**) Nonmetric multidimensional scaling ordination (2D, minimum stress 0.1) of assemblages in KWR derived from a Bray–Curtis resemblance matrix of species capture rates [log(x + 1)] for each survey at each site. Size of bubbles scaled to the capture rate (number of individuals of the species/number of harp trap nights) of each of the three species to illustrate their spatiotemporal variability and contribution to the ordination. *KL* Kuala Lompat, *LB* Lubuk Baung, *KS* Kuala Serloh, *KG* Kuala Gandah, *JS* Jenderak Selatan. Number following locality indicates year of survey, e.g., *KL02* survey of Kuala Lompat in 2002 (T Kingston, unpublished data)

Interestingly, temporal variation in the relative abundance of the forest-interior insectivorous bats was far greater than any seen in those from other Palaeotropical and Neotropical sites, and overall, Palaeotropical species show significantly greater temporal variation than their Neotropical counterparts (Meyer et al. 2010). Unfortunately, this will likely make it very difficult to achieve sufficient power to detect subtle population declines that can be indicative of a species on the cusp of vulnerability. For example, simulations suggest that it may require >20 years of monitoring with at least two surveys a year to pick up even a modest 10 % decline in species abundance at one of the Krau study sites (Lubuk Baung) (Meyer et al. 2010).

9.3 Roosting Ecology, Assemblage Composition, and Forest Disturbance

In Southeast Asian bat faunas, it is apparent that the availability and distribution of cave systems greatly influences the composition of the local bat fauna. For example, none of the 13 species of cave-roosting Hipposideridae and Rhinolophidae found in a karst area of East Kalimantan (Suyanto and Struebig 2007) were captured in comparable surveys of oligotrophic forests in Central Kalimantan that lacked cave systems (Struebig et al. 2006). As suggested above (Sect. 9.2), much of the landscape variability within the intact system at Krau Wildlife Reserve is driven by cave availability and distributions. In fact, one major cave-roosting resource had a significant influence on bat assemblage composition, within both continuous forest and fragment sites, over a distance of up to 11 km (Struebig et al. 2009). Thus, it is important to be aware that species representation in surveys of pre-disturbance habitat may simultaneously reflect the quality of the local habitat as well as the availability (presence and proximity) and quality of critical landscape resources (caves).

Further ensemble subdivisions, based on roosting ecology, have demonstrated the influence of local and landscape habitat quality on species composition of rainforest bats (e.g., Rossiter et al. 2012; Struebig et al. 2008; Turner 2011). Forest-interior insectivores have been divided into those that roost in local forest structures (e.g., cavities in live trees, hollows in dead trees which may be standing or fallen, living foliage, clusters of dead foliage, bird nests) and hereafter termed "forest-roosting" (equivalent to Struebig et al.'s (2008) foliage-roosting subgroup), and those that forage in the forest but roost in caves—"cave-roosting" species. Forest-roosting species are influenced primarily by local habitat quality as it affects both roost availability and foraging success, whereas cave-roosting species are influenced by both the quality of the local foraging environment and the quality/availability of cave systems in the surrounding landscape. Seemingly, this would make the cave-roosting species more vulnerable to anthropogenic disturbances, but in fact most cave-roosting species are more vagile than their forest-roosting counterparts, with higher wing loading and aspect ratios facilitating faster, more efficient, nightly commuting from caves to foraging grounds (Kingston 2000). They may thus be able to adapt to changes in local habitat quality by switching foraging grounds. In contrast, recapture and radio-tracking data (Allen 2005; Fletcher 2006) from KWR suggest that forest-roosting species forage in close proximity to their roosts (Fig. 9.4) and, as might be expected from their wing morphologies and echolocation call designs (Kingston et al. 2003), do not appear to forage in the open environments beyond the forest edge (Allen 2005). Forest-roosting species are consequently predicted to be less resilient overall and show more immediate responses to declines in forest quality and availability.

A large-scale comparison of 7 forest-interior assemblages from KWR with those from 15 fragments in the surrounding agricultural landscape (c. 56,200 km^2 total area) provides good support for these predictions. Diversity losses in the forest fragments were driven primarily by a decrease in species richness of forest-roosting species with fragment area (Struebig et al. 2008); there was no significant relationship between fragment size and species richness or abundance of cave-roosting bats, which may in fact have been augmenting overall fragment diversity (Struebig et al. 2009).

Of course, the resilience of the cave-roosting forest-interior ensemble will only continue to hold true in landscapes where cave disturbance is minimal, and unfortunately this is not the case in much of Southeast Asia (Kingston 2010), where many cave-roosting species are hit with the "double whammy" of disturbance at their roost

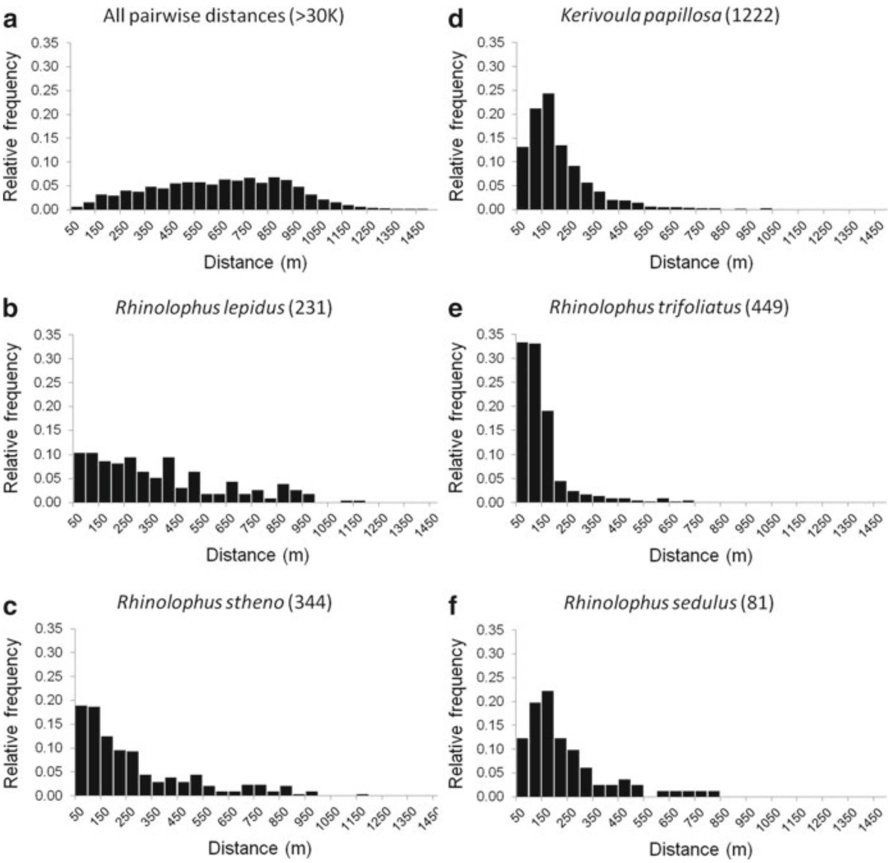

Fig. 9.4 Relative frequency of intra-grid recapture distances derived from spatially explicit capture data of two species of cave-roosting bats (**b**, **c**) and three forest-roosting species (**d**–**f**) on the five study grids of Krau Wildlife Reserve, Malaysia. Data are pooled across years and grids; number of recapture distances shown in *parentheses* after species name. Minimum possible recapture distance is 0 (if an individual is recaptured in the same trap in subsequent years), and maximum distance on grid is ~1,450 m. Distribution of all possible pair-wise inter-trap distances from one study grid shown in (**a**). Forest-roosting species (**d**–**f**) show substantial and greater skew in distribution towards shorter distances and smaller maximum distances than do cave-roosting species (**b**, **c**). This suggests that forest-roosting species forage close to their roosts. *Kerivoula papillosa* roosts in small (2–15) mixed-sex groups in small tree hollows and individuals tend to switch roosts every few days. *Rhinolophus trifoliatus* roosts alone under foliage in the understory and *Rhinolophus sedulus* either singly or in pairs in fallen hollow logs or occasionally under foliage (Fletcher 2006; Kingston et al. 2006). Slight skew to shorter distances in **b** and **c** (compared to **a**) suggests individual fidelity to a hunting territory in cave-roosting species (T Kingston, unpublished data)

(e.g., tourism, mining and quarrying, direct hunting) and degradation/loss of their foraging grounds. Even in the event of cave protection or high cave availability, persistent loss and degradation of foraging habitat is likely to erode population viability as the energetic costs of commuting progressively greater distances, or foraging in

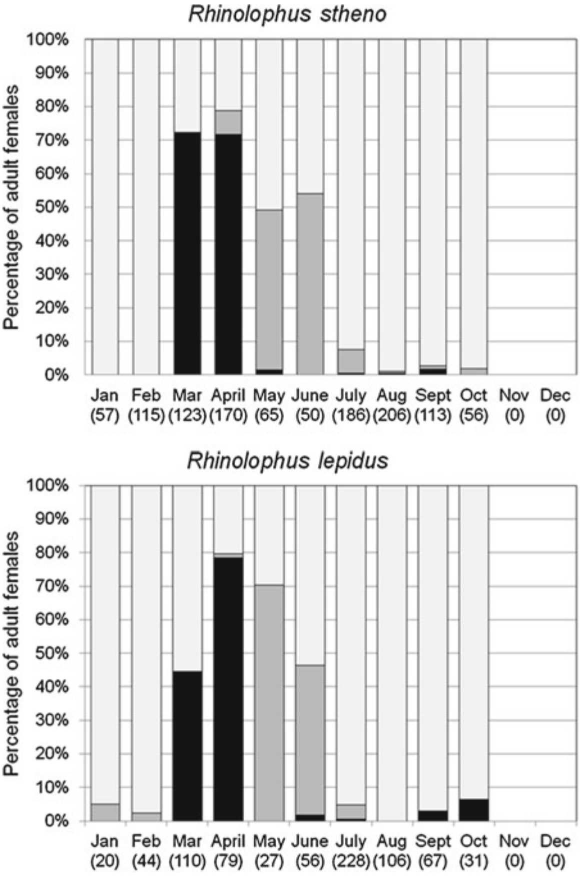

Fig. 9.5 Reproductive phenology of adult females of two cave-roosting forest-interior species captured on the five study grids of Krau Wildlife Reserve, Malaysia (2002–2009). Data are pooled across study grid and years; no trapping was conducted in the main monsoon months of November and December. Reproductive activity denoted by *black bars* (pregnant) and *dark gray bars* (lactating) and inactivity by *pale gray bars* (individuals either nulliparous or post-lactating). Reproductive categories based on external assessments of the condition of nipples and pubic nipples and abdominal palpation (see Kingston et al. 2006; Racey 2009 for details). Numbers in *parentheses* indicate total number of adult females assessed for each month. Both species exhibit a pronounced peak in pregnancy in March–April, followed by a peak in lactation in May–June, with reproductive activity low to nonexistent throughout the rest of the year. Low activity in September, October, January, and February suggests that November and December are similarly low-activity months (T Kingston, unpublished data)

poorer habitats, reduce individual fitness. Resource-dependent energetic constraints on reproduction in forest-dependent cave bats are indicated by strong seasonality of reproductive activity (Fig. 9.5; Furey et al. 2011), and it is possible that apparently healthy cave-roosting bat populations in modified landscapes may be living on borrowed time. Support for this comes from surveys of modified habitats in the karst regions of Vietnam, where local bat populations are unlikely to be limited by cave availability (Furey et al. 2010). Although Furey et al. (2010) found that species richness of cave-dependent species of the Rhinolophidae and Hipposideridae was only slightly reduced in disturbed and degraded forests, the abundance of individuals was less than a third that found in the primary forest, despite comparable effort. As habitat modification intensifies and logged or fragmented forests are converted to plantations or intensive agriculture, the resource base for forest-interior species is reduced still further, precipitating actual species losses. In Thailand, dramatic abundance declines were matched with extensive loss of cave-roosting species (as well as forest-roosting species) in the heavily modified habitats of rubber plantations (Phommexay et al. 2011), and this was attributed at least in part to reduced insect biomass in the plantations compared to paired forest plots.

Rossiter et al. (2012) further characterized the forest-interior bats by their roosting ecology and associated social organization, and subdivided the forest-roosting species into those that form small social groups in tree cavities or leaf clusters and solitary species roosting under foliage or in fallen hollows. The objective was to provide a predictive framework to explore gene flow and genetic structure of seven syntopic bat species in the unmodified habitat of the KWR study area. As predicted, the vagile, cave-roosting colonial species exhibited no genetic correlation across the study area. In contrast, forest-roosting species that formed small social groups in limited roosts exhibited significant positive genetic structure, with weaker structure detected in those species that roosted alone in widely available roosts. Critically, these findings explained patterns of allelic diversity loss in a comparison of three of the species in the forest fragments of the surrounding landscape (Struebig et al. 2011). Allelic richness of the fragment populations of *Rhinolophus lepidus*, a cave-dwelling colonial species which exhibited no significant structure in the unmodified habitat, did not differ from that of the KWR populations. In contrast, *Rhinolophus trifoliatus* is a solitary species that roosts under leaves in the understory and showed modest genetic structure in KWR. Allelic richness in 7/9 fragment *R. trifoliatus* populations was significantly less than that of populations from the continuous forest. *Kerivoula papillosa* roosts in small (2–15) groups in tree cavities and exhibited the highest positive genetic structure in the reserve. Fragment populations of this species not only experienced a significant decrease in allelic richness but richness declined significantly with fragment size (Struebig et al. 2011).

9.4 Summary and Future Directions

The great taxonomic and ecological diversity of bats in tropical assemblages has intrigued community ecologists for decades and fueled the continuing quest to find or refute nonrandom patterns of species composition in assemblages indicative of structuring processes (Kingston et al. 2000; Moreno et al. 2006; Schoeman and Jacobs 2011). Here I have tried to illustrate how studies of bat assemblage structure and dynamics in Southeast Asia can provide insights into diversity responses to disturbance gradients, which I believe can ultimately lead to the development of predictive frameworks of comparative vulnerability. Briefly, it was first proposed by Kingston et al. (2003) that the ecomorphological adaptations that enable some insectivorous bats to forage in dense vegetation of the forest interior would preclude them from foraging successfully in more open habitats and, as a consequence, the forest-interior insectivore ensemble was predicted to be particularly vulnerable to human disturbance and degradation of forests that create such habitats. As detailed above, there is now good support for this from empirical research, and recent work has highlighted the utility of distinguishing between cave-roosting forest-interior species and forest-roosting species, with the interaction between roost type and social structure within the forest-roosting species providing additional predictive power. However, studies remain very few in number and conclusions based largely on work in the KWR landscape in Malaysia. Further studies from other localities are needed to test the generality of these findings, and detailed studies on the response of the forest-interior ensemble to the dominant industrial plantations [e.g., oil palm (*Elaeis guineensis*), rubber (*Hevea brasiliensis*), coffee (*Coffea* spp.)] from across the region are urgently needed. Studies are also needed that explicitly examine the tolerance of cave-roosting species to forms of disturbance that differ in intensity and frequency (e.g., quarrying, tourism, guano extraction) as well as the interaction between disturbance at the cave and loss or degradation of surrounding foraging habitats.

The forest-interior insectivorous ensemble is just one of the five ensembles in Southeast Asian bat assemblages (Table 9.1). The open-space and edge/gap insectivorous bats were predicted to be less vulnerable to disturbance by Kingston et al. (2003), because their natural foraging habitat is more comparable to that which arises from disturbance. Some support for this comes from an increase in the representation of edge/gap species in harp trap captures in forest fragments in the Krau Landscape (Struebig et al. 2008), although harp traps underrepresent edge species in old-growth forest (Table 9.1). However, these two ensembles include at least 35–40 species in Peninsular Malaysia alone, and it is probable that species can be grouped by factors that are likely to predict their success in modified landscapes, such as their ability to utilize anthropogenic structures (e.g., the "house bats" *Scotophilus* spp.) or crops (e.g., *Myotis* spp., *Glischropus* spp. in furled banana leaves) as roosts, and to forage effectively over large distances. Studies of these ensembles have been hampered by the difficulties of capturing them at foraging grounds (Table 9.1), but they can be surveyed acoustically (e.g., Phommexay et al. 2011), although full-spectrum recordings are advisable for species' characterization and discrimination in such species-rich systems, and there is currently a dearth of reference calls (but see Hughes et al. 2011). The costs of full-spectrum real-time passive detectors have dropped substantially in recent years, making comparative acoustic surveys across disturbance gradients and in different agricultural/plantation land uses a profitable research direction, particularly as part of a multifaceted approach that can simultaneously capture changes in forest-interior ensembles.

The outstanding problem area for quantified assessments of responses to disturbance remains the plant-visiting or phytophagous ensembles (Table 9.1). This is primarily because temporal variation in abundance of some species can make it extremely difficult to characterize the baseline assemblage composition in old-growth forests. Of 8 species captured over 3 years in a rotated system of 24 stacked-net rigs (Hodgkison et al. 2004a) in Krau Wildlife Reserve, only three (*Balionycteris maculata*, *Chironax melanocephalus*, and *Cynopterus brachyotis*) were resident and relied on a wide range of asynchronously fruiting "steady state" resources (Hodgkison et al. 2004b). The remaining five species all depended on ephemeral, widely distributed "big bang" food resources typically associated with large canopy trees and strangler figs (Hodgkison et al. 2004b) and either showed significant temporal variation in abundance (*Cynopterus horsfieldii* and *Megaerops ecaudatus*) or were too rare for analysis (*Dyacopterus spadiceus*, *Eonycteris spelaea*, and *Rousettus amplexicaudatus*). Although the responses of resident forest species to disturbance might therefore be reliably determined, those of the more nomadic, wide-ranging species will be hard to document without substantial effort in both the baseline site and the disturbed sites.

This chapter has focused on efforts to predict and understand assemblage responses to anthropogenic disturbance of forests, but the consequences of assemblage restructuring for ecosystem functioning remain unexplored. Southeast Asian bats play key ecosystem roles as predators of insects, pollinators, and seed dispersers and are critical for forest regeneration (Hodgkison et al. 2003), the sustainability of commercial fruit crops (Bumrungsri et al. 2008, 2009), and control of insect crop pests (Leelapaibul et al. 2005). Declines in local species richness and abundance and the concomitant changes in assemblage composition are likely to have profound effects on these services and compromise the integrity of both natural and production ecosystems. Studies that quantify the ecological and economic consequences of assemblage changes induced by anthropogenic activities are urgently needed.

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Chapter 10
Modeling the Colonization of Hawaii by Hoary Bats (*Lasiurus cinereus*)

Frank J. Bonaccorso and Liam P. McGuire

Abstract The Hawaiian archipelago, the most isolated cluster of islands on Earth, has been colonized successfully twice by bats. The putative "lava tube bat" of Hawaii is extinct, whereas the Hawaiian Hoary Bat, *Lasiurus cinereus semotus*, survives as an endangered species. We conducted a three-stage analysis to identify conditions under which hoary bats originally colonized Hawaii. We used *FLIGHT* to determine if stores of fat would provide the energy necessary to fly from the Farallon Islands (California) to Hawaii, a distance of 3,665 km. The Farallons are a known stopover and the closest landfall to Hawaii for hoary bats during migrations within North America. Our modeling variables included physiological, morphological, and behavioral data characterizing North American Hoary Bat populations. The second step of our modeling process investigated the potential limiting factor of water during flight. The third step in our modeling examines the role that prevailing trade winds may have played in colonization flights. Of our 36 modeling scenarios, 17 (47 %) require tailwind assistance within the range of observed wind speeds, and 7 of these scenarios required <10 m s^{-1} tailwinds as regularly expected due to easterly trade winds. Therefore the climatic conditions needed for bats to colonize Hawaii may not occur infrequently either in contemporary times or since the end of the Pleistocene. Hawaii's hoary bats have undergone divergence from mainland populations resulting in smaller body size and unique pelage color.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_10,

10.1 Introduction

The Hawaiian archipelago is the most isolated cluster of islands on planet Earth. Among terrestrial animals, the only groups to reach the archipelago and flourish with significant radiations are birds, land snails, spiders, and a variety of insects that include flies, beetles, katydids, and moths (Zimmerman 1970). That's it. No rodents, amphibians, or reptiles other than marine sea turtles have ever established on any Hawaiian island without anthropogenic association. There are only two living endemic mammals in Hawaii, one is the Hawaiian Monk Seal, *Monachus schauinslandi*, and the other is the animal the Hawaiians call Ōpe'ape'a (*oh-pay-ah-pay-ah*), the Hawaiian Hoary Bat. This bat currently is recognized as a subspecies of the hoary bat, *Lasiurus cinereus semotus*, although historically it has been given full species status and reduced to a subspecies several times (see Tomich 1986). Subfossils of *L. c. semotus* are known from at least 5,500 YBP (Olson and James 1982), and it is believed that its ancestors colonized the Hawaiian Islands in the early Holocene or the late Pleistocene. Another bat, now extinct, successfully colonized Hawaii even earlier, flourished in the Hawaiian Islands since at least 130,000 YBP (Olson and James 1982), and probably survived until at least 1,760–1,460 YBP. Despite ample skeletal remains (Olson and James 1982), this bat has yet to be described officially, but appears to be related to lasiurine bats. This now extinct bat, the putative "lava tube bat," was slightly smaller in cranial and forearm measurements than the extant Hawaiian Hoary Bat (N. Simmons, personal communication). It has been suggested that the ancestral stock may have been *Lasiurus borealis*. Thus, two species of bats, probably both lasiurines with origins in North America, have successfully colonized Hawaii.

Lasiurine bats are the only bats found in the Americas that seem to have a proclivity for successful colonization of distant oceanic islands. Both *L. cinereus* and *L. blossevillii* (as the subspecies *brachyotis*) occur on the Galapagos Islands, 925 km W of Ecuador (McCracken et al. 1997). *Lasiurus cinereus*, *L. seminolus*, and *Lasionycteris noctivagans* regularly occur on Bermuda (Hall 1981; Van Gelder and Wingate 1961; Yates et al. 1976). The distance from North Carolina to Bermuda overwater is an impressive 1,064 km. Even more impressive *Lasiurus cinereus* occasionally appears on Iceland, and there is a single record of this bat from the Orkney Islands north of Scotland (Nowak 1999). From Newfoundland, the northeastern limit of distribution for the hoary bat in North America to Iceland is 2,428 km. Despite the extreme distance that would need to be covered in a single uninterrupted flight to Iceland, hoary bats have apparently made such flights on multiple occasions. However, the longest overwater flights with subsequent successful colonization for any bat (or dispersal by a land mammal) are from mainland North America to the Hawaiian archipelago. The shortest distance from San Francisco to Hawaii is 3,665 km, with this landfall at the eastern tip of Maui Island. Of course even this flight would be considerably longer if immigrating bats did not maintain the minimum straight line flight distance or if they made landfall on a different island.

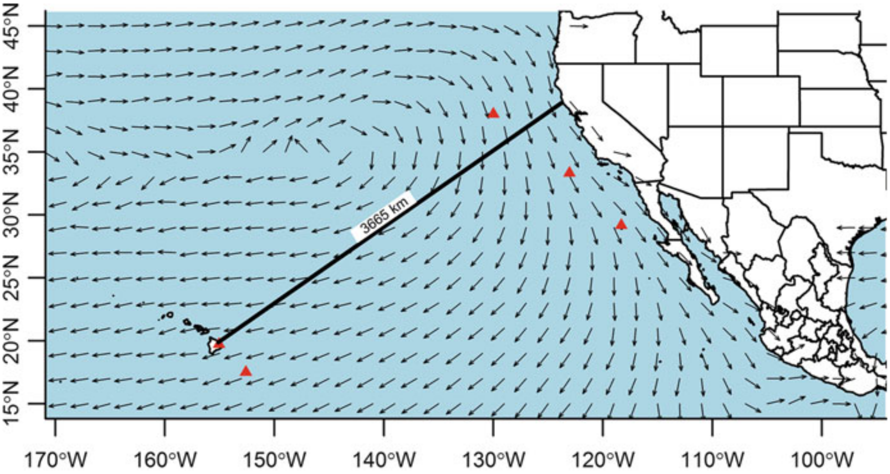


Fig. 10.1 Prevailing wind currents in the northeastern Pacific Ocean with five NOAA weather stations used for historical wind speed values indicated by *red triangles*. The shortest distance from North America to Hawaii is indicated by the *black line*. Note that prevailing winds off coastal northern California lead directly to the Hawaiian archipelago

We have timed Hawaiian Hoary Bats flying directly between two points while foraging and calculated their flight speed as 11 m s^{-1}. At that speed, a bat would take 3.86 days to reach Hawaii. To a flying animal's advantage, the prevailing trade winds in the North Pacific usually would assist flight along the projected course from California to Hawaii. In present times, trade winds between North America and Hawaii typically are about 7 m s^{-1} and blow from the NE in this region about 90 % of the time (Fig. 10.1). Furthermore, it is not unusual for periods of higher wind speed to occur. Consequently it is plausible that a bat departing from the West Coast of North America, following the trade winds, would achieve a moderate tailwind assistance directed towards the Hawaiian archipelago. In a storm, it is easily possible that winds could reach speeds of 25 m s^{-1} or more. We can imagine ancestral hoary bats flying near San Francisco or even more likely the Farallon Islands (see below) getting swept on a southwesterly direction by a wind storm. Such a scenario is easily imaginable given the autumn aggregation of hoary bats of both sexes on Southeast Farallon Island, 48 km offshore from San Francisco (Cryan and Brown 2007). As the winds abate, these bats are far at sea and are committed to continue flying in the direction of least resistance with the normal trade winds. The main volcanic islands of the Hawaiian archipelago stretch 620 km in an alignment nearly perpendicular to the approach of a bat that would be flying from California with the trade winds. With rain forest and a rich biomass of nocturnal flying insects, Hawaii would therefore present an obvious and welcome sight to an exhausted hoary bat.

To go beyond simple speculation of a colonization flight from the Farallon Islands to Maui, it is necessary to examine in more detail key physiological constraints for a bat of this size and the particular wing morphology and flight mechanics of the hoary bat. Could a hoary bat fly that long and that far from mainland North America to Hawaii without running out of two vital resources: energy and water? In this chapter we input the best available data into a series of models and calculations from a viewpoint of energy and water storage and expenditure, flight dynamics, and meteorological conditions to suggest how hoary bats may have managed the successful colonization of Hawaii some 10,000 years ago.

We conducted a three-stage analysis to determine the conditions under which hoary bats may have originally colonized Hawaii. The first, and most obvious, question is whether hoary bats could store enough fat to provide the energy necessary to fly from California to Hawaii. To answer this and subsequent questions discussed below, we used the software program *FLIGHT* [v 1.2, and the companion guide, *Modeling the Flying Bird* (Pennycuick, 2008)]. Though primarily designed for migratory birds, the principles involved work equally well for any flying vertebrate (birds, bats, pterosaurs) and the software has been used successfully for studies of bats (e.g., Grodzinski et al. 2009; McGuire et al. 2012). Users input basic morphometric and body composition values and the program can then be used to model flight, including long-distance migration, from aerodynamic principles. The program simulates a migration in 6-min interval, calculating the fuel requirement and hence mass change in each interval, as well as several other factors such as wingbeat frequency, flight speed, and distance traveled. At each 6-min interval, the remaining fuel stores and body mass are recalculated, repeating until the fat store is completely exhausted.

The second step of our modeling process investigated the potential for water to become a limiting factor in the flight range of hoary bats. If nutrient stores are completely exhausted, the animal will no longer be able to power flight and will fall into the ocean without successfully colonizing Hawaii. Similarly, if the animal becomes excessively dehydrated, unable to maintain water balance, it will not be possible to continue flying. The output of the *FLIGHT* simulations considers only the former scenario. Therefore, we used these simulations as a base model and developed our own calculations to determine if water balance may become a limiting factor. We considered various scenarios of water loss to determine under which conditions water may be a more limiting resource than fat.

The final component of our calculations is determining what role the prevailing trade winds may have played in the original colonization flights. Our energy and water balance models assume the animal is flying in still air. Such an assumption is clearly not valid. As described above, the prevailing trade winds blow in such a manner that a bat departing from California would likely receive some degree of tailwind assistance (and guidance by following the path of least resistance) on a flight to Hawaii. For each model, we calculated the minimum tailwind speed required (or headwind that could be tolerated) for a bat to fly 3,665 km to Hawaii before either fat or water was depleted. We know that bats have successfully colonized Hawaii at least twice and thus our objective was not to determine if such flights are possible, but rather to provide a context to the likelihood of such flights and the role of environmental conditions. Could a bat only reach Hawaii if blown by gale force storm winds, or could such a flight be reasonably expected to occur under regularly expected environmental conditions? If the predicted environmental conditions are not exceptional, there may be implications for repeated colonization events and a continued influx of genetic material to the population.

10.2 Methods and Assumptions

As with all models, assumptions must be made; however, there is a solid body of data for many of the variables that contribute to a robust modeling process. The following outlines the data we have used as well as the explicit assumptions and approximations we have made to arrive at all the inputs into our model. Additional assumptions are made implicitly by accepting the default configurations of the *FLIGHT* software. Our first assumption was that the hoary bats that first colonized Hawaii were morphologically similar to modern North American Hoary Bats (*L. c. cinereus*). Therefore, all morphological and physiological parameters are determined from North American Hoary Bats. The values of the various parameters input into our models and calculations are given in Table 10.1.

Table 10.1 Values used for FLIGHT models and water loss calculations

Parameter	Values	References
Wing span (m)	0.398	Norberg and Rayner (1987)
Wing area (m^2)	0.0196	Norberg and Rayner (1987)
Fat fraction (fat mass × body mass^{-1})	0.0756, 0.1147, 0.1525, 0.2869	McGuire (2012)
Fat-free mass (body mass–fat mass)(g)	17.5859, 20.8364, 26.4152	McGuire (2012)
Flight muscle fraction (pectoralis mass × body mass^{-1})	0.0518	McGuire (2012)
Cruising altitude (m)	500	n/a
Water vapor density deficit (g m^{-3})	3, 10, 20	Gerson and Guglielmo (2011)
Cutaneous water loss (g)	10 % of respiratory water loss	Carmi et al. (1992)
Initial total body water (g)	0.734 × initial body mass	McGuire (2012)
Critical water balance threshold (g)	70 % of initial body water	Carmi et al. (1992)
Water from protein catabolism (g kJ^{-1})	0.155	Jenni and Jenni-Eiermann (1998)
Water from fat catabolism (g kJ^{-1})	0.029	Jenni and Jenni-Eiermann (1998)
Respiratory frequency (breaths × wingbeat^{-1})	1	Suthers et al. (1972)
Wingbeat frequency (Hz)	Output from FLIGHT model	n/a
Tidal volume (mL)	1.025	Canals et al. (2005)

Perhaps the most crucial information input into *FLIGHT* are the morphometric dimensions of the subject, specifically wing span and wing area, from which aspect ratio is calculated. Aspect ratio determines aerodynamic and energetic efficiency in flight (Norberg and Rayner 1987) and thus is a crucial measure when modeling an extreme flight. We used values specific to North American Hoary Bats (*L. c. cinereus*), obtained from Norberg and Rayner (1987).

The remaining critical values for our simulations pertain to body composition. Flight range is largely determined by the amount of fat as a proportion of total body mass (how much fuel is in the tank?). The *FLIGHT* model calculations assume that fat is the primary fuel source with ~5 % of the total energy coming from protein sources, consistent with observations of migratory birds (Jenni and Jenni-Eiermann 1998; McWilliams et al. 2004). While the proportion of fat determines the energy available for flight, the fat-free mass is more important to consider for water balance. Fat is stored nearly anhydrously while lean tissue contains 70–80 % water by mass. Therefore, fat-free body mass was another important component of our model.

In the course of other research, one of us (LPM) has gathered information about the body composition of North American Hoary Bats. Hoary bats were collected in late summer and during spring migration. To avoid potentially confounding effects of reproductive physiology, we limit ourselves here to only male bats. For details on collection methods, see McGuire (2012). Total body mass was recorded from each bat before the pectoralis muscles were carefully dissected and weighed. The carcass was dried to a constant mass at 70 °C to determine total body water and then extracted with petroleum ether for 6 h in a Soxhlet apparatus to determine total fat mass (correcting for tissue subsamples). We determined the minimum, mean, and maximum fat fraction (fat mass/total body mass), fat-free mass (total body mass–fat mass), and flight muscle fraction (pectoralis mass/total body mass) among all bats sampled. The range of fat fraction in *L. c. cinereus* was relatively small, so we also considered the greatest fat fraction [measured by quantitative magnetic resonance (McGuire and Guglielmo 2010)] observed in a recent study of fall migrating Silver-Haired Bats (*Lasionycteris noctivagans*; McGuire et al. 2012) which was substantially greater than any values observed in our sample of hoary bats. Such a large fat fraction likely represents the extreme range of what may be expected to occur in a migrating hoary bat. We found that varying flight muscle fraction had negligible effects on the outcome of the model and conservatively used the minimum observed flight muscle fraction, thus reducing the number of variable parameters in the analysis.

FLIGHT allows the user to input starting and cruising altitudes. A bat departing from the California coast would begin flying at approximately 0 m asl, and we estimated a cruise altitude of 500 m asl. In practice, for long-distance flights (i.e., the 3,665 km required to reach Hawaii), the flight altitude does not dramatically affect the results of the simulation. Assuming a cruising altitude of 500, 1,000, or even 2,000 m merely changes flight range approximately 1 % of the total flight range. Therefore, although estimates of cruising altitude in migrating bats are lacking, the actual altitude chosen has little impact on the conclusions of our models.

A bat flying over the Pacific Ocean would have no exogenous source of fresh water. Assuming that endogenous metabolic water production is the only water input once a flight has begun, we calculated respiratory water loss based on different water vapor density deficits. Total evaporative water loss is the sum of respiratory water loss and cutaneous water loss (assumed to be 10 % of respiratory water loss; Carmi et al. 1992). Thus, when an animal cannot drink free water, water balance is maintained as evaporative water loss is replaced by metabolic water production.

Sophisticated and detailed calculations of total evaporative water loss require far more detailed information than is currently available for flying bats. One method of calculating respiratory water loss would be to know to what temperature the bats are able to cool their expended breath (then assume saturation) and the temperature and relative humidity of the ambient air. With each breath, bats will inspire a lung volume of ambient air and exhale a lung volume of saturated air. Based on the temperature of those gases, one could determine the water vapor densities in g m^{-3} and the difference is the respiratory water loss. To avoid assumptions regarding ambient temperatures and humidities and the ability to cool expended breath, we ran calculations at three different water vapor density deficits which cover a range of ecologically relevant scenarios. In a study of evaporative water loss in flying Swainson's Thrushes (*Catharus ustulatus*), Gerson and Guglielmo (2011) ran water vapor density deficits of 3.2 and 13.2 g m^{-3}, arguing that such conditions are relevant to the flight of migrating Swainson's Thrushes. Songbirds migrating across the Sahara desert experience deficits of approximately 23 g m^{-3} (Schmaljohann et al. 2008, 2009). Based on this range, we evaluated water balance at deficits of 3, 10, and 20 g m^{-3}. The 20 g m^{-3} scenario is likely far more extreme than would be experienced over the ocean (more comparable to flying over a desert), but provides context to the two other scenarios which are more realistic.

We calculated total initial body water based on the mean of the proportion of total body water in the hoary bats described above (McGuire 2012). We assumed the critical water balance threshold (below which dehydration precludes survival/continued flight) was 30 % loss of the total body water pool (Carmi et al. 1992). Hoary bats have been reported to survive water loss equivalent to 28 % total body mass with no ill effects (Shump and Shump 1982), so 30 % water loss should be a realistic limit. Shump and Shump (1982) also reported that hoary bats have lower rates of evaporative water loss than several other bats in the family Vespertilionidae, perhaps due to their dense pelage and to the presence of pelage on the wing and tail membranes (an intriguing observation which, if water loss is a limiting factor to long-distance flight, may partially explain why lasiurines appear more likely to colonize remote landmasses than other vespertilionids). Finally, we took tidal volume from Canals et al. (2005), which reports lung volume for South American *L. c. villosissimus*, and we therefore assume that lung volume is similar for *L. c. cinereus*.

In each 6-min interval of the *FLIGHT* model, the number of breaths can be determined given the wingbeat frequency (output by the model) and the respiratory frequency (breaths × wingbeat^{-1}). We assumed the respiratory frequency to be 1 (as reported in Suthers et al. 1972). Therefore, given the number of breaths and the respiratory water loss per breath (plus 10 % to account for cutaneous water loss), we calculated the total water loss in each interval. To calculate the total metabolic water production, we determined the water that would be produced based on the catabolism of fat and protein. Fat catabolism produces 0.029 g H_2O kJ^{-1} and protein produces 0.155 g H_2O kJ^{-1} (Jenni and Jenni-Eiermann 1998). The amount of each fuel burned (in kJ) is reported by the software for each interval. Based on these values, we calculated metabolic water production (and hence, net water loss) and the remaining total body water at each interval. It was then possible to determine if/when water balance became a limiting factor.

Ultimately our models will be affected importantly by (1) initial proportion fat (how much energy is available to burn), (2) initial fat-free mass (how much body water is available), and (3) water vapor density deficit (rate of evaporative water loss). There are 4 estimates of fat proportion and 3 estimates of initial fat-free mass, hence 12 base models (fat and fat-free combinations) that use the FLIGHT simulations to predict flight range assuming no winds and no water limitation. After running the 12 base models and the 3 water loss conditions for each (36 conditions total), we evaluated which conditions were energy or water limited. If the simulation exhausted fat reserves before reaching Hawaii (3,665 km), or if total body water pool dropped more than 30 %, we increased airspeed by adding a tailwind, optimized such that the bat reached Hawaii exactly as fat/water stores were exhausted.

10.3 A Model for Colonization of Hawaii Hoary Bats from North America

10.3.1 Is Fat Limiting?

From the base *FLIGHT* models, we examined how long and how far a hoary bat could fly nonstop until fat stores were completely exhausted (temporarily ignoring the effects of water balance and wind). The flight range is determined strictly by fat fraction. Potential flight distance is largely independent of the size of the bat, although the flight speed and hence duration will vary (Pennycuick 2008). Assuming neutral winds, energy is limiting for most conditions, but at the highest fat proportions in our models, bats could reach Hawaii before fat stores were exhausted (Fig. 10.2). The model predicts that a bat with a fat fraction of approximately 0.24 could just reach Hawaii without tailwind assistance (assuming water is not limiting, below). In our models we only considered the body composition of males, but female hoary bats regularly carried larger fat stores than males (McGuire 2012). During spring migration, one-third of the females examined by McGuire (2012) had fat fractions >0.17, suggesting the upper limits of this curve represent realistic body composition expectations.

Even at fairly modest fat proportions, the model predicts the bats could fly for long durations (Fig. 10.3). Maximum flight duration ranged from 27.2 to 126.3 h

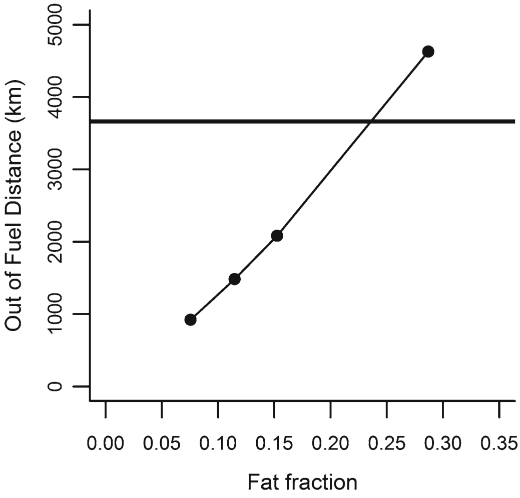
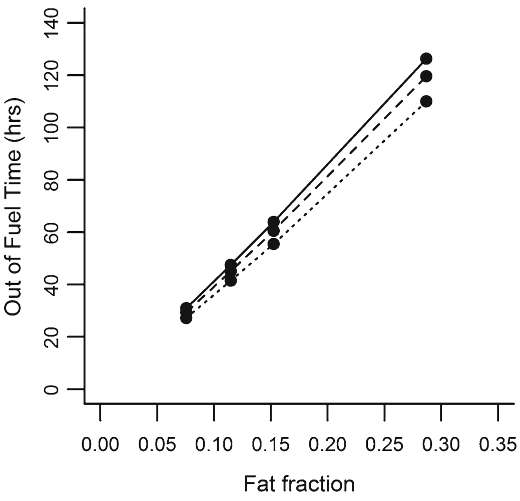


Fig. 10.2 Total possible flight distance based on energy stores without considering wind or water limitations. The *solid horizontal line* indicates the distance from southern California to Hawaii. At the highest fat fractions (>0.24), hoary bats could reach Hawaii without any tailwind assistance (assuming water is not limiting). Only the highest fat-free mass scenario is plotted here for simplicity. At the highest fat fractions, lower fat-free masses would be able to fly a few tens of kilometers farther

Fig. 10.3 Predicted maximum flight durations for hoary bats with varying body composition. The three lines indicate the low (*solid*), mid (*dashed*), and high (*dotted*) estimates of fat-free mass included in the models (see Table 10.1 for values)



(5.26 days!) depending on the initial proportion fat. Flight duration was somewhat variable depending on the size of the hypothetical bat considered. There is little difference in flight duration when fat stores are slight; at a fat fraction of 0.076, flight duration ranged from 27.2 to 30.9 h. However, as the proportion of fat increases, the differences in flight duration corresponding to variation in fat-free mass are more exaggerated. At the highest fat proportion, maximum flight duration was between 110.0 and 126.3 h. Holding fat fraction and wing morphometry constant, but varying body mass, both drag and wing loading will be affected, resulting in variable flight duration predictions. These models assume fat stores are completely exhausted (i.e., absolutely zero fat remaining at termination) which is probably not realistic so perhaps these are slight overestimates.

Our model also predicts airspeed from aerodynamic principles. In the model, true airspeed varies within a flight based on rules regarding maximum power speed in the initial climb, maintaining constant muscle work in the early period of level flight and ultimately maintaining the maximum range speed as fat stores and hence body mass declines. Predicted airspeed varied from 5.9 to 12.5 m s^{-1}, and mean airspeed among flights ranged from 6.1 to 10.8 m s^{-1}. Such estimates are reasonable compared to direct observations of hoary bats. Foraging Hawaiian Hoary Bats averaged 11 m s^{-1} (range 5.9–15.2 m s^{-1}) (Bellwood and Fullard 1984; Jacobs 1996; FJB unpublished data). North American Hoary Bats average 7.7 m s^{-1} in foraging flight, and some individuals fly as fast as 12 m s^{-1} (De la Cueva Salcedo et al. 1995). These authors did not control for the effects of wind speed, which may increase or decrease the apparent flight speed depending on direction, and thus are not directly comparable to the flight speeds estimated by our models. Furthermore, optimal flight speed theory (Hedenstrom and Alerstam 1995) predicts different speed when foraging and migrating. Consistent with this theory, commuting bats do indeed fly faster than foraging bats (Grodzinski et al. 2009). Thus, while previous hoary bat flight speed observations are not directly comparable, they indicate that the flight speeds predicted by the model are reasonable and may in fact represent underestimates.

10.3.2 Is Water Limiting?

Equally important to depletion of energy stores in considering the likelihood of a bat to successfully continue prolonged flight over an ocean barrier is depletion of body water. Might a hoary bat run out of adequate stores of body water before it ran out of energy? Which would be the more limiting during a flight from San Francisco to Hawaii?

We considered whether water balance was more limiting than energy balance (i.e., would water run out before fat, regardless of whether the bat could reach Hawaii or not?). Energy balance was more limiting than water balance at 3 g m^{-3} deficit. Conversely, water was always more limiting than energy at 20 g m^{-3}. At 10 g m^{-3} the limiting resource was related to the combination of fat-free mass and fat fraction. At lower fat proportions (0.076, 0.115), water was more limiting than energy when fat-free mass (largely the size of initial water stores) was low (17 and 20 g, respectively). At higher fat proportions, water was always more limiting than energy.

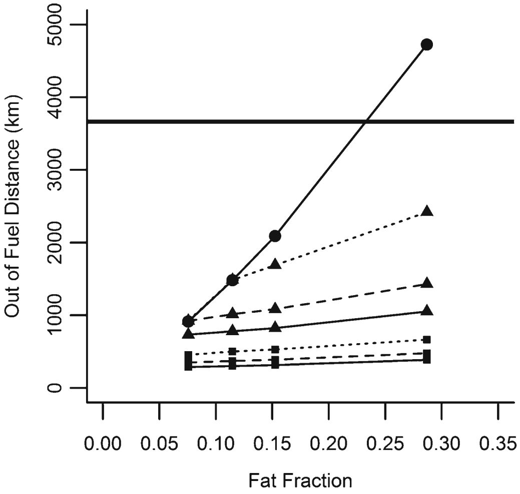


Fig. 10.4 Flight range accounting for energy and water limitations assuming neutral winds. *Solid*, *dashed*, and *dotted lines* represent low, mid, and high fat-free mass values. The symbols indicate the respiratory water loss scenarios: low water loss (*circles*), mid (*triangles*), and high water loss (*squares*). See Table 10.1 for values of fat-free mass and water loss rates. Higher water loss scenarios limit flight range, but higher initial fat-free mass provides a larger starting water pool which increases the potential flight range before water becomes limiting. Water balance was never limiting for the low water loss scenario and therefore the three lines are overlaid and equivalent to Fig. 10.2. The *horizontal black line* at 3,665 km represents the minimum distance from California to Hawaii

Accounting for scenarios where water was more limiting than energy, we reevaluated the potential flight range (Fig. 10.4). Comparing Fig. 10.4 with Fig. 10.2 clearly illustrates the potential of water balance to limit flight range. Only bats with the highest fat fraction and lowest water vapor density deficit had sufficient energy and water reserves to reach Hawaii in neutral winds. At high respiratory water deficits, water has a much greater impact on defining flight range than fat. With a 20 g m^{-3} deficit, flight range was limited to approximately 500 km, well short of Hawaii.

10.3.3 Tailwind Assistance

The next step in the modeling process is the role of tailwind assistance. For each of our 36 model scenarios, we know the theoretical limit to flight range (Fig. 10.4) and the minimum required distance (3,665 km to Hawaii). We determined the minimum required tailwind assistance for the bats to reach Hawaii. Or to put it another way, what tailwind speed would be required to compensate for given energy or water limitations? For practical purposes we assumed a constant wind speed for the duration of the flight and that the wind and flight direction were parallel, both leading a wayward bat on the shortest distance to Hawaiian refuge. If one or the other is off-angle, the vectors will change and for the same wind/airspeed, the ground speed would be lower. Given the prevailing trade winds in the region (Fig. 10.1), such an assumption is reasonable.

To find the minimum required tailwind, we took a fixed wind speed and added it to the airspeed in each 6-min interval of the model output. Adding wind speed and airspeed gives ground speed and thus a new distance estimate can be made for each time interval. We solved for the minimum wind speed necessary to reach 3,665 km in the total flight time possible for a given scenario. In other words, our models assume the bats reach Hawaii exactly as fat and water reserves are exhausted. We determined the range of observed wind speeds in this region of the Pacific Ocean from five weather stations, two island stations, and three environmental buoys [Fig. 10.1; data obtained from National Oceanic and Atmospheric Administration (NOAA) Climate Services http://www.climate.gov/#dataServices]. The five stations had variable temporal coverage, with the most extensive records obtained from Hilo International Airport (1943–present). We constrained the records to only include the months of September–October to coincide with autumn migration and the arrival of hoary bats in California as this is the most likely period when a bat may be swept out to sea and forced to fly to Hawaii. Weather records from these stations include mean and maximum sustained wind speeds recorded at daily intervals. To be conservative, we considered the mean wind speeds (mean 4.51 m s^{-1}, max 25.8 m s^{-1}), rather than maximum sustained wind speed (mean 7.0 m s^{-1}, max 36.0 m s^{-1}). We obtained wind direction information from the Comprehensive Ocean–atmosphere Dataset (COADS, Woodruff et al. 1987) which we obtained through the National Virtual Ocean Data System (NVODS). As for wind speed, we considered the long-term average wind direction for the months of September and October. Required tailwind assistance is highly variable depending on the modeling scenario. Bats with the highest fat proportions and lowest rates of respiratory water loss don't require wind assistance (Figs. 10.5 and 10.6), rather could reach Hawaii even in the face of a slight headwind! At the opposite end of the range, bats facing a 20 g m^{-3} water deficit would require tailwinds of at least 45 m s^{-1} (162 km h^{-1}), corresponding to a category 2 (or higher) cyclone. Therefore, if bats face 20 g m^{-3} water deficit, colonizing Hawaii represents a truly rare and remarkable occurrence. At a more moderate 10 g m^{-3} deficit, required tailwinds ranged from 6 to 32 m s^{-1} (22–115 km h^{-1}), not far off of our originally speculated 25 m s^{-1} storm winds in some cases, and perhaps even much less.

In Fig. 10.6, we have overlaid a histogram of the required tailwind speeds for each of our modeling scenarios and a boxplot of the historically observed range of wind speeds. With the exception of the high water loss scenario, many of our models require tailwinds that could be reasonably expected in the region. Of our 36 modeling scenarios, 17 (nearly half) require tailwind assistance within the range of observed wind speeds. Considering maximum sustained wind speeds, 24 (two-thirds) of our models fall within the range of observed values. Furthermore, seven scenarios required <10 m s^{-1} tailwinds as may be regularly expected due to the easterly trade winds.

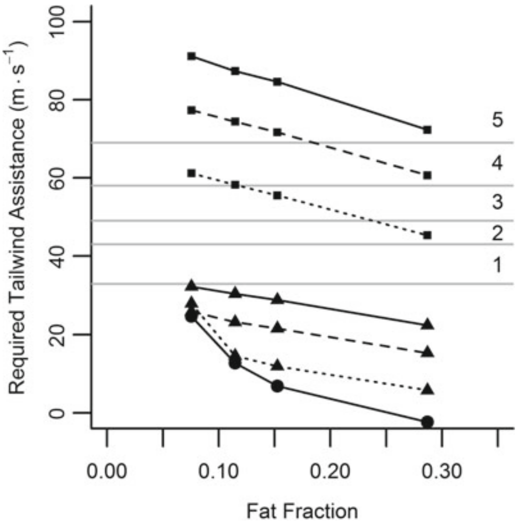


Fig. 10.5 Required tailwind assistance to reach Hawaii before water or energy reserves are exhausted. *Symbols* and *line* styles indicate the various modeling scenarios as in Fig. 10.4. The *horizontal gray lines* indicate the wind speed thresholds for cyclone categories *1*–*5*. At the highest water loss estimates, our models predict that bats could only have reached Hawaii under extreme cyclone winds (category 2 or greater)

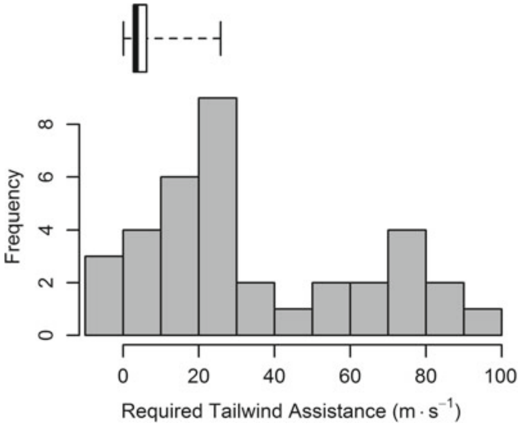


Fig. 10.6 Distribution of required tailwind speeds, replotted from Fig. 10.5 to provide alternate perspective. The horizontal *boxplot* indicates the historical range of sustained wind speeds (data from 5 NOAA weather stations indicated in Fig. 10.1). Note that observed wind speed (from NOAA) is always positive, independent of direction, whereas model scenarios include negative wind speeds to indicate possible headwinds. It is clear that many of our modeling scenarios, accounting for both energy and water limitations, are plausible given the typical wind speeds in the regions

Therefore, the climatic conditions needed for bats to colonize Hawaii may not be exceptionally infrequent in occurrence either in contemporary times or at least since the end of the Pleistocene, ~11,000 YBP. During the Pleistocene, prevailing winds would have fluctuated greatly between glacial and interglacial times.

10.4 Modeling Conclusions

The modeling exercises we conducted for this chapter have examined the physiological, behavioral, and morphological mechanisms and adaptations that we judge were present in the ancestral population in North America from which hoary bats colonized Hawaii. Most of these attributes probably remain relatively unchanged in North America, while the Hawaiian Hoary Bat populations have undergone some noted changes either due to genetic drift (such as founder effects) or natural selection in a novel environment.

Our model provides insights into specific questions crucial to a 3,665 km one-way flight by the ancestral bats that traveled to Hawaii. Can hoary bats carry enough fat to sustain them on a flight to Hawaii? Our model suggests they could. Would dehydration be an issue on that flight? It is apparent that certain dehydration scenarios (Figs. 10.4 and 10.5) have a greater impact on colonization likelihood than energy balance. The extreme variation in the water balance models arises from greater uncertainty in the respiratory water loss processes of flying bats. We have reliable, empirically measured data regarding body composition and can model energy expenditure based on established aerodynamic principles. How bats regulate and compensate for respiratory water losses is poorly understood. How much wind assistance would the bats need to reach Hawaii? Depending on the scenario, some hoary bats could have arrived in Hawaii with light wind assistance or even with slight headwinds; however, prevailing trade winds are likely to have assisted flights with wind speeds in the range of 7–25 m s^{-1} common throughout much of the year.

While our model predicts that hoary bats can store adequate fat to power continuous flight up to 5 day duration, such flights are well beyond the scope of flight behavior known in any other bat species. Even large pteropodid bats have not been successful in colonizing water gaps greater than 1,000 km (Bonaccorso and McNab 1997) across the Pacific Ocean. However, comparable flights do occur regularly in bird migration. Barnacle Geese (*Branta leucopsis*) fly 14 h nonstop during migration (Butler et al. 1998). Great Knots (*Calidris tenuirostris*) fly 4 days nonstop from Australia to China (Pennycuick and Battley 2003). Pacific Golden Plovers (*Pluvialis fulva*) fly nonstop from Alaska to Oahu (Hawaii) taking an average of 4 days over the 4,900 km passage (Johnson et al. 2012). The champions of continuous flight among avian migrants are Bar-Tailed Godwits (*Limosa lapponica*) which make nonstop flights from Alaska to New Zealand that regularly take >7 days of continuous flight (Battley et al. 2012). Impressive transoceanic flights are also known from smaller songbirds. The 25 g Northern Wheatear (*Oenanthe oenanthe*) is similar in body size to North American Hoary Bats and is suspected of making a nonstop migratory flight lasting 4 days from Baffin Island in the Canadian Arctic, across the Atlantic Ocean to the British Isles (Bairlein et al. 2012). Even tiny hummingbirds are known to migrate across the Gulf of Mexico (Lasiewski 1962). Therefore, while such extreme endurance flights are not known to regularly occur among bats, such flights are certainly possible.

10.5 Post-colonization Ecology and Evolution of Hoary Bats in Hawaii

10.5.1 Hawaiian Founder Population

Neither the size of the original founder population nor the number of subsequent colonizing events for hoary bats arriving in Hawaii is known; however, estimation of each can possibly be inferred from future examination of molecular genetics in modern populations of Hawaiian Hoary Bats. Theoretically, a population can be founded by a single pregnant female, but the loss of diversity would be severe, and inbreeding depression in such a small founding population would make extinction much more likely (Amy Russell, personal communication). Looking at the founding of *Triaenops rufus* (Hipposideridae) populations in Madagascar from a source population on the African continent, Russell et al. (2008) found their simulation data to be consistent with a founding population size as small as ~10–25 individuals which subsequently led to speciation events in Madagascar in this genus.

Generally from late August to the end of October each year, hoary bats (both in North America and in Hawaii) swarm in social groups during evening flights, during which time copulations occur (Cryan and Brown 2007, Christopher Todd, personal communication). A single colonization event, if originating during a period of *L. cinereus* autumnal swarming along the North American coast or on offshore islands such as the Farallons, possibly could have resulted in the arrival of individuals in Hawaii sufficient for a founder population of similar size to the model demonstrated for *Trianeops* (Russell et al. 2008). Subsequent arrivals of individuals or small groups in separate migration events would have enriched the genetic diversity of the population, particularly early in the founding process.

10.5.2 Morphological Divergence of Modern Hawaiian Hoary Bats

Hawaiian Hoary Bats have undergone significant character displacement from their mainland counterparts in a number of physical traits. Phenotypic divergence since arrival in Hawaii has included appreciable reductions in body size compared to the presumed ancestral stock in North America. These traits include smaller body mass, skull size, and forearm length (Jacobs 1996; Tomich 1986; FJB personal observation). Jacobs (1996) found that adult female *semotus* were on average 45 % smaller in body mass and 8.4 % smaller in forearm length than *cinereus*. Although he made no conclusion regarding males, our own recent data (FJB, personal observation) indicates similar proportionate reductions in male body size between these two subspecies.

The smaller body mass of Hawaiian compared to North American Hoary Bats also has resulted in a lesser wing loading (Jacobs 1996) in the Hawaiian subspecies

Fig. 10.7 Comparison of pelage coloration in adult *Lasiurus cinereus semotus* from Hawaii Island (female *upper* and *lower left*, male *lower right*) and *L. c. cinereus* from North America (*upper right*); *L. c. semotus* adult males from the island of Maui more closely resemble *L. c. cinereus* (Photos of *L. c. semotus* by Jack Jeffrey and *L. c. cinereus* by Adam Miles)

which in turn makes possible a more maneuverable and acrobatic flight. Because of the dominance of fast flying moths in the nocturnal insect fauna of Hawaii (Belwood and Fullard 1984; Bonaccorso, personal communication), the ability to chase and intercept the evasive flight of moths is of critical importance to hoary bats in Hawaii. In all habitats from sea level to 1,600 m, moths are the most abundant taxa in Hawaiian Hoary Bat diets (Bonaccorso et al. 2013).

Given that hoary bats have been resident in Hawaii for at least 5,500 years, one may wonder if there is significant movement between island populations within Hawaii or if there is little gene flow between islands. Comparing skins and skulls of *semotus* from Kauai, Maui, and Hawaii Islands, there are striking differences in pelage coloration between islands (FJB, personal observation). Bats from Maui have much more distinct fur tipping to render the typical "hoary" appearance similar to mainland *cinereus* (Fig. 10.7). Despite the small water gap (46 km) between the closest portions of coastline separating Maui and Hawaii, the bats from the island of Hawaii in general possess a bright red-brown under coat with much less white tipping on the fur. The lack of white tipping on adult male *semotus* is particularly noticeable on the crown of the head (Fig. 10.7). In fact bats of Hawaii Island were more similar in appearance to those from the most distant major island, Kauai. The similarity of Maui bats to mainland *cinereus* in body size and pelage color, and their distinct difference from bats on Hawaii Island in these characteristics, suggests the possibility of multiple colonization events for separate island groups in

Hawaii, with relatively little genetic exchange among islands post-colonization. Future molecular genetics studies sampling these different islands may resolve such questions.

10.5.3 Present Hawaiian Distribution and Habitat Use

Hawaiian Hoary Bats presently occur on Kauai, Molokai, Oahu, Maui, Lanai, and Hawaii (Tomich 1986). They probably occur on Kahoolawe; however, no effort at ascertaining this is shown in the published literature. Furthermore, Hawaiian Hoary Bats are recorded from sea level to virtually the summit of Mauna Loa Volcano, which is 4,169 m above sea level. We have observed this bat flying over almost every conceivable habitat found in Hawaii including over saltwater embayments, rivers, forests (including both virgin native forest and forest dominated by nonnative trees), grasslands and pastures, eucalyptus plantations, fruit orchards, within deep gulches, as well as over suburban and urban landscapes. Occasionally, Hawaiian Hoary Bats fly and forage over almost barren lava flows despite the apparent scarcity of insect prey that might be expected. Conducting echolocation surveys on both the Big Island of Hawaii and Kauai, foraging activity is common on both windward and leeward sides of these islands. The windward coasts and mountain slopes of the major Hawaiian Islands are incredibly wet and are (or were) covered by rain forests where rainfall in many locations exceeds 4,000 mm year^{-1}, whereas the leeward slopes and coasts of these same islands are much drier. Thus, Hawaiian Hoary Bats truly are extreme habitat generalists.

The subfossil record substantiates that hoary bats arrived in Hawaii at least 5,500 YBP and perhaps as much as 130,000 YBP (Olson and James 1982). Molecular data Morales and Bickham 1995), as well as zoogeographic considerations, and wind patterns suggest that hoary bats arrived in Hawaii from an origin somewhere along the coastline of North America between northern California and southern Alaska. DNA analyses may confirm the region of origin by comparing Hawaiian and North American populations. Our modeling scenarios suggest that the conditions necessary for hoary bats to successfully colonize Hawaii may be reasonably expected and thus provide suggestive evidence that hoary bats may have reached Hawaii more than once. Molecular evidence from DNA may also shed insights into whether more than one colonization event in Hawaii occurred and the degree of continuing exchange among islands. Since their arrival in Hawaii, hoary bats have diverged from the ancestral stock. Both physical and behavioral characteristics distinguish this bat from their North American counterparts. Furthermore, present-day *L. c. semotus* from the neighboring islands of Maui and Hawaii have striking differences in cranial measurements and pelage coloration. Could these two islands have been colonized independently with low subsequent gene flow between them? Further investigation is required to fully answer the question of how bats became the only terrestrial mammals to colonize the Hawaiian archipelago, one of the most isolated insular landmasses on Earth.

Acknowledgments We thank Peter Donaldson, Brock Fenton, Alex Gerson, Jens Rydell, and Amy Russell for thoughtful discussions on the topics of bat and bird migrations, animal physiology, population genetics, and meteorology. We thank Brian McNab and Michel Genoud for critical and constructive review leading to improvements in the manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. Bonaccorso was supported by the Pacific Island Ecosystems Research Center, US Geological Survey, for work on this project.

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Chapter 11
Learning and Memory in Bats: A Case Study on Object Discrimination in Flower-Visiting Bats

Georgina Ross and Marc W. Holderied

Abstract Bats are an exceptionally successful mammalian order, with a wide range of physiological, morphological and behavioural adaptations to the niche of an actively flying nocturnal vertebrate. Many aspects of their life histories suggest bats also possess remarkable learning and memory abilities, which renders them particularly interesting subjects for cognitive research. Yet compared to some other mammalian orders, relatively little is known about their cognitive abilities. Here we will review current knowledge on how bats use spatial learning to navigate during migration and foraging, how they use vocal learning for individual and group recognition, and how they employ social learning to glean information from others. Finally, discrimination learning (learning to use stimulus differences for classification) is explored for nectar-feeding bats. These bat species pollinate plants that have evolved a suite of floral traits associated with bat pollination (chiropterophily), including echoacoustic signatures. We present and discuss an experiment on the individual- and sex-specific cognitive abilities of captive *Glossophaga soricina* comparing their speed of discrimination learning, long-term retention of discrimination ability and relearning flexibility. The aim of this chapter is to provide glimpses into the cognitive abilities of these fascinating mammals to explore how their learning and memory abilities may have contributed to their success.

11.1 Introduction

Learning (the change in state resulting from experience) and memory (the storage, retention and retrieval of information) (Shettleworth 2010) are key areas for investigation in cognitive research. Whilst we have a fairly strong base of information on

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_11,

learning and memory strategies in primates, rodents and birds (Pearce 2008), there is a gap in our knowledge about bats. Despite being the second-largest mammalian order, with species that show a wide range of physiological, morphological and behavioural adaptations for a diverse array of habitats and diets, we know relatively little about their comparative cognitive abilities. The aim of this chapter is to illustrate the diversity of cognitive abilities of these fascinating mammals and how this may have contributed to their success. In particular, we will concentrate on spatial memory, vocal and social learning and echoacoustic discrimination learning.

11.2 Spatial Memory

Spatial learning and memory may be important for bats for several reasons including migration (Fleming and Eby 2003), pup relocation in maternity colonies (McCracken 1993) and for revisiting known food sources, e.g. flowering plants (Carter et al. 2010). Echolocating bats have been shown to rely heavily on spatial memory and are spatially accurate over large distances in long-range migrations (e.g. Richter and Cumming 2008), as well as at very fine scales in laboratory tasks (Ulanovsky and Moss 2008). Spatial memory may even dominate over external sensory cues. For example, in *Megaderma lyra* individuals that had been trained to fly through a narrow gap continued to pull in their wings when the hole was widened (Möhres and Neuweiler 1966) and avoided the positions of obstacles even when these were removed (Neuweiler and Möhres 1967). Similarly, *Phyllostomus discolor* continued to attempt to land on a dummy perch that could no longer be used even when other nearby perches were available (Holler 1995; Holler and Schmidt 1996). In a relatively stable environment, supplementing echolocation with spatial memory seems highly adaptive in a number of contexts. Long-term spatial memory over several months in bats has also been demonstrated experimentally, where memory for a rewarding maze arm persists over periods of hibernation in *Myotis myotis* (Ruczynski and Siemers 2011).

11.2.1 Large-Scale Navigation

Many species of bat show impressive annual migrations, for example, around 2,500 km in *Eidolon helvum* (Richter and Cumming 2008) or over 1,000 km in *Lasiurus borealis* and *Nyctalus noctula* (Fleming and Eby 2003). Relocated bats (tens to hundreds of km) show homing behaviour to a familiar roost site (Holland et al. 2006; Mueller and Emlen 1957). Spatial memory may be particularly important for this type of large-scale navigation because most species' dominant sense of echolocation operates over ranges that are smaller by several orders of magnitude (e.g. Holderied and von Helversen 2003). Some bats show site fidelity and return annually to the same feeding areas (Martinoli et al. 2006), roost area (Hillen et al. 2010; Veilleux and Veilleux 2004) or particular tree (Lewis 1995).

For long-distance migrations and homing, spatial maps may be anchored using visual landmarks such as mountains (Williams et al. 1966) and directional cues such as the post-sunset glow (Buchler and Childs 1982). The magnetic field also helps at least some bats to navigate (Holland et al. 2006, 2008; Holland 2007). There is also some evidence that bats follow large-scale linear landmarks such as rivers and riverbanks (Serra-Cobo et al. 2000; Verboom et al. 1999) or use large-scale map-like navigation based on distant visual landmarks (Tsoar et al. 2011).

11.2.2 Foraging

Spatial memory for routes to foraging sites may be important for bats that regularly revisit known areas of high food density. Whilst travelling from roost to foraging sites, bats have been shown to follow certain 'flyways' and use these consistently over several nights (Racey and Swift 1985; Williams and Williams 1970). An impressive example of the importance of spatial memory for foraging can be seen in phyllostomid nectar-feeding bats (Carter et al. 2010; Thiele and Winter 2001, 2005). Some flowering systems create predictable profitable resource patches that, if remembered, can reduce the time spent searching (Gentry 1974; Tschapka and von Helversen 2007). Accordingly, laboratory studies in the long-tongued bat *Glossophaga soricina* have indicated that spatial memory is particularly important for relocation of previously rewarded feeding areas, whilst other cues (echoacoustic or scent) are used if this strategy fails (Carter et al. 2010; Thiele and Winter 2005). The use of spatial cues appeared to provide some advantage to bats when making decisions, because bats were more efficient finding targets when they chose spatially rather than searching with echolocation (Thiele and Winter 2005). The importance of spatial memory has also been shown in other nectar foragers, particularly in hummingbirds (Hurly and Healy 1996; Sutherland and Gass 1995) and bees (Janzen 1971; Orth and Waddington 1997).

11.2.3 Small-Scale Landmarks

Small-scale spatial memory might be gathered through an individual's own movements (idiothetic orientation) or through exogenous information such as landmarks. The limited available evidence suggests that the importance of landmarks appears to differ between species and sexes. In *Cynopterus sphinx* landmarks did not improve the speed of building spatial memory of a rewarded feeder location, and the performance in locating feeders did not decline when these landmarks were removed (Zeng et al. 2010). In *Rousettus aegyptiacus* memory for a perch location was not influenced by changes in landmarks in the vicinity of the perch, indicating that the perch was remembered as a location in space as opposed to in relation to other objects, both visually and echoacoustically (Holland et al. 2005).

For other species, however, landmarks do appear to play an important role in the memory of a spatial task (Jensen et al. 2005). *Eptesicus fuscus* was trained to fly through a 35 cm hole in a mist net to receive a food reward on the other side, and a landmark was placed 10 cm from the hole. Once bats had learned to fly through the hole, both landmark and hole were moved either together or separately. When landmark and hole were moved but the relationship between them was preserved, bats still performed the task well. However, if the relationship was not preserved, the bats repeatedly crashed into the net where the hole would have been expected in relation to the landmark. Bats echolocated whether or not they crashed and there were no obvious differences in the echolocation behaviour between crash and successfully trials, indicating that bats favoured the use of landmarks over conflicting sensory cues (Jensen et al. 2005). In this study, it was however not investigated whether the learning performance was affected by the presence of landmarks.

More recent research suggests sex differences in the use of landmarks for spatial orientation (Schmidtke and Esser 2011). *P. discolor* bats were first trained to fly a direct route through an empty flight tunnel to a landing platform. Then four large acrylic glass panes were added that forced bats on a detour route. Modifying the configuration of these landmarks affected performance of females more than that of males indicating that females relied more on landmarks. This result falls in line with findings from landmark studies in visually orientating mammals including humans (Sandstrom et al. 1998), rhesus macaques (Lacreuse et al. 2005) and rodents (Jacobs et al. 1990; Roof and Stein 1999), where females also relied more on landmarks. The selection of respective studies is small and it is difficult to make direct comparisons of species because differences in experimental setups might greatly vary task performance. It would be remarkable, however, if spatial memory did play a role in laryngeal echolocators, but not in the Pteropodidae.

11.2.4 Spatial Memory and the Brain

In bats, much is already known about the relationship between relative brain size and a variety of life history traits. For example, Ratcliffe et al. (2006) demonstrated that behaviourally flexible foragers (in this case, bats which both caught prey in midair and gleaned prey) had larger relative brain sizes than bats using just one dominant foraging strategy. The hippocampal region of the brain has been implicated in the navigational abilities of many birds and mammals, e.g. across both small (e.g. Morris et al. 1982) and large scales (e.g. Gagliardo et al. 1999). In bats, hippocampal size is positively related to increased cognitive demands for spatial processing, for example, in more complex foraging habitats, species have larger relative hippocampi (Safi and Dechmann 2005). The dynamics of hippocampal spatial coding is considered one basis for spatial learning (e.g. Wilson and McNaughton 1993). Hippocampal place cells have been found in *E. fuscus*, where echolocation suffices to elicit location-specific neural activity (Ulanovsky and Moss 2007). Recently, grid cells in the entorhinal cortex have been shown to contribute to the encoding of spatial memory (Yartsev et al. 2011). Interestingly, adult neurogenesis (the proliferation of neurons in the hippocampus), which is considered an important aspect of spatial learning and memory in some mammals, is either at very low levels or completely absent in the bat species studied. This is remarkable considering the long life spans of bats (Amrein et al. 2007).

11.3 Vocal Learning

Whilst many mammals have been shown to have versatile vocalisations (e.g. dolphins; Reiss and McCowan 1993; Richards et al. 1984), there is very little evidence in support of full vocal learning, such as through active modifications of call pitch or structure as a result of learning, in taxa other than birds, humans and chimpanzees (Janik and Slater 1997; Crockford et al. 2004). In bats, sound communication plays an important role in social interactions and so they are ideal candidates for investigating vocal learning. Whilst social calls, which are generally longer than echolocation pulses, audible and multi-harmonic (Fenton 2003), presumably evolved solely for communication purposes, echolocation calls have a dual role. Echolocation calls are primarily utilised to collect spatial information for orientation and foraging but as they are broadcast loudly, they can be overheard and interpreted by nearby bats (Jones and Siemers 2011). The communication potential of echolocation calls may be quite substantial as they can carry information regarding sex (Kazial and Masters 2004; Neuweiler et al. 1987; Siemers et al. 2005), age (Jones and Ransome 1993; Masters et al. 1995) and size (Russo et al. 2001) of a particular bat as well as the behavioural context in which a call is made (Bohn et al. 2008). Differences in both social and echolocation calls can be perceived and used as cues for conspecific (Schuchmann and Siemers 2010), individual (Yovel et al. 2009) and group (Boughman 1997) recognition.

11.3.1 Individual Recognition

Vocalisations have been described as important for the perception of individual identity in mammals, with many calls having individual-specific components (e.g. Janik et al. 2006; Rendall et al. 1996). There is considerable evidence that both echolocation calls (Fenton et al. 2004; Kazial et al. 2008; Yovel et al. 2009) and social calls (Arnold and Wilkinson 2011; Carter et al. 2008; Melendez and Feng 2010) can have individual-specific components. Individual signatures may be beneficial to both the calling individual (to reduce jamming with other bats; Ulanovsky et al. 2004) and to the receiver of the call as a basis for reciprocal interactions in a stable social group (Siemers and Kerth 2006). Although other cues, such as olfaction, are used to recognise individuals (Caspers et al. 2008), acoustic information is more reliable over greater distances in dynamic contexts, e.g. whilst in flight, and so can be useful for discriminating familiar individuals from strangers (Voigt-Heucke et al. 2010) or maintaining contact with affiliated individuals (Carter et al. 2009; Chaverri and Gillam 2010).

Recent studies provide evidence that bat species can learn such individual-specific call characteristics. This was first demonstrated in *Desmodus youngi* by Carter et al. (2008) where foraging individuals exchange social calls in a duet-like fashion (also known as antiphonal calling). Playbacks of social calls could elicit antiphonal calling, and such experiments indicated that these social calls attract conspecifics. Bats showed individual variation in call structure, and they were consequently able to discriminate between individuals using their specific calls (Carter et al. 2008, 2009). In *Noctilio albiventris* females form stable roosting and foraging groups. In playback experiments these bats showed the strongest behavioural responses to calls of non-familiar conspecifics. They responded with more frequent wing stretching and honk calling to non-familiar compared to familiar conspecifics, indicating that they recognised the calls of individuals from their own social group (Voigt-Heucke et al. 2010). In two-alternative forced-choice experiments with *M. myotis*, bats were able to classify individuals based on their calls and to transfer this habituation to unknown calls of the same individuals. The authors suggested distribution of energy over the frequency spectrum as candidate cues for this discrimination (Yovel et al. 2009).

11.3.2 Vocal Learning in Bat Pups

Mother-infant recognition in large bat colonies is crucial but may be a difficult task and can be aided by individually distinct contact ('isolation') calls made by pups (Bohn et al. 2007; Gelfand and McCracken 1986). Mothers may respond to such isolation calls with directive calls (e.g. Balcombe and McCracken 1992). In some cases, duetting occurs between mother and pup, e.g. in *Rhinolophus ferrumequinum* (Matsumura 1981), which may further help mothers to identify and localise pups. It appears that bat pups can learn their mother's vocal signature and adapt their own isolation calls to incorporate these individual-specific components (Esser and Schmidt 1989). For example, in *P. discolor*, hand-raised pups presented with a recorded maternal call adapted their isolation call structure to this stimulus in contrast to pups reared in the absence of calls (Esser 1994). In *R. ferrumequinum* there is a transmission of resting constant frequency (RF) between mother and pup (Jones and Ransome 1993). Although not tested explicitly, there appears to be a learned component to this transmission, as pups born to mothers whose RF has declined with age, themselves had lower RF values (Jones and Ransome 1993). Recent research has indicated that bat pups show babbling behaviour and also possess the ability to imitate the vocal repertoire of adult conspecifics indicating that auditory experience is required for vocal development (Knörnschild et al. 2006, 2010).

11.3.3 Group Recognition

Recognising group membership may be important if bats show reciprocal altruism with group mates (*Desmodus rotundus*, Wilkinson 1984), coordinate group foraging (*Phyllostomus hastatus*, Wilkinson and Boughman 1998) and/or share information about foraging sites (*Nycticeius humeralis*, Wilkinson 1992). Several bat species show differences in calls between social groups (e.g. Boughman 1997; Pearl and Fenton 1996; Voigt-Heucke et al. 2010), but *P. hastatus* provides a particularly good example of vocal group recognition. Unrelated females in this species live in stable social groups and give audible broadband (4–18 kHz) contact (or 'screech') calls, which are group but not individual specific (Boughman 1997). When bats changed group affiliation, not only did their own call type change to become more like that of the new group but the existing group members responded with reciprocal call modifications (Boughman 1998). This demonstrates that group signatures are acquired through vocal learning as opposed to other mechanisms such as low gene flow or call matching (Boughman 1998). The benefits to forming and maintaining these female groups appear to lie in sharing information about food sources, the coordination of foraging and the mutual defence of feeding and roosting sites (Boughman and Wilkinson 1998). The calls appeared to be particularly important when bats were feeding on a concentrated resource where group defence can be most effective (Wilkinson and Boughman 1998).

11.4 Social Learning

Bats are highly social mammals with a diverse range of social systems (Kerth 2008). They show a wide variation in group size (up to millions in a cave), group stability and timing (Bradbury 1977; Wilkinson and Boughman 1999). Groups may be very flexible in fission–fusion societies (Kerth and König 1999; Willis and Brigham 2004), similar to those seen in primates, carnivores and cetaceans (Packer et al. 1990; Whitehead et al. 1991). The reason for the evolutionary origin of sociality in bats is not fully understood but possible explanations include the limited availability of suitable roost sites and social thermoregulation (Kerth 2008). Additionally, both longevity and natal philopatry support formation of a stable group structure, which can lead to cooperation in female bats (Kerth 2008). In male bats, groups may have formed for food resource information transfer (Safi and Kerth 2007). Bats are ideal candidates for investigating social learning because of widespread sociality (communal roosting), because they are long lived and because their echolocation calls may provide public information (Wilkinson and Boughman 1999). Despite these benefits, there are relatively few studies that investigate or demonstrate social learning in bats (Wilkinson and Boughman 1999).

11.4.1 Feeding Resource Information

Feeding resource information can be passed on in various ways, through actual interactions with bats that have fed, by following to profitable foraging sites or by eavesdropping on feeding bats. Information transfer through roosting interactions has been shown in the short-tailed fruit bat (*Carollia perspicillata*) in the laboratory (Ratcliffe and ter Hofstede 2005). In this study bats were either 'observers' or 'demonstrators'. Observer bats could learn about novel food types through roosting interactions with their experienced demonstrator and indeed they then preferred the food flavour their demonstrator had consumed. This learning was reversible. The authors suggested two adaptive functions for social learning of food preferences (1) bats encounter unfamiliar odours on roost mates and then search for that odour rather than following the roost mate and (2) a familiar odour on a roost mate indicates that this food source is available and then the bat can fly to the known location of this food source using spatial memory (Ratcliffe and ter Hofstede 2005).

Social learning about food resources has also been shown in frog-eating bats (Page and Ryan 2006). The fringe-lipped bat (*Trachops cirrhosus*) uses the courtship songs of its frog prey to detect and locate them and to assess features such as palatability and size (Tuttle and Ryan 1981). When bats had to learn a novel association (calls of the poisonous toad *Bufo marinus* paired with an edible fish reward), they mastered the task quicker if they were paired with an experienced 'tutor' bat than if they were paired with a naive bat or when they foraged alone. It is currently unclear how naive bats learned the task from the tutors, whether it be eavesdropping on the echolocation behaviour of tutors or directly following them whilst they foraged (Page and Ryan 2006). Likewise, in *E. fuscus*, naive bats appear to be able to learn from experienced demonstrators (Wright et al. 2011). Observer bats had to locate a mealworm suspended on a string at different positions in a room. Observer bats (including juveniles with little or no prior foraging experience) paired with demonstrators were significantly more likely to direct feeding buzzes towards and attack mealworms than those paired with other inexperienced observers. Bats that attacked and captured mealworms flew closer to demonstrators and showed more following/chasing behaviour than did those which did not attack mealworms (Wright et al. 2011).

11.4.2 Eavesdropping

Because ultrasound is absorbed strongly by air, echolocation calls are usually intense to achieve the operational range needed for spatial orientation and foraging. As a consequence, these calls are often also audible to other bats in the vicinity, who might exploit this public information (Jones and Siemers 2011). Eavesdropping on both social and echolocation calls may aid bats in finding roosting sites. Bats are strongly attracted to roosts from which social calls are broadcast (Furmankiewicz et al. 2011), and they locate roosts faster when eavesdropping on broadcasts of conspecific calls (Ruczynski et al. 2007, 2009). In a foraging context, eavesdropping may be very important because echolocation calls are task specific (search phase calls vs. feeding buzzes). This means that bats may communicate information to eavesdroppers on not only their current feeding behaviour (Fenton 2003) but also the success of a feeding attempt (Britton and Jones 1999). Therefore, paying attention to the foraging calls of conspecifics may allow bats to assess the quality of a particular feeding ground and locate patches rich in prey. In playback experiments, bat foraging activity indeed has been shown to be significantly higher when conspecific feeding buzzes (as opposed to other call phases or backward played buzzes) were played, e.g. in *Tadarida brasiliensis* (Gillam 2007), *Myotis lucifugus* (Barclay 1982), *L. borealis* (Balcombe and Fenton 1988) and *N. albiventris* (Dechmann et al. 2009). Additionally, *M. lucifugus* responds to the calls of *E. fuscus*, which has an overlapping diet but different echolocation calls (Barclay 1982). Bats may also eavesdrop on incidental rustling noises created by prey to locate and capture them (e.g. Marimuthu and Neuweiler 1987; Ryan and Tuttle 1987). Other bat species locate prey by their courtship songs, such as *Myotis blythii* hunting tettigonid bush crickets (Jones et al. 2011) and *T. cirrhosus* hunting and making prey quality assessments on frog calls (Tuttle and Ryan 1981), all of which requires associating these sounds with prey.

11.5 Discrimination Learning in Flower-Visiting Bats

Discrimination learning, i.e. associating one response to one stimulus and a different response to another, involves the perception of differences between stimuli, which allow stimulus classification (Shettleworth 2010). The ability to discriminate between stimuli is an essential part of an organism's success in finding food and avoiding predators. For the rest of this chapter, we will concentrate on an example of discrimination learning and memory in echolocating nectar-feeding bats, which have evolved an intimate relationship with the bat-pollinated flowers they visit in the tropics. These bats are thought to be important players in tropical pollinator webs with around 50 species worldwide pollinating over 1,000 plant species in 270 genera (von Helversen and Winter 2003). Both bats and the plants they visit have evolved a suite of traits associated with bat pollination, which is often referred to as the 'syndrome of chiropterophily'. In nectar-feeding bats, commonly seen traits include hovering flight, long tongues and excellent spatial memory to relocate flowers (von Helversen and Winter 2003). In plants, traits include nocturnal anthesis (Lemke 1984) and flowers which are often bell shaped (von Helversen et al. 2003), drab or pale coloured (Tschapka et al. 2006), with strong sulphurous scents (Bestmann et al. 1997), and which protrude or hang down into the forest canopy (Heithaus et al. 1974).

Learning of floral signals impacts of foraging energetics and hence is a key factor governing plant-pollinator coevolution (Heinrich and Raven 1972). Glossophagine bats have higher average basal metabolic rates than other bats, and their daily energy expenditures are among the highest in mammals (Winter and von Helversen 2001; von Helversen and Reyer 1984). Given these specific energetic requirements of their pollinators, bat-pollinated plants achieve high visitation and hence pollen transfer rates by adequately high nectar production (Heinrich 1975) but also by improving the bats' search efficiency by becoming more conspicuous (von Helversen and Winter 2003). Hence, the conspicuousness and uniqueness of flower echoes should be selected for to match the perceptual and cognitive abilities of their echolocating pollinators. The characteristic bell shape of chiropterophilous flowers is believed to facilitate detection and recognition (Simon et al. 2006; von Helversen et al. 2003). Striking examples of acoustic signalling can be seen in the vines *Mucuna holtonii* (von Helversen and von Helversen 1999) and *Marcgravia evenia* (Simon et al. 2011).

Differences in flower shape and size give rise to different echo reflectance, of which the bat can analyse intensity, temporal differences and/or spectral patterns for echoacoustic discrimination (Simon et al. 2006). Although we know that nectar-feeding bats can make accurate discriminations based on acoustic characteristics (Thiele and Winter 2005; von Helversen 2004; von Helversen and von Helversen 2003), little is known about how well they can remember these differences, how flexible they are to changes and whether other factors such as individual differences in learning ability or strategy play any role in foraging performance. A recent study by Ross and Holderied (in preparation) exemplified a nectar-feeding bat's (*G. soricina*) learning and memory capabilities in a simple echoacoustic object discrimination task. These small nectar-feeding bats are particularly well suited for behavioural experiment because their natural foraging behaviour (making hundreds of flower visits per night) can easily be replicated in a laboratory setting. In a two-alternative forced-choice (2-AFC) size discrimination task, initial learning performance and memory over time (after 1, 7 and 28 nights) were tested. Using two computer-controlled artificial feeders that can give a 10 μl sugar water reward, bats were trained to choose the larger of two wooden spheres (10 vs. 15-mm radius) that alternated pseudo-randomly between the two feeders. Learning and memory performance was measured through speed (number of visits to reach a criterion level of 75 % correct) and accuracy (% correct choices once criterion reached) (Fig. 11.1a).

11.5.1 Initial Learning and Memory

Bats learned to choose the larger of two spheres in 513 ± 213 visits ($n = 12$, Fig. 11.1b), which is consistent with previous research in these bats (von Helversen 2004; von Helversen and von Helversen 2003; Thiele and Winter 2005). Bats could remember the size discrimination task even 28 nights after their previous test, but remembered best after 1 night (Fig. 11.1b), which links in with the predictable long-term flowering system of chiropterophilous plants. The persistence of discrimination memory over 28 days (and likely much longer; personal observation GR) may also be advantageous because some chiropterophilous plants show more than one flowering peak throughout the year (Hopkins 1984). Task accuracy was relatively

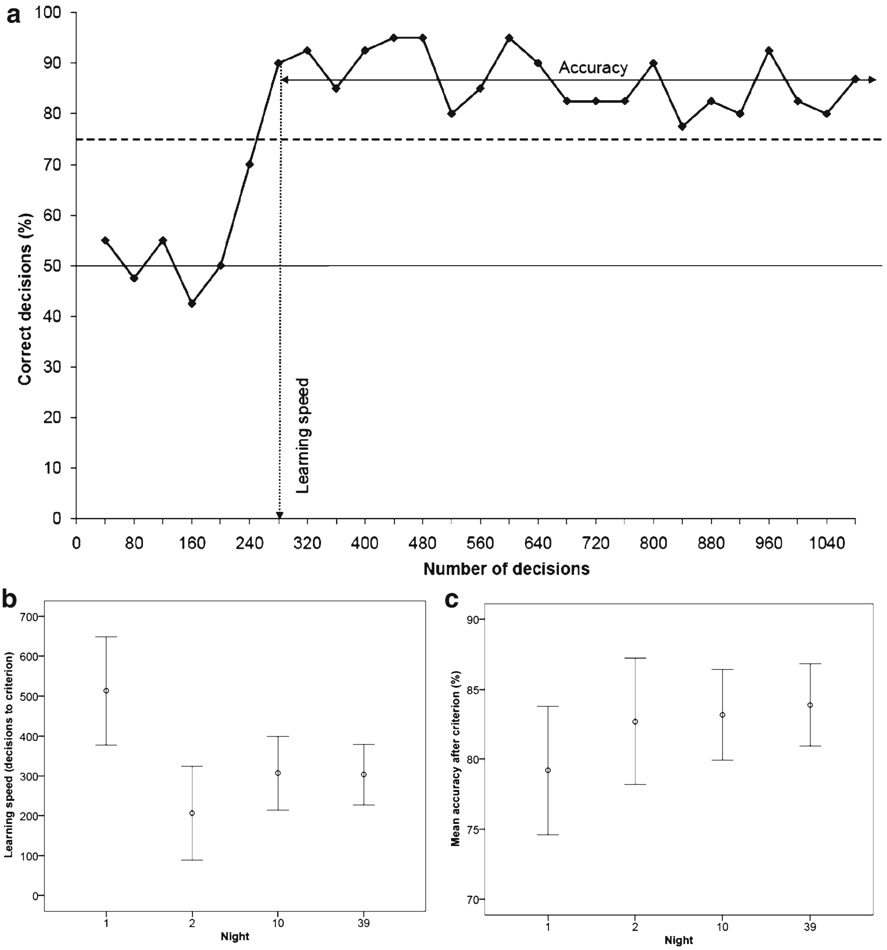

Fig. 11.1 Discrimination learning in *G. soricina*. (**a**) Example learning *curve* on the first night of testing, *dashed horizontal line*: 75 % criterion level. 'Learning speed' denotes number of decisions to reach criterion (280 here). *Line* labelled 'Accuracy' shows the average accuracy once the criterion level was reached (86.6 % here). (**b**) Learning speed measured as mean number of decisions that bats ($n = 12$) required to reach criterion level (75 %) and (**c**) mean accuracy after criterion level was reached for all bats (n = 12) for the 4 test nights. Night 1: initial discrimination, night 2: starts 12 h after end of night 1, night 10: 7-day break after night 2, night 39: 28-day break after night 10. Error bars indicate 95 % confidence intervals

constant throughout testing, but only at 80 % success rate (Fig. 11.1c), which might reflect a foraging strategy of monitoring currently non-profitable sources. This strategy can be adaptive because bats that base decisions exclusively on the previously rewarded contingency may lose out if relative profitabilities change. The slight increase in accuracy from 79 % on night 1 to 83 % on night 39 might indicate that repeated training resulted in greater confidence in the stability of the environment.

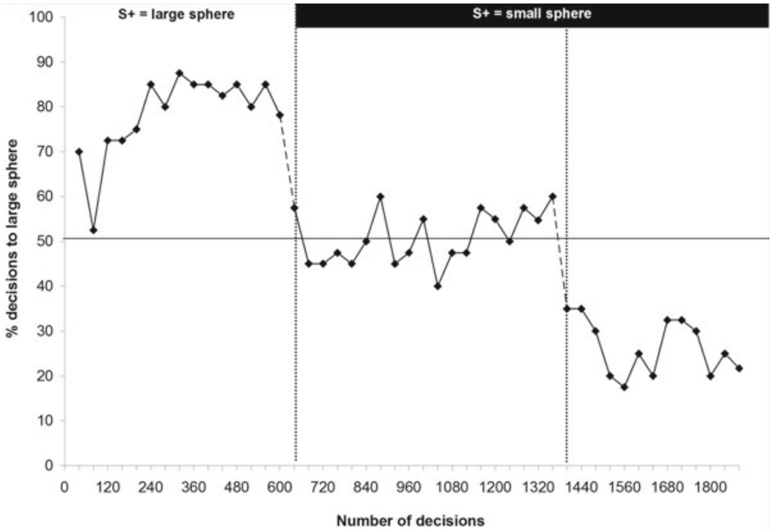

Fig. 11.2 Example of preference reversal over 3 nights. *Dotted vertical lines* separate the 3 nights. The first is night 39 where the larger sphere was rewarded S+. In the following 2 nights, the large sphere became S− and the small sphere the S+. The bat responded by a 50:50 strategy in the first night and reversed its preference only on the second reversal night

11.5.2 Flexibility, Sex and Individual Differences

To test learning flexibility, bats were trained to reverse their preference to the smaller sphere, which appeared to be a difficult task (Fig. 11.2). Only half of the bats ($n=6$) switched their preference within the 2 days of testing, and they required 1,787 ± 521 visits to achieve this. This was surprising because we expected bats to be flexible to maximise their foraging success. However, bats were not punished (other than by a lack of reward) for an incorrect decision and could receive a reward in 50 % of cases by using a random strategy (as adopted in the first reversal night; see Fig. 11.2). Hence, the poor reversal performance might not truly reflect the limits of their relearning capability.

Although sex differences in learning and memory had not been previously tested in nectar-feeding bats, there was anecdotal evidence that males outperform females, resulting in a preference for males as subjects (Simon et al. 2006; Stich and Winter 2006). Sex differences for spatial tasks have been shown in other mammals including humans (Driscoll et al. 2005), other primates (Ng et al. 2009), rodents (Frick and Gresack 2003; Galea et al. 1996; Ricceri et al. 2000) and most recently bats (Schmidtke and Esser 2011). We, however, failed to find any significant difference between the sexes in learning or memory performance. Presumably the pressures for males and females to memorise and remember flower profitabilities are similar. Yet, there were distinct individual differences between bats with consistently ‘quick and accurate’ and ‘slow and inaccurate’ individuals. It appears that spending more trials learning about the reward contingencies did not aid accuracy; in fact the opposite was true as of our 12 individuals, the three fastest learners were also most accurate and the three slowest learners the least accurate. This suggests individual cognitive differences rather than speed-accuracy trade-offs in learning.

11.6 Conclusion

In this chapter we have presented a review of recent research on the learning and memory capabilities of bats and how these may have aided bats in becoming such an exceptionally successful mammalian order. This review covers three key areas: spatial memory, vocal learning and social learning. Bats use spatial memory for navigation over both large and small scales, and it is particularly important for bats foraging on spatially predictable resources. An interesting direction for future research would be to study the differences between species with different feeding ecologies in their use of internal and external spatial reference points. We have further presented evidence that bats' calls can have individual and group signatures, which may be learned to allow for individual, group and mother-pup recognition. It would be interesting to discover differences in vocal learning of echolocation versus social calls. Bats are social mammals and so may learn about resources by following other bats or by eavesdropping on their vocalisations. It would be interesting to see further work on the influence of 'tutors' on naive bats, and how information is passed between the two. Our case study on flower-visiting bats revealed individual cognitive abilities in echoacoustic object discrimination. Recent discoveries on echoacoustic plant signalling provide fascinating first glimpses into sensory and evolutionary aspects of discrimination learning.

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Chapter 12
Cooperation and Conflict in the Social Lives of Bats

Gerald G. Carter and Gerald S. Wilkinson

Abstract To be evolutionarily stable, cooperative behavior must increase the actor's lifetime direct fitness (mutualism) or indirect fitness (altruism), even in the presence of exploitative, noncooperative "cheaters." Cooperators can control the spread of cheaters by targeting aid to certain categories of individual, such as genetic relatives or long-term social partners. Without such discrimination, cheaters could gain the reproductive benefits of cooperation without paying the same costs and eventually outbreed cooperative phenotypes. Here, we review evidence for cooperative behaviors in bats and the possible mechanisms that might prevent cheating. Cooperative behavior in bats is shaped by ecology, life history, and social structure. Altruism without kin discrimination is unlikely to evolve through population viscosity in bats because dispersal leads to low-average relatedness in the colony or social group. On the other hand, mutually beneficial cooperation, often between unrelated individuals, is found in several bat species. Examples include social thermoregulation, male cooperation for defense of female groups, female cooperation for defense of food and pups, social grooming, and food sharing. Many forms of cooperation in bats likely involve both direct and indirect fitness benefits. Some group-living tropical bat species provide intriguing examples of costly helping behavior between unrelated individuals, but the exact mechanisms that prevent cheating remain to be tested.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_12,

12.1 Introduction

Natural selection favors various forms of cooperation at every level of life from genes to cells to individuals to social groups. Cooperative behaviors increase the fitness of recipients and are adaptive because they promote the actor's own direct fitness, increase indirect fitness by helping a genetic relative (kin selection), or some combination of both (Table 12.1). All forms of social life from microbial biofilms to insect and human societies involve mechanisms that maintain cooperation and suppress conflict. Without these mechanisms, helping behaviors or public goods would be exploited by noncooperative "cheaters" that can gain the reproductive benefits without paying the costs. Here, we describe evidence for cooperative behaviors in bats and discuss what behaviors might prevent cheating. Before discussing specific examples, we briefly review the underlying social evolution theory. Further review of social evolution is provided by West et al. (2007a), Wenseleers et al. (2010), and Bourke (2011).

12.2 Inclusive Fitness Theory

Hamilton (1964) solved the long-standing puzzle of why natural selection sometimes rewards organisms that sacrifice their own lifetime reproductive success to help others. Within evolutionary constraints, organisms should evolve to maximize a quantity he called inclusive fitness, which includes the transmission of genes via both personal reproduction (direct fitness) and by helping genetic relatives reproduce (indirect fitness). Hamilton's concept of inclusive fitness updated the original concept of fitness based on offspring production: adaptations evolve because they increase inclusive fitness, not just offspring production. Thinking of individual organisms as "inclusive fitness maximizers" has provided among the most testable and successful hypotheses in social behavior (Davies et al. 2012). Hamilton showed that altruism evolves when the reproductive benefits to others (B), scaled by the coefficient of relatedness (r), exceed the reproductive costs to the helper (C), i.e., $rB > C$, an inequality now known as Hamilton's rule.

To distinguish inclusive fitness theory from early, flawed theories of group selection, paths to cooperation via indirect fitness benefits were confusingly termed "kin selection" by Maynard-Smith (1964), despite kin selection not really being a form

Table 12.1 Categories of adaptive social behavior

Effect on actor's direct fitness	Effect on recipient's direct fitness	
	Increase	Decrease
Increase	Mutualism or mutual benefit	Selfishness or parasitism
Decrease	Altruism	Spite
	Cooperation	**Conflict**

Adapted from Hamilton (1964) and West et al. (2007b). Effects on direct fitness are measured in average lifetime offspring production. Altruism and spite are maintained solely by indirect fitness (kin selection) of selection and not requiring shared familial ancestry as would be observed in a pedigree (e.g., "greenbeards," Hamilton 1964; West and Gardner 2010). Kin selection does not even require kin recognition, because it can occur passively via limited dispersal. Yet, the term "kin selection" still leads to the common semantic misunderstanding that kin selection and modern forms of group selection (i.e., multilevel selection) are alternative competing hypotheses, rather than alternative ways of modeling the same biological process (e.g., Wade et al. 2010). Inclusive fitness models can implicitly account for the effects of competition between groups even when those groups are not explicit in the model, just as multilevel selection models can implicitly capture the effects of relatedness even when relatedness is not explicit in the model. In this way, the two approaches are mathematically equivalent (Wenseleers et al. 2010; Bourke 2011), as both models can involve the same nonrandom association of cooperative genotypes.

Although originally developed to explain altruism in insect societies, social evolution theory is now routinely applied to fields as diverse as microbiology, medicine, agriculture, and the social sciences (e.g., Denison et al. 2003; Foster 2005; Kümmerli et al. 2009). The diversity of applications reflects the central role of cooperation at every level of life, wherever reproducing entities cooperate in groups: genes within genomes, genomes within cells, cells within organisms, organisms within groups, and members of different species in interspecific mutualisms. At all these levels, cooperation is potentially vulnerable to exploitative "cheating" that occurs when individuals benefit from defecting from cooperation. Examples of cheating include genes that transmit themselves at the expense of the rest of the genome, cancer cells that replicate at the expense of the individual, social group members that receive aid but do not help others, eusocial insect workers that lay their own eggs rather than only tending the eggs of the queen, or flowers attracting pollinators without providing a nectar reward. Social evolution can often be seen as a coevolutionary arms race between cooperative and cheating strategies.

Inclusive fitness theory explains why cooperation evolves, but does not specify the mechanisms that maintained or enforce it. The benefits of receiving help (B) and costs of giving help (C) in Hamilton's rule are determined by the social and ecological circumstances individuals typically encounter in their natural environment and are often frequency dependent since they may be influenced by the conditional behavior of social partners. Moreover, the relative importance of direct and indirect benefits and the particular mechanisms that prevent cheating are still open questions across a wide range of organisms, including bats. To estimate the fitness costs and benefits of social behaviors in bats, it is crucial to know about both kinship and social structure.

12.3 Kinship and Social Structure in Bats

The social and mating systems of the roughly 1,260 species of Chiroptera have greatly diversified over the last 50 million years. Bats are primarily tropical (~75 % of species) and follow the general mammalian social pattern: females aggregate around important resources—including food, roosts, or conspecifics—whereas males compete for access to females (Clutton-Brock 1989). Although most tropical bats are polygynous, overall bats display a diverse spectrum of mating systems, ranging from social monogamy to promiscuity. McCracken and Wilkinson (2000) categorized 66 species (~5 %) into mating systems based on seasonality, sex composition of groups, and whether mating occurs within or outside roosts. More recent reviews of social and mating systems include Wilkinson (2003), Zubaid et al. (2006), and Kerth (2008a).

Limited dispersal, including natal philopatry, can result in high relatedness among social group members and thus has important implications for social evolution. Kin selection via limited dispersal occurs when individuals cooperate indiscriminately with neighboring individuals that are genetically related on average (Hamilton 1964). However, evidence suggests that conditions of high-average relatedness within groups or colonies are rare or absent in most bats.

Natal philopatry can lead to competition among kin that reduces or completely negates the indirect fitness benefits of helping relatives (Hamilton and May 1977; West et al. 2002). In other words, the easiest way to benefit kin might be to disperse and avoid competing with them (Hamilton and May 1977; Kümmerli et al. 2009). Kin competition can be reduced in theory if subsets of relatives leave the natal colony as a group (i.e., budding dispersal, Gardner and West 2006; Kümmerli et al. 2009), and interestingly, this pattern has been found in at least one bat species (*Eptesicus fuscus*, Metheny et al. 2008a; Kerth 2008b).

Natal philopatry of both sexes can lead to inbreeding. In bats, the typical pattern is female philopatry and male dispersal resulting in social groups with matrilineal kin, where immigration and visitation by foreign males reduce average group relatedness and inbreeding (e.g., Wilkinson 1985; Kerth and Morf 2004). In species where dominant males typically remain resident longer than the age of female sexual maturity, females tend to disperse to avoid mating with their fathers (*Lophostoma silvicolum*, Dechmann et al. 2007; *Saccopteryx bilineata*, Nagy et al. 2007; *Rhynchonycteris naso*, Nagy et al. 2013; other mammals, Lukas and Clutton-Brock 2011). The same conditions probably explain the complete female dispersal found in *Phyllostomus hastatus* (McCracken and Bradbury 1981) and female movements between different social groups in *Artibeus jamaicensis* (Morrison and Handley 1991).

Average colony relatedness in bats remains low even when pairwise relatedness values between certain individuals are high. For example, *Myotis bechsteinii* live in closed female groups with 75 % of females roosting with close relatives ($r = 0.25$ or greater), yet mean colony relatedness is still quite low ($r = 0.02$, Kerth et al. 2002). Similar patterns are found in other species (e.g., Wilkinson 1985; Burland et al. 2001; Metheny et al. 2008b; Boston et al. 2012; Patriquin et al. 2013). Even unusually high levels of pairwise relatedness do not translate to high-average colony relatedness. For example, in *Rhinolophus ferrumequinum*, some females mate with the same male across years and other females mate preferentially with the same male as their mother. As a consequence, pairwise relatedness between resulting progeny is elevated two to five times above normal (Rossiter et al. 2005). Yet, mean colony relatedness remains low ($r = 0.03$, Rossiter et al. 2006).

In social species where pairwise relatedness is on average low within groups but highly variable among pairs, as is the case in most bats, individuals are expected to actively discriminate kin rather than rely on indiscriminate altruism and limited dispersal (Cornwallis et al. 2009). Kin discrimination based on phenotypic matching of olfactory or vocal cues would benefit individuals whenever kin and non-kin intermingle. For example, in the group-living *Desmodus rotundus* where individuals are surrounded by non-kin (average, $r = 0.02–0.11$), females groom and regurgitate food with close relatives more than expected by chance (Wilkinson 1984, 1986). Similarly, average relatedness with roostmates is low in *Myotis septentrionalis*, but related pairs were more likely to associate and switch roosts together than unrelated pairs (Patriquin et al. 2013). Unlike cooperatively breeding birds and mammals, bats rarely, if ever, live in extended family groups; therefore, helping behaviors in bats that are directed indiscriminately within groups are likely mutually beneficial rather than altruistic.

Patterns of mating, dispersal, and survival determine kinship structure, but except for maternal care, kinship is not always a strong determinant of cooperation in bats. Non-kin cooperation is found in several species (e.g., *D. rotundus*, Wilkinson 1984; *M. bechsteinii*, Kerth et al. 2011; *P. hastatus*, Wilkinson and Boughman 1998; Bohn et al. 2009), and, on the other hand, highly related female *R. ferrumequinum* do not demonstrate obvious strong cooperative behaviors (Rossiter et al. 2002, 2006). Ecological factors, which determine costs and benefits of cooperation, are likely to shape the occurrence of cooperative behaviors in bats.

12.4 Cooperation and Conflict as a Coevolutionary Arms Race

For some cases of cooperation, there appears to be no cost to cooperating and hence no potential for cheating (by-product mutualisms). Consider, for instance, bats making drinking passes at watering holes from a single direction to avoid collisions (Adams and Simmons 2002). However, once a by-product benefit is established, investments in a mutualism may evolve that yield higher returns (Connor 1986). For example, in species where young cluster together in crèches (Fig. 12.1a), females

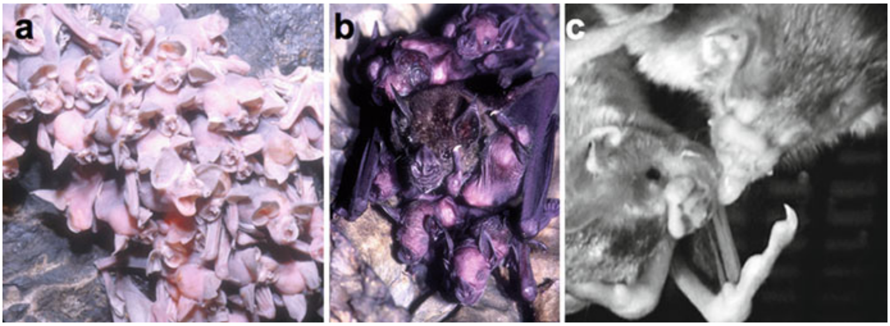

Fig. 12.1 Examples of cooperative behavior in bats. (**a**) Pups like these *Rhinolophus darlingi* may mutually benefit from clustering together for warmth. (**b**) A female *Phyllostomus hastatus* roosting with several non-offspring pups in Trinidad. (**c**) Regurgitated food sharing between unrelated adult *Desmodus rotundus*

might benefit from helping non-offspring pups survive if their own pups gain thermoregulatory benefits with larger crèche sizes (an example of a hypothesis called "group augmentation," Kokko et al. 2001). Besides thermoregulation, bats may associate for the direct benefits of avoiding predators (*Pteropus poliocephalus*, Klose et al. 2009) or learning about roosts and feeding sites (e.g., Wilkinson 1992b, 1995; Kerth and Reckardt 2003; Ratcliffe and Hofstede 2005; Safi and Kerth 2007; Dechmann et al. 2009, 2010). The significance of social information on the evolution of sociality is suggested by the finding that male sociality in temperate bats evolved more often in lineages that forage on ephemeral insects in open habitats—conditions where eavesdropping is most likely (Safi and Kerth 2007).

Opportunities to cheat can be subtle. For instance, huddling for thermal benefits (e.g., Pretzlaff et al. 2010) is often regarded as a by-product mutualism that does not require enforcement; yet, cheating is possible if an individual can maintain a lower body temperature than its neighbors, saving energy while still being kept warm by others ("the huddler's dilemma," Haig 2008). Similarly, social information about food can lead to frequency-dependent selection between "producer" strategies that invest in finding food and "scrounger" strategies that instead follow others (Barnard and Sibly 1981). For example, aerial-hawking bats might eavesdrop on hunting conspecifics and steal their prey (Chiu et al. 2010) or vampire bats might drink from wounds opened by other individuals (GGC and GSW, personal observations).

To prevent exploitative strategies from invading, cheating prevention is required. Kin discrimination can prevent cheating by ensuring that helpers aid primarily genetic relatives. Individuals can enforce non-kin cooperation through various forms of conditional reward or punishment. Such enforcement might involve individuals actively punishing cheaters, decreasing cooperative investments, or switching away from social partners that cheat (Trivers 1971; Noë and Hammerstein 1994; West et al. 2007a; Fruteau et al. 2011) and are described by many terms in the literature including reciprocal altruism, reciprocity, sanctions, rewards, partner control, or partner choice (West et al. 2007b; Bshary and Bronstein 2011).

Such mechanisms of enforcement are necessary to explain the persistence of cooperative behaviors that pose energetic costs with potential negative fitness consequences (Table 12.2). Below, we describe evidence for such helping behaviors in bats. We illustrate how altruistic parental care is exploited by foreign pups, how conditions of intense competition between unrelated males can select for alliances between related males, how competition between groups for food might select for cooperative foraging within groups, how infanticide can lead to pup guarding, and how the need to enforce cooperation might lead to partner fidelity and long-term social bonds.

12.5 Cooperative Behaviors in Bats

12.5.1 Alloparental Care

There are many anecdotal observations of bats nursing non-offspring pups in the wild (*Tadarida brasiliensis*, *Nycticeius humeralis*, *Pipistrellus pipistrellus*, *Miniopterus schreibersi*, *P. hastatus*) or in captivity (*Eidolon helvum*, *Rousettus*

Table 12.2 Cooperative behaviors in bats that pose costs to the helper

Behavior	Key references	What might prevent cheating?
Alloparental nursing	McCracken and Gustin (1991), Wilkinson (1992a)	1. Kin discrimination: help is targeted to genetic relatives 2. By-product mutualism: cheating is impossible if alloparental nursing leads to higher milk yield, increased milk fat, or reduced mastitis 3. Group augmentation: increases in colony size benefit mother or offspring by increasing social information
Food sharing	Wilkinson (1984), Carter and Wilkinson (2013)	1. Kin discrimination 2. Reciprocity: bats share with reciprocators and withhold sharing with non-reciprocating cheaters
Social grooming	Wilkinson (1986), Kerth et al. (2003), Kerth (2008a), Ancillotto et al. (2012), Carter and Wilkinson (2013)	1. Kin discrimination 2. Reciprocity 3. Exchange: bats reciprocate social grooming for other services within a long-term social bond
Cooperative mate guarding	Ortega et al. (2008), Nagy et al. (2012)	1. Kin discrimination 2. Helpers more likely to inherit dominant status
Pup guarding	Bohn et al. (2009)	1. Group augmentation 2. Exchange

aegyptiacus, *Macrotus californicus*, *P. pipistrellus*, *T. brasiliensis*, *D. rotundus*, *Diaemus youngi*), as well as some observations of conspecifics helping with birth (*Pteropus rodricensis*) or adopting pups in captivity (*D. rotundus*, *Diphylla ecaudata*, *Cynopterus brachyotis*, Kunz et al. 1994, LeBlanc 2001, and refs therein, Delpietro and Russo 2002). However, in most cases, it is unknown if such helping behaviors are evolved adaptations or simply by-products of maternal care.

Prior to inclusive fitness theory, such behaviors were thought to occur for the good of the colony or species. For example, nursing female *T. brasiliensis* were thought to “act as one large dairy herd delivering milk passively…to keep the milk supply distributed among the caves in proportion to demands for it” (Davis et al. 1962). Wild female *T. brasiliensis* nurse pups that are not their own offspring in at least 17 % of observed cases (McCracken 1984), but this behavior is best understood as successful milk parasitism by unrelated pups. Given the chance, *T. brasiliensis* pups will attempt to nurse from non-mothers that respond by hitting, scratching, and biting them (McCracken and Gustin 1991).

Milk parasitism likely occurs in other species as well. *S. bilineata* pups respond to non-maternal female calls (Knörnschild and von Helversen 2008), and *N. humeralis* pups attempt to nurse from non-maternal females with increasing frequency starting at 8 days of age (Wilkinson 1992a). During two summers of observation of *N. humeralis*, females almost always allowed nursing by offspring, but rejected at least 23 % of attempts by non-offspring pups by covering their nipple, moving away, or biting at persistent pups (Wilkinson 1992a).

Yet, *N. humeralis* mothers also nursed offspring that were not their own in at least 20 % of nursing bouts, and two hypotheses can explain how alloparental nursing might be adaptive for nursing females. First, alloparental nursing might be explained as "milk dumping" to decrease weight and improve foraging ability. As in other mammals, incomplete suckling might decrease milk yield, decrease percentage of fat in milk, and increase rate of mastitis. Also, the peak of alloparental nursing coincided with the annual peak in prey density, when mothers would be predicted to have excess milk (Wilkinson 1992a). This by-product mutualism hypothesis predicts that alloparental nursing would correlate with higher post-foraging mass gain.

A second group augmentation hypothesis is that alloparental nursing increases colony size, and subsequently, social information available to mother and offspring (Wilkinson 1992a, b). *N. humeralis* preferentially nursed non-offspring pups that were female and hence philopatric, even those mothers with male offspring. This hypothesis predicts that groups with more female pups should yield greater pup survival.

12.5.2 Cooperative Male Defense of Females

In bats, there are no known cases of cooperative breeding, where subordinates help a dominant female breed. However, there is evidence of male bats forming cooperative alliances to monopolize females, similar to the male alliances found in dolphins (Connor et al. 1992) and chimpanzees (Watts 1998). In polygynous species, dominant males often tolerate the presence of other less dominant males, especially relatives, and these subordinate males often appear to help ward off foreign males (Ortega et al. 2003). In *A. jamaicensis*, dominant males lose paternity to both subordinates and visiting foreign males, but dominants are able to defend larger female groups with the help of subordinates, and also benefit indirectly if subordinates that sire offspring are related. In return, subordinate males gain greater access to females, increased chances to acquire dominant status, and indirect benefits if they are related to dominants (Ortega et al. 2003, 2008). Such alliances between dominants and subordinates can last more than 2 years (Ortega et al. 2008). In *S. bilineata*, dominant males benefit from subordinate males that queue for dominant status (Voigt and Streich 2003; Nagy et al. 2012). In this species, dominant male tenure duration is not correlated with number of females or forearm size (a proxy of body size), but tenure duration does increase with the number of subordinate males on their territory (Nagy et al. 2012).

12.5.3 Cooperative Female Defense of Food and Pups

Whereas access to females is the key limit on male fitness, access to food and roosts is the equivalent limitation on female fitness. Not surprisingly then, there is also evidence that females cooperatively monopolize and defend food resources from other females. Female greater spear-nosed bats, *P. hastatus*, live and cooperate with unrelated groupmates. Since both sexes disperse from their natal group, the 10–22 adults in a group are unrelated (McCracken and Bradbury 1981). On the island of Trinidad, between 4 and 40 groups coinhabit various caves, each group located in a ceiling alcove guarded by a resident male. Female groupmates give birth synchronously (Porter and Wilkinson 2001) and cooperatively forage and defend food patches such as large fruiting trees (Wilkinson and Boughman 1998). Group members coordinate their movements using socially acquired, group-specific “screech calls” that convey group membership but not individual identity (Boughman 1998; Boughman and Wilkinson 1998). These calls also appear to facilitate cooperative mobbing of predators (Knörnschild and Tschapka 2012). In contrast, pups produce individually distinct “isolation calls” (Bohn et al. 2007).

Isolation calls allow mothers to find, recognize, and retrieve their pups that have fallen to the ground (Bohn et al. 2007, 2009). Falls are surprisingly common; Bohn et al. (2009) observed 85 pups (~4 % of all non-volant pups) fall to the cave floor during 50 h of observation. And these fallen pups will die quickly if not retrieved. In one cave, 17 females visited and inspected fallen pups as many as 342 times (Bohn et al. 2009). Mothers will retrieve their pups, but females from other groups will often bite pups, and sometimes carry away and kill them. Females from the same social group, on the other hand, while not retrieving the pup themselves, will often guard pups from attacking non-groupmate females (Bohn et al. 2009). Males ignore fallen pups.

Female *P. hastatus* appear to cooperate with unrelated groupmates and compete with non-groupmates. But given that all offspring disperse, what do adult females gain from the survival of pups in their social group? One possibility is group augmentation (Kokko et al. 2001): that females guard pups to ensure that warm bodies surround their own pup when they are out of the cave foraging. This hypothesis predicts that pup guarding increases or helps maintain pup numbers in the group towards an optimum (Kokko et al. 2001) and is consistent with the unusual synchrony of births within groups (Porter and Wilkinson 2001; Bohn et al. 2009). Yet, data on group size and pup fitness show either no effect (Boughman 2006) or a negative linear correlation between group size and pup condition (Bohn et al. 2009). However, an optimal group size might produce a unimodal, rather than linear, relationship between group size and pup survival. Furthermore, long-term field studies of cooperatively breeding birds and mammals show that the effects of cooperation on the fitness of young can be subtle and slow acting; hence lifetime fitness benefits cannot be discounted even when no short-term benefits are obvious (Hatchwell et al. 2004; Russell et al. 2007; Brouwer et al. 2012). More data are therefore needed to determine the relationship between lifetime reproductive success and group size in *P. hastatus* and other bats.

A second possibility is that females somehow enforce the direct fitness benefits of pup guarding. Bohn et al. (2009) found no evidence for direct reciprocity, that mothers guard pups to enforce guarding of their own pup. There was no correlation between guarding pups and having one’s own pup guarded, or between time guarding and time having one's own pup guarded (Bohn et al. 2009). It is possible that females "exchange" pup guarding for other cooperative services, such as some of the behaviors described above or the possibility of "babysitting" when adults stay behind with several pups (GSW personal observation, Fig. 12.1b), but documentation of such exchanges, or exclusion of less cooperative individuals, remains to be obtained.

12.5.4 Food Sharing

When adult female or young *D. rotundus* miss a nightly meal, female roostmates will typically regurgitate some of their own blood meal to feed them (Wilkinson 1984). Female donors regurgitated blood mostly for their own offspring (70 % of cases) but also for other hungry adult females (Fig. 12.1c). Adult food sharing correlates independently with both relatedness and roosting association (Wilkinson 1984). Reciprocal food sharing in *D. rotundus* is frequently cited as an example of reciprocity, but it also demonstrates kin discrimination because more than 95 % of food sharing occurred between close relatives ($r < 0.25$) despite the majority of possible donors being unrelated (Wilkinson 1984). On the other hand, simulations demonstrate that, if reciprocity exists, the resulting direct fitness benefits would greatly exceed the indirect fitness benefits (Wilkinson 1988). Hence, it is possible that *D. rotundus* base their helping decisions on past social experience rather than cues to relatedness. Others have proposed that food sharing between non-kin only occurs due to kin recognition errors (Hammerstein 2003), harassment of potential donors (Clutton-Brock 2009), or group augmentation (Davies et al. 2012).

To test these alternative hypotheses, we induced food sharing in *D. rotundus* under controlled captive conditions of varying relatedness and equal roosting association (Carter and Wilkinson 2013). We found that the majority of donations were initiated by donors and hence could not be explained by harassment. The dyadic patterns of food sharing were reciprocal, correlated with grooming, and stable over time. Reciprocal donations were eight times more important than pairwise relatedness estimates for predicting food donations under conditions of equal association (Carter and Wilkinson 2013).

A multilevel selection model (Foster 2004) assumes that donors do not discriminate between unfed bats when giving blood and suggests that food sharing could in theory evolve without kin discrimination or reciprocity. However, this model ignores several important factors: the possibility of cheating, the frequent roost switching and fluid fission–fusion dynamics that make *D. rotundus* social groups unstable in membership (Wilkinson 1985), and the evidence that food is not shared indiscriminately among group members (Wilkinson 1984; Carter and Wilkinson 2013).

Reciprocity can exist in *D. rotundus* assuming they remember past social experiences and invest accordingly, which is consistent with this species possessing the largest brain and neocortex relative to their body size among bats (Baron et al. 1996). To demonstrate reciprocity, however, one must show that the bats will punish cheaters by switching partners or decreasing investment to those partners who provide poor returns.

Regurgitated food sharing is found in all three vampire bat species (Wilkinson 1984; Elizalde-Arellano et al. 2007; Carter et al. 2008), but has not been found in other bats. Work on primates suggests that the evolution of adult food sharing is predicted by several factors: the difficulty of obtaining and processing the diet, parental feeding of young, and opportunities for partner choice in other contexts, such as mating or other mutualisms (Jaeggi and van Schaik 2011). Whereas some of these conditions do occur in some other bat species (e.g., parental food sharing from cheek pouches in *Noctilio albiventris*, Brown et al. 1983, or transfer of a captured prey from adult to young in some carnivorous bats, *Megaderma lyra*, Raghuram and Marimuthu 2007; *Vampyrum spectrum*, GSW personal observation), *D. rotundus* satisfy all the conditions.

12.5.5 Social Grooming

Mothers routinely groom their pups (e.g., McLean and Speakman 1997), but social grooming also occurs between adult bats in some species (*D. rotundus*, *D. youngi*, *D. ecaudata*, *A. jamaicensis*, *P. hastatus*, *M. bechsteinii*, and *Pipistrellus kuhlii*, Wilkinson 1986; Kerth 2008a; Ancillotto et al. 2012, GSW and GGC personal observation). In some cases, social grooming appears nepotistic and explained by kin selection. Kerth et al. (2003b) found that social grooming between adult female *M. bechsteinii* was not significantly reciprocal but was correlated with kinship, occurring mostly between adult mothers and daughters, sometimes between sisters, and rarely between non-kin. Kin-biased social grooming also occurs in female *D. rotundus* (Wilkinson 1986).

In primates, social grooming provides a social purpose beyond its hygienic function and has become co-opted over evolutionary time as an important signal of social investment. A similar process may have occurred in some bat species. Consistent with this hypothesis, patterns of social grooming do not correlate with patterns of self-grooming or amounts of parasites (Wilkinson 1986; Kerth et al. 2003), and reciprocal social grooming remains common among captive-born *D. rotundus* that completely lack observable external parasites (Carter and Wilkinson 2013). Although *D. rotundus* groom themselves less than *M. bechsteinii*, female *D. rotundus* groom their roostmates about twice as often as female *M. bechsteinii* (Wilkinson 1986; Kerth et al. 2003). When controlling for kinship, social grooming among female *D. rotundus* is predicted by roosting association and food sharing and is elevated immediately prior to food sharing (Wilkinson 1986). When mother-offspring pairs are excluded, the effect of roosting association and food sharing on mutual grooming exceeds that of relatedness (Wilkinson 1986; Carter and Wilkinson 2013).

The correlation between social grooming and food sharing in *D. rotundus* highlights the possibility that cooperative behaviors can lead to cooperative social bonds (Carter and Wilkinson 2013). For example, one scenario for the evolution of cooperation in vampire bats is that regurgitated food sharing evolved initially as part of maternal care and was then co-opted as a form of altruism towards other close relatives. Natural selection would reward individuals that preferentially fed those relatives that were not only more related but also more likely to reciprocate. Smaller investments, such as social grooming between adults, could then act as costly signals for larger social investments (e.g., Roberts and Sherratt 1998; Fruteau et al. 2011) such as food sharing. Since partner fidelity makes cooperative investments less risky, the continuation of investments should promote long-term social bonds and vice versa.

12.6 Social Bonds and Complex Cooperation

The ages of females in social groups suggest that similarly aged cohorts form roosting associations in many bats (e.g., McCracken and Bradbury 1981; Wilkinson 1992a; Brooke 1997; Porter and Wilkinson 2001), but little is known about the development and formation of social relationships among individuals. When *P. kuhlii* pups were raised together for 6 weeks in separate groups and then allowed to freely interact in a flight chamber, young raised in the same groups showed significantly higher rates of clustering, social grooming, and roosting near each other (Ancillotto et al. 2012).

Once associations are formed, they might be maintained by eavesdropping on echolocation calls (e.g., Jones and Siemers 2011; Schuchmann and Siemers 2010; Voigt-Heucke et al. 2010), but this is limited by call amplitude (Hoffmann et al. 2007; Ruczyński et al. 2009). Social calls are lower in frequency, more variable, and travel farther distances. Contact calls allow isolated individuals to find and identify roostmates at a distance (*D. youngi*, Carter et al. 2008, 2009; *D. rotundus*, *D. ecaudata*, Carter et al. 2012; *Thyroptera tricolor*, Chaverri et al. 2010; Gillam and Chaverri 2011; *M. bechsteinii*, *M. nattereri*, Schoner et al. 2010; *Antrozous pallidus*, Arnold and Wilkinson 2011; *Nyctalus noctula*, Furmankiewicz et al. 2011) and may also carry information about genetic relatedness (*A. pallidus*, Arnold 2011) or group affiliation (*P. hastatus*, Boughman and Wilkinson 1998). At closer range, recognition, and perhaps bonding, is likely mediated through social grooming or mutual scent marking (*D. rotundus*, Wilkinson 1986; *N. leporinus*, Brooke 1997; *M. bechsteinii*, Safi and Kerth 2003; *D. youngi*, GGC personal observation).

Females are known to form long-term associations in both tropical and temperate bat species. For example, unrelated pairs of female *P. hastatus* have been recorded roosting together 16 years later (Wilkinson and Boughman 1998), and pairs of female *D. rotundus* have been found together after 12 years (Wilkinson 1985). *M. bechsteinii* is a temperate species that maintains social bonds that last over 5 years and span categories of age, size, reproductive status, and kinship (Kerth et al. 2011). *M. septentrionalis* form significant associations for at least 3 years (Patriquin et al. 2010). In both species, relationships persist despite frequent roost switching and annual disintegration of group structure during winter. Given that bats are unusually long-lived for their size (Wilkinson and South 2002), we suspect that long-term social bonds occur in many other bat species.

There are several good reasons to maintain long-term rather than short-term mutually beneficial social relationships. Partner fidelity reduces the cost of searching for and learning about partners, allows one to invest gradually in cooperation to limit losses from cheating, and bundles the benefits from multiple forms of cooperation into a single relationship. For example, affiliated female *D. rotundus* might in effect exchange and monitor multiple forms of social investment including social thermoregulation, social grooming, information transfer, and food sharing. According to this hypothesis, social bonds permit fitness benefits to be mutually exchanged and enforced. This hypothesis predicts that change of a social bond by increase or decrease of one cooperative behavior will lead to the same change in other cooperative behaviors by the social partner. Furthermore, a decrease in one's capacity to perform a helping behavior (e.g., food sharing) could lead to an increase in other cooperative behaviors (e.g., grooming) to compensate and maintain the social relationship.

12.7 Future Avenues of Research

Many important questions remain regarding social evolution and social behavior in bats. Can we predict the patterns of social cooperation across different species based on ecological factors and life history traits? Is kinship less important for shaping cooperation among bats compared to other social taxa, and if so, why? What mechanisms prevent cheating in helping behaviors such as food sharing and pup guarding? To what extent do bats form long-term social bonds or follow particular conspecifics when foraging, dispersing, or migrating? Have social bat species converged on behaviors found in other highly cognitive social mammals? How does social behavior correlate with brain morphology? Do social networks among bats predict social transmission of pathogens and parasites?

The social lives of bats often play out in dark, elusive places, and the extent of direct observations of bat social behavior therefore pales in comparison to those of group-living primates, birds, and diurnal mammals. For bats, occasional observations of roosting aggregations may not be sufficient to reveal the full extent of social relationships. For example, fission–fusion dynamics lead to highly associated bats being in different roosts (e.g., Wilkinson 1985; Patriquin et al. 2010; Kerth et al. 2011), and large cave colonies might conceal smaller cryptic social networks or groups of highly associated individuals. Further advances in the tracking of individuals through PIT and GPS tags will surely lead to great advances in quantifying social network structure in bats. However, direct observation of the social behavior of marked individuals, albeit difficult, can lead to significant insight into cooperation and conflict in bats.

Acknowledgments GGC thanks the support of a Ford Foundation Predoctoral Fellowship. GSW thanks the National Science Foundation for supporting studies on bat social behavior conducted by himself and his past students, JW Boughman and KM Bohn.

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Chapter 13
Decision-Making and Socioemotional Vocal Behavior in Bats

Jagmeet S. Kanwal, Zhenzhen Zhang, and Jiang Feng

Abstract Emergent technologies and ecological concerns have expedited studies of the secretive life of bats in ways that were not possible in the recent past. Here we review some of the results of studies on decision-making behavior in bats with respect to foraging, roost selection, and mate choice and show how the economics of decision-making influences some of these choices. Individual bats make choices based upon immediate sensory feedback from their microenvironment as well as social considerations. Many species of bats emit a rich variety of social calls to communicate with conspecifics within large coherent societies that can undergo fission and fusion on a daily as well as long-term basis. These calls are typically associated with the expression of emotional as well as motivational behavior elicited by the activation of specific brain circuits by social calls and other stimuli. Therefore, we

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_13, © Springer Science+Business Media New York 2013

include a brief description of the putative neural circuits underlying these behaviors. Within bat colonies, newborn pups babble until they learn to mimic the vocal signature of their mother's call and produce complex phrases with a well-defined syntax. Vocal syntax is also observed in singing, courting, and territorial signaling accompanied by postural displays in some species of bats. Mustached bats also use "prosodic" variations within a single call type in association with its usage in different behaviors. Together, the findings reported here challenge the notion that bats and other "primitive" mammalian species express behaviors that are largely innate and reflexive in nature rather than being conscious, insightful, and highly plastic.

13.1 Introduction

As the only truly flying mammals, bats hold a special fascination for mankind. Together with their amazing ability to fly quietly in the dark, many species of bats also possess the exotic ability of echolocation. They emit short, high-frequency sound pulses and make decisions about head turns as well as their flight path (Valentine et al. 2002) based upon the information contained within the returning echoes. In this way, they are able to "visualize" the shape and texture of large objects, such as walls and openings of caves as well as of specific targets such as fish, frogs, and insects that they pursue while foraging. The ability of echolocation is particularly well developed in insectivorous bats. Fruit-eating bats generally are not able to echolocate but have enhanced vision allowing them to discern fruits from vegetation under low-light conditions (Suthers and Wallis 1970). All species also likely have well-developed, but rarely studied, olfactory and tactile sensitivity used in both foraging and social interactions. Here, we focus on bats' ability to gather information from their highly sensitive sensory systems for action-selection or decision making during various behaviors, particularly roost selection and socioemotional behaviors. Some of the topics we touch upon here are dealt with in more detail elsewhere in the book. In the latter half of this chapter, we provide details of the types of sounds used during social interactions and of the motivational-structural rules governing behavior modification in response to social calls that make actions more predictable. Decision making may be informed by acoustic communication indicating potential beneficial or aversive consequences via responses of colonymates. For a better understanding of this ability, we dig deeper by defining the neuroscientific underpinnings of decision-making and socioemotional communication behavior, including a brief description of the brain regions that are involved in its expression and modification. Our research, together with that of others, provides new insights into the cognitive and emotive capacities of bats. We support the idea that, like humans, bats too have emotions given the rich variety of socioemotional behaviors they engage in and the relative uniformity in the organization of the neurobiological emotive substrate. The evolutionary diversification of bats as a major taxonomic group and their success in interacting with their environment is a direct result of the specialization of their sensory structures in conjunction with neural plasticity that allows their relatively tiny brains to rapidly processes sensory information and elicit adaptive behavior.

13.2 Decision Making

13.2.1 The Economics of Decision Making

The behavior of all animals, including humans, may be considered as a series of decisions that are based on genetically determined neural circuitry, past experience, and the immediate ecological and social context. Nature is profoundly unpredictable, and all animals have to find ways to counteract the negative consequences of this unpredictability. In many ways, e.g., via morphology and physiology, they are evolutionarily adapted to do so. Here, we review how immediate sensory feedback from their microenvironment as well as social considerations impacts the economics of decision making in bats. We discuss some of the behavioral adaptations accompanied by morphological, sensory, and neural features that allow information gathering and decision making at the level of the individual and the group. These decisions are of course constrained by the ecological niche and habitat in which the bats have to survive and adapt. For example, habitat use may vary seasonally as resource availability fluctuates over space and time (Wang et al. 2010).

13.2.2 Prey Selection via Echolocation

Microchiropteran bats have minimized the unpredictability of predator attacks by adapting to a nocturnal lifestyle and using sounds instead of light to “visualize” their surroundings. The ability to orient using sound is referred to as echolocation. As a brief overview, many species of bats emit sound pulses and listen carefully to the acoustic distortions and delays of respective frequencies in the returning echoes. This is an energetically economic way to obtain a lot of precise information about the surroundings in the absence of light. Each species of bats has different morphological and physiological adaptations to maximize information gathering during echolocation. These acoustic–behavioral adaptations enable each species to efficiently forage within its ecological niche.

During echolocation, bats produce a sound signal designed to interact with the environment. Because the same individual is the emitter and the receiver, echolocation is sometimes considered as a form of auto-communication. Here, information about an object is imparted to the signal when it interacts with the environment. For example, wing beats of an insect modulate both the amplitude and frequency of the echo that can be later decoded by the auditory system.

The echolocation pulses of insectivorous bats can range anywhere from 5 to 140 kHz. Acoustically, echolocation signals can be classified into four types—the

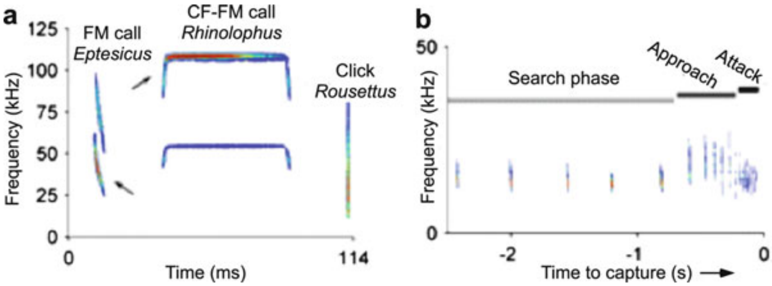

Fig. 13.1 Echolocation behavior in four genera of echolocating bats. (**a**) Spectrograms of echolocation calls of three bat species: big brown bat (*E. fuscus*), which produces FM echolocation calls; lesser horseshoe bat (*Rhinolophus hipposideros*), which produces CF–FM calls; and Egyptian fruit bat (*Rousettus aegyptiacus*), which uses clicks for echolocation. *Red color* indicates maximal intensity. *Arrows* point to the dominant harmonic: first harmonic in *Eptesicus* and second harmonic in *Rhinolophus*. The last two calls were recorded in Israel, courtesy of B. Fenton (University of Western Ontario, London, ON, Canada) and A. Tsoar (Hebrew University of Jerusalem, Jerusalem). (**b**) Spectrogram of a sequence of FM calls produced by a European free-tailed bat as it chased an insect. *Gray bars* denote the three echolocation phases of insect pursuit: search → approach → terminal phase (attack) (recorded in Israel by N. Ularnovsky and B. Fenton). Reproduced from Ulanovsky and Moss (2008)

laryngeal constant frequency (CF) pulses, frequency modulated (FM) pulses, CF–FM composites, and the lingual wideband clicks (Fig. 13.1a). Clicks produced by the Egyptian fruit bat (*Rousettus aegyptiacus*) are >50 kHz in bandwidth. One of the few species that emits a CF pulse is the so-called bumblebee bat also known as Kitti's hog-nosed bat (*Craseonycteris thonglongyai*)—it is arguably the smallest mammalian species (30–40 mm in length and weighing ~2 g) and lives in western Thailand in Sai Yok National Park in the Kanchanaburi Province. Its usual habitat is small holes or crevices formed by stalactites in caves, and it is most active at dusk (Hill and Smith 1981). Individuals of this species fly above the bamboo and teak trees to feed on insects, either in flight or on the trees.

In comparison to communication sounds (discussed later), echolocation sounds are relatively stereotypic (Kanwal et al. 1994). Nonetheless, bats can alter the physical properties of their echolocation pulses to maximize information gain. For example, mustached bats (*Pteronotus parnellii*) reduce the length of the CF portion of their CF–FM echolocation pulse as they switch from the search to the approach phase of foraging and eliminate it altogether during the final buzz produced just before insect capture (Novick and Vaisnys 1964). European free-tailed bats exhibit a similar pattern (Fig. 13.1b). These changes are precise and systematic to fit foraging-related ecological constraints as well as the design of echo-image processing neural mechanisms. In addition, five species (*Chaerephon johorensis*, *Mops mops*, *Cheiromeles torquatus*, *Hesperoptenus blanfordi* and *Pipistrellus stenopterus*) belonging to two families (Molossidae and Vespertilionidae) of aerial-hawking bats from Malaysia switch between high- and low-frequency pulses when flying at high altitudes (>10 m). The echolocation pulses of different species may also differ in duration and sound pressure level (Kingston et al. 2003).

Most echolocation pulses contain two to four harmonics, and some species, such as the mustached bat, use a hetero-harmonic, pulse–echo combination for extraction of information about the target (Esser et al. 1997). Since the echolocation behavior of bats has been extensively studied and described elsewhere (Griffin et al. 1960; Simmons 1973), we do not go into extensive details here. An echolocating bat gives away information about its position and flight path by the timing and structure of its echolocation pulses. In this and a few other instances, the respective echolocation pulses can be used for information transfer between individuals (Wilkinson 1992). Echolocation pulses may also play a possibly unintended communicative role when they are used for eavesdropping on individuals of the same species in order to locate a food source (Balcombe and Fenton 1988; Gillam 2007; Jones and Siemers 2011). For example, high intensity echolocation pulses may reveal the identity of the emitter and possibly the presence of a food source to an attentive conspecific (Gillam 2007).

In horseshoe bats, *Rhinolophus ferrumequinum*, individuals may use the echolocation pulses of conspecifics to obtain positional information about preferred roostmates and facilitate group cohesion in Spix's disc-winged bat, *Thyroptera tricolor* (Chaverri et al. 2012). Similarly, little brown bats, *Myotis lucifugus*, use the number of echolocation pulses emitted by conspecifics as an indicator of local food resources. In some larger species, bats use echolocation pulses produced by conspecifics to achieve optimal spacing of at least 50 m during foraging (Wilkinson and Boughman 1998).

In their studies, Koselj et al. (2011) designed a behavioral experiment that controlled availability (rate) and quality of prey items and tested whether bats monitored the supply of prey categories in the environment to make informed foraging decisions. Horseshoe bats adjusted their selectivity for the more profitable prey by ignoring small prey items in sessions with a high presentation rate of large prey, as predicted by foraging theory and never rejected profitable prey. The idea tested in this study was that in a foraging context, predators should use those sensory cues about a prey type that are connected to its profitability, i.e., energy gain per unit time of handling. They make foraging decisions that benefit fitness, for example, switching to a prey-selection strategy that maximizes net energy intake. The authors found that all bats achieved efficient prey selection. Their selection criteria matched the central prediction of foraging theory. First, the probability of rejecting less profitable prey decreased with an increase in the average time between successive presentations of profitable prey. Second, the animals seldom rejected large, profitable prey items. In this way, Koselj et al. were able to show that horseshoe bats make adaptive prey-selection decisions that are informed by echolocation. In other words, bats were choosing their prey economically, dependent on the supply in the environment. Furthermore, they reached their decisions from a distance, based solely on the echolocation information available to them. It is highly likely that many species of bats can associate prey-specific information with great profitability and use it to inform foraging decisions.

13.2.3 Roost Selection

Roost selection is an important decision-making process for individuals of any bat species. The decision criteria are more complex in gregarious or social species because they frequently involve group decisions. Animal societies that are social tend to break into subgroups when making roosting decisions. Such societies have been referred to as fission–fusion societies (Kerth et al. 2006). Bechstein's bats engage in group decision making when selecting a roost after a subset (Kerth and Konig 1999) of individuals has been disturbed (Kerth et al. 2006). In the absence of visual cues, roosting locations influence the nature of social interactions between conspecifics. These decisions can be critical for reproductive success and are based upon a majority rule or individual decisions, depending on the prior experience and preferences of the individual. Decision making may involve information transfer between experienced and naïve individuals (Kerth et al. 2006) besides being influenced by sensory information about the physical environment of the roost (Clement and Castleberry 2013a, b; KlugBrandon et al. 2012).

Related bats may also split into subgroups when establishing new colonies (Metheny et al. 2008). Most bat species breed communally (Kerth 2008b), and studies in Bechstein's and big brown bats (*Eptesicus fuscus*) suggest that closely related females may jointly decide to leave an existing colony to form a new one (Kerth and Petit 2005; Metheny et al. 2008). The study on big brown bats presented detailed observations of joint movements of individually marked females to a new area in combination with genetic data on their relatedness. This process of colony formation can take a relatively long time, in this case 4 years. While colony formation may be slow, bats switch among day roosts in tree cavities almost daily and frequently form subgroups that fuse again later (Kerth 2010). Fission–fusion behavior is widespread among bats. One thought is that this type of behavior allows females to adjust group sizes to changing environmental conditions, such as ambient temperature and parasite loads, while maintaining the social relationships within a colony (Kerth 2010). Several species of bats exhibit nonrandom roosting associations despite regularly changing subgroup compositions (Gillam et al. 2011; Kerth and Konig 1999). One explanation for why related females form new colonies comes from the need for social warming of newborn pups by non-lactating females (Kerth 2008a). When adult females are related, they may gain indirect benefit from contributing to social warming of pups that grow faster and are healthier. Another possibility is that females founding new colonies simply share alleles imparting a common exploratory drive as reported in other species (Kerth 2010). This could provide an evolutionary advantage to those species, including humans, who are both social and highly mobile. Spreading into new habitats could ensure reproductive success when local climactic conditions become unfavorable.

Many species of bats roost in harem-like social structures. Colonies of the greater sac-winged bat, *Saccopteryx bilineata*, consist of nonterritorial males and males that defend harem territories containing roosting females (Voigt et al. 2008).

In this species, a male hierarchy is present in and around the roost. In greater sac-winged bats, nonterritorial males gain access to a harem via a predetermined queue depending on genetic or prior social interactions (Voigt and Streich 2003). The Jamaican fruit-eating bat *Artibeus jamaicensis* exhibits a similar social structure (Ortega et al. 2003) as do mustached bats, *Pteronotus parnellii* (Clement and Kanwal 2012). One advantage of a stable roosting structure is that individuals within the colony may identify others based on olfactory and auditory cues. Female greater spear-nosed bats, *Phyllostomus hastatus*, are able to discriminate among calls from different caves using screech calls, but not individuals within the colony (Wilkinson and Boughman 1998). Evidence from other species indicates that bats can also discriminate between individuals. Some species even use echolocation signals to recognize and court potential mates (Knornschild et al. 2012; Yovel et al. 2009).

Roosting patterns in mustached bats are very stable over the short term (Clement and Kanwal 2012). Individuals maintain the same location, relative to the roost and to each other (Fig. 13.2a, b). In our study, males stayed in an average area of just 6.1 cm^2, commanding more exclusive space than females and sharing on average an area of 1.0 cm^2 with other males and 3.2 cm^2 with females. Females roamed across 19.5 cm^2 and typically shared an area of 5.0 cm^2 with males and 16.4 cm^2 with other females. Both sexes engaged in nonvocal social behaviors such as scent marking and grooming (Fig. 13.2c–e).

13.2.4 Choosing a Mate

Important decisions also have to be made during social interactions with conspecifics. These decisions can be critical for aggression avoidance, mate selection, and parental care. In bats, many of these interactions are "negotiated" largely using sounds, as in mother–infant interactions (discussed later in the chapter). Mate choice has been studied much more extensively in birds compared to mammals, especially bats. Some interesting observations, however, have been made in a few species of bats.

Males of greater sac-winged bat, the Mexican free-tailed bat, *Tadarida brasiliensis*, and the false vampire bat, *Megaderma lyra*, perform a hovering flight and produce songlike vocalizations to impress females during courting (Leippert 1994). Sac-winged bats live in a harem-polygynous social system in which males mate with females within their harem group only in 30 % of the successful copulations. For males, the harem territory is a site to advertise and attract females from outside the harem, enabling them to copulate with more females than non-harem males (Voigt et al. 2008). Given the small size of males, female choice and cooperation is very important in this mating system.

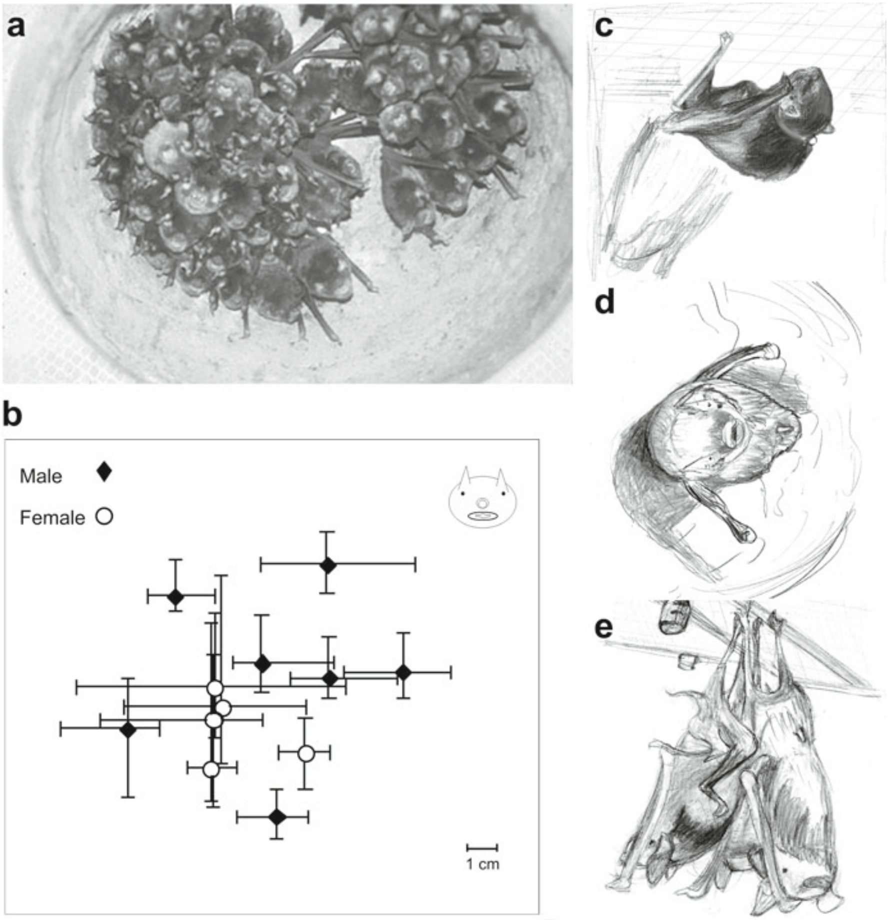

Fig. 13.2 Roosting and nonvocal social behaviors. *Left*: (**a**) a group of approximately 65 captive mustached bats roosting within an upside-down clay pot mounted in the ceiling of a flight room. (**b**) The scale of roosting positions by sex for mustached bats, over a 2-week period. A bat's head (*top right*) is drawn to scale to indicate that the farthest extent of roosting location is typically the size of the body width of an individual. *Symbols* (*solid diamonds* for males and *unfilled circles* for females) are located at the mean location of an individual within the roost and *the bars* indicate the range of individual-specific roosting locations. *Right*: Drawings of individuals traced from images acquired with an infra-red camera showing common postures [(**c**) Crouching, (**d**) Marking, (**e**) Grooming] that are not typically associated with any vocalizations; adapted from Clement et al. (2006). These likely play a role in maintaining the colony structure

13.2.5 Neural Substrate for Decision Making

Exactly how a bat's brain converts sounds into images for navigation, insect capture and decision making in the context of roosting, foraging, mating, and other socioemotional behaviors is still not completely clear. Here socioemotional refers to the

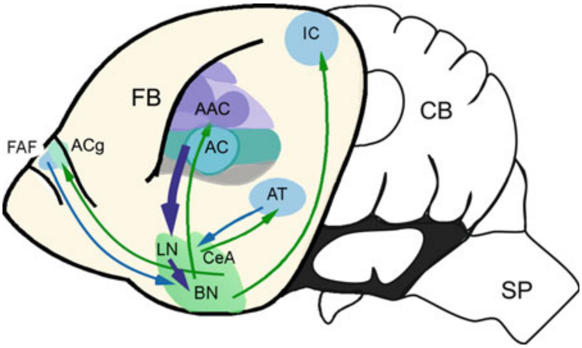

Fig. 13.3 Socioemotional auditory communication and decision-making circuit on a sagittal schematic of the mustached bat brain. Reciprocal connections between the frontal cortex and the amygdala are of key importance in decision making. The basolateral amygdala projects to the auditory association cortex (AAC) and receives input from the auditory cortex (AC). The basolateral amygdala also receives projections from the dorsomedial prefrontal cortex (McDonald et al. 1996) where a frontal auditory field (FAF) is located (Kanwal et al. 2000). The central amygdala (CeA) projects to the auditory thalamus (AT) as well as back to the anterior cingulate (ACg) (Ghashghaei and Barbas 2002), and the basal nucleus (BN) projects to the inferior colliculus (IC) in the midbrain (Marsh et al. 2002). Sensory structures are shown in *blue*, and premotor/motivational structures are shown in *green*. *CB* cerebellum, *FB* forebrain, *SP* spinal cord

expression of emotions, such as fear and aggression, within a social context. We do know that specialization of brain regions allows many areas in the auditory cortex of mustached bats to process both echolocation (target identification and tracking decisions) and communication sounds (social decisions).

Tremendous progress has been made on unraveling echolocation behavior, but social and emotional processes are just beginning to be tackled in a rigorous manner in any species. To date, these studies are limited to <1 % of the echolocating species. The brain areas and mechanisms that process communication sounds and coordinate various social interactions in the mustached bat are just beginning to be discovered (Kanwal 2006; Kanwal et al. 2004; Medvedev and Kanwal 2004). Figure 13.3 illustrates some of the key players in the brain-based understanding of decision making that is influenced by both cognitive (cortical) and emotive (limbic) processing. Reciprocal projections and interactions between the frontal cortex and amygdala are particularly relevant in this context (LeDoux et al. 1990, 1991). These findings, together with work on other species, have the potential to help us understand our own ability to communicate using emotionally relevant sounds, such as speech and music.

Cortical neurons of at least some bat species, such as mustached bats, are sharply tuned and highly specialized in that they respond well to specific parameters in the second harmonic of the echo. Interestingly, the same neurons are not so sharply tuned to the frequency of the fundamental in the echolocation pulse (Suga 1990), which is known to vary with sex and across individuals (Jen and Kamada 1982). These neuronal properties may allow mustached bats to monitor echolocation pulses of others as well as track variations in their own, e.g., during Doppler-shift compensation (Riquimaroux et al. 1992).

13.3 Social and Vocal Behavior

Most species of bats are highly social—some of them mate for life, and others live in a harem of thousands of individuals surrounding the alpha male hanging from its favorite spot deep inside a cave. Vampire bats, *Desmodus rotundus*, are the most social of them all and, like some birds, regurgitate their bloody meal to feed their young. In fact, if an individual does not share its meal with others, conspecifics will tend to shun it and avoid any social interactions. All of the small insectivorous bats rely on complex sounds for most of what they do—to echolocate, to find food, impress another bat, court, show affection, warn others of danger, and fight with each over their preferred mates.

The social arena provides individuals with a particularly rich environment from which to extract information from the behavior of others (Brown and Laland 2003). In animals, such as bats that live in the dark, vocal signals provide an excellent means to obtain this information quickly and unobtrusively from a distance. The act of extracting information in this manner is termed as social eavesdropping when more than two animals are involved (McGregor 1993), in contrast to the public advertisement and receiver-directed vocalizations. Thus, vocal signals provide an important means of communicating social, cognitive, and emotive information.

In contrast to the relatively stereotyped calls used for echolocation, social communication among conspecifics by its very nature involves a structurally wide variety of sounds (Bohn et al. 2008). The same call type may even be used in different contexts, as in some nonhuman primates, or modified in different contexts to carry a different meaning (Snowdon 1982). Moreover, call parameters may fluctuate depending on the identity (physical characteristics) and motivational state of the emitter (Miller et al. 2005; Yovel et al. 2009). Whether these subtle variations are used to convey information to other bats of the same and other species remains to be determined, but there is some evidence of the former possibility in at least one species (Clement and Kanwal 2004).

Most insectivorous bats use sounds to echolocate as well as communicate, whereas the fruit-eating megachiropteran species have good vision, feed during the day, and make sounds mainly to communicate with each other. Pallid bats (*Antrozous pallidus*), living in deserts in the western United States, use both passive hearing and echolocation cries to seek their food at night (Brown 1976; Razak et al. 1999). Individuals of another species, the mustached bat with ratlike whiskers, abound in the hot and steamy climate of the Caribbean, Central and South America (Herd 1983). This is one of the most abundant species and yet difficult to observe because of its timid nature and residence within complete darkness in caves.

13.3.1 Acoustic Structure of Social Calls

Most bat species rely heavily on communication sounds for their everyday social interactions (Kanwal et al. 1994; Porter 1979). The structure of communication sounds has been described in reasonable detail in several species, including

Megaderma lyra, *Pteronotus parnellii*, *Carollia perspicillata*, and *Phyllostomus hastatus* as well as in the greater white-lined bat, *Saccopteryx bilineata*. Individuals in at least some species use modified echolocation sounds together with other calls in a communicative context, as in mother–infant interactions (Schmidt-French et al. 2006). They may also indulge in eavesdropping to either gather information on or locate a food source during foraging (Fenton 2003) or be alerted to social activities, such as mating, carried out by other individuals (Jones and Siemers 2011). Like *Desmodus*, a genus of vampire bats, mustached bats are highly social, living in colonies of thousands to possibly millions of individuals.

Mustached bats emit at least 33 different types of communication sounds or "calls." The general pattern of call structure and function in this species is largely consistent with empirical findings from other bat species (Fig. 13.4). Mustached bats have a vocabulary of at least 19 so called simple syllabic calls that have been recorded in captivity as well as 14 composites for social communication (Kanwal et al. 1994). There may be additional sounds used in natural settings that have not been observed in captive bats. This means the variety of mustached bat calls approach the number of phonemes produced by humans. Unlike humans, however, the number of combinations of these sounds in composites is severely limited, to <20 in bats. Nevertheless, it has been shown that bats can combine their simple syllables into more complex sounds, not unlike the phonetic syntax we see in all human languages. This statistically defined classification of each syllable is based on acoustic characteristics of the call types. Each syllable type is not stereotypic; rather, it may vary in different acoustic parameters within the multidimensional boundaries of each category. This variation may depend on and therefore signal the sex, identity, and/or the emotional state of the emitter.

The precise acoustic structure of vocal signals, like all other animal signals, may be influenced by multiple factors during the production and transmission of sound by the sender. Similarly, decision making by the receiver depends on the detection, identification, and mood (physiological state) of the receiver. These factors have not been clearly delineated as most studies on signaling systems focus exclusively on the sender and often ignore the receiver side and the ecological conditions under which signals evolve during different stages of communication.

13.3.2 Babbling and Prosody

Development of vocal signals has been studied both in captivity (Esser and Schmidt 1989; Esser 1994) and in the wild (Knörnschild et al. 2006). In the Asian particolored bat, *Vespertilio sinensis*, vocalizations emitted after birth usually are either of long pulse duration (isolation calls) or of short pulse duration resembling adult echolocation calls (Jin et al. 2012). As the pups grow, the short calls become more like the brief FM echolocation calls emitted by the mother. From these and other studies, the authors concluded that precursors of echolocation calls of young bats might serve a communication function during the first week prior to its modification and

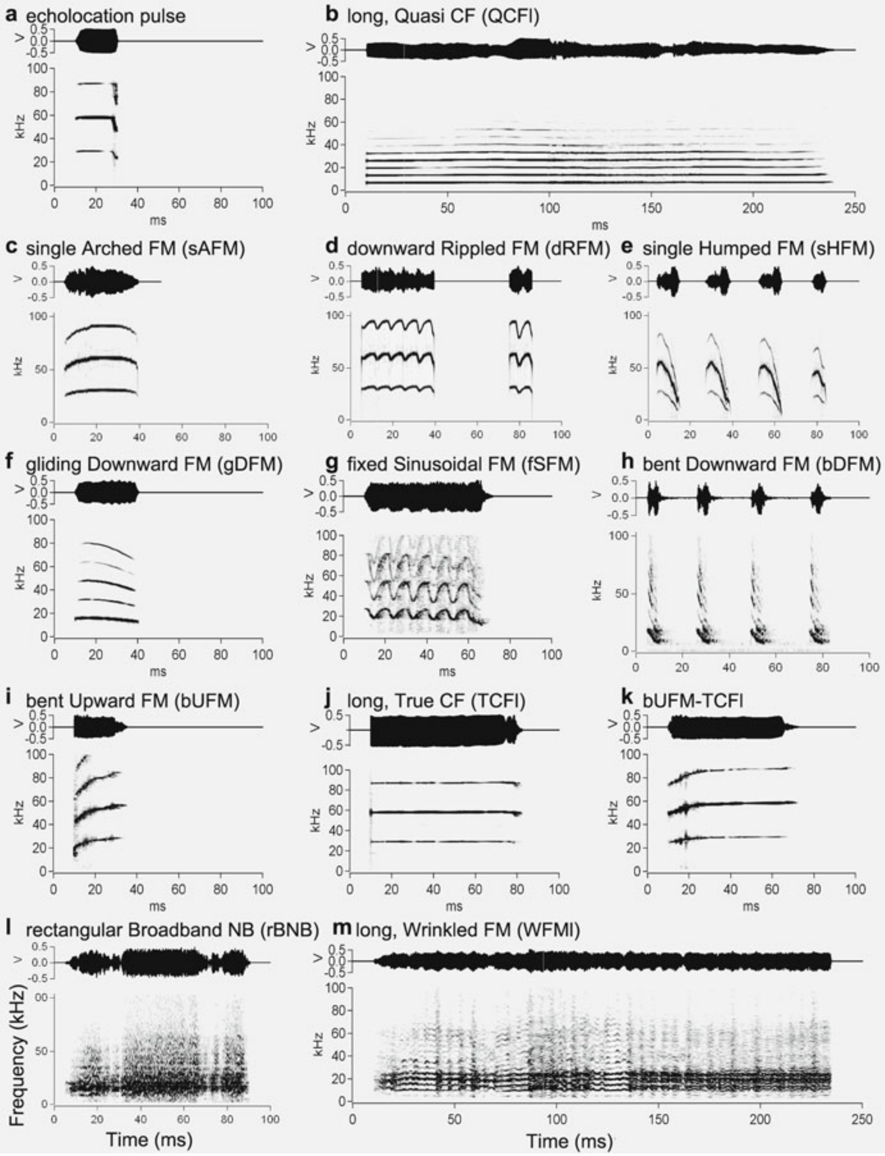

Fig. 13.4 Simple syllabic call types and a composite emitted by mustached bats, *P. parnellii*. Amplitude envelopes (*above*) and spectrograms (*below*) of 12 different call types. Calls are broadly classified into CF, FM, and noise burst (NB) type of calls. The frequency-time patterns of FMs makes the calls especially diverse in their acoustic structure, and the calls are named and classified according to these patterns. Call shown in "k" is a classified as a composite

thereafter be used for orientation and navigation, which becomes increasingly important for the survival of young bats. Pups of mustached bats emit echolocation-like pulses a few days after birth as well as a few communication calls similar to the long quasi-CF call type (personal observation). Within a few weeks, their echolocation pulses change from a multi-harmonic signal dominated by the fundamental to the adult-like signal with an attenuated fundamental as the pups grow older (Vater et al. 2003). The pups' communication repertoire becomes gradually enriched by the addition of single arched FM, trapezoidal, quasi CF followed by sinusoidal FM and single syllables of checked, downward FM call types (Vater et al. 2003).

The occurrence of babbling (syllable jumbling) in some species attests to the humanlike development of audio-vocal communication in bats. The sac-winged bat feeds on tiny insects and remains solitary when not engaged in reproductive activities. Pups of this species were discovered recently to babble (Knörnschild et al. 2006). Four- to eight-week old pups of both sexes make long strings of barks, chatters, and screeches that resemble jumbled-up adult-like calls. These bats could be the first animals, besides some primates and birds, documented to babble as babies. As with infant babbling in humans, these pups produce renditions of all known adult call types during vocalization. These vocalizations appear to be independent of a distinct social context and provide evidence of training to communicate effectively. Eventually, the pups learn complex vocalizations through vocal imitation. This auditory experience is considered essential for normal vocal development, not unlike what has been extensively shown in songbirds and earlier in bats of the genus *Phyllostomus* (Boughman 1998; Esser and Schmidt 1989; Esser 1994). From a neural and functional perspective, babbling may be equivalent to play behavior; in bodily play, pups frequently jumble elements of prey capture (e.g., in dog pups, and leopard and bear cubs) with elements of reproductive and parental care type behaviors. Similarly, when babbling, pups may mix up vocal elements that belong to aggression with fear and isolation calls eliciting care. Thus, babbling and play may be common strategies that facilitate appropriate wiring of the nervous system or simply reflect developmental wiring and transient "miswiring" whose output needs to be played out to gain feedback for proper wiring, compartmentalization, and coordination of each behavior.

In humans, auditory communication consists of two distinct components—speech sounds and individual laryngeal structure and/or mood-driven inflections within speech segments, also termed as prosody. Whereas the representation, processing, and production of speech sounds have been extensively studied, less is known about the prosodic variations in speech. More importantly, there is no clear animal model in which the prosodic features of auditory communication are well recognized and studied. There is some evidence, however, for prosodic variation in the mustached bat (Clement and Kanwal 2004) and the false vampire bat (Bastian and Schmidt 2008). Several species are also known to sing during mating and territorial behaviors (Behr et al. 2009; Bohn et al. 2009; Behr and Helversen 2004; Davidson and Wilkinson 2004; McWilliam 1987; O'Donnell et al. 1999). Singing involves repetition of the same set of syllables while incorporating variation in the timing and acoustic structure of these syllables. Per this canonical definition of singing, several species of nonhuman animals, including whales and bats, produce songs appropriate to a behavioral context, such as during mate selection.

We have attempted to identify, describe, and measure fine variations within a call type (Fig. 13.5). The long, quasi-CF (QCFl) call exhibits a significant amount of

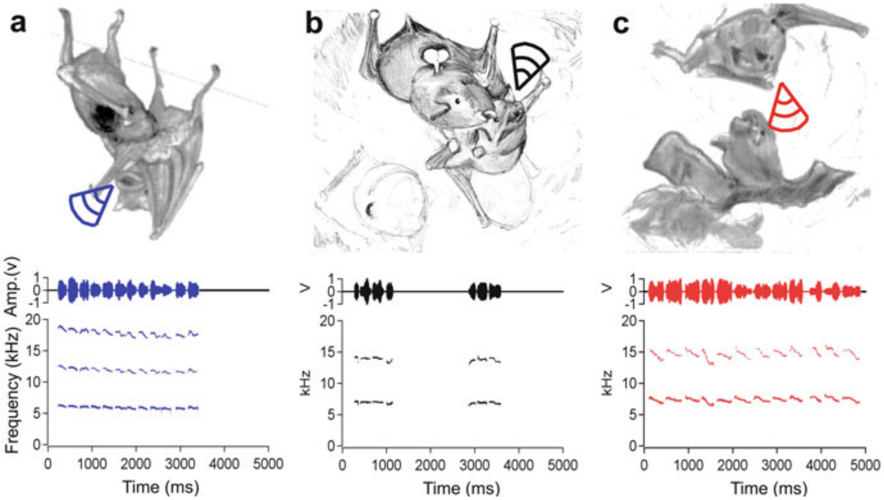

Fig. 13.5 Context-driven syllabic variation in the QCFl call type. Three behaviors and representative spectrograms of trains of QCFl syllables are illustrated in *P. parnellii*. (**a**) During inspection, a male sniffs a female's genital region, and she emits a QCFl train. (**b**) During a nose touch, two males appear to "kiss," emitting a QCFl train each time. (**c**) During an outcast event, an unwelcome male issues a stream of QCFl calls as resident males attack him (Clement and Kanwal, unpublished data)

inter- and intraindividual variation with social context and sometimes with the identity of the emitter (Clement and Kanwal 2004). One individual may emit a variety of syllables as a long sequence within QCFl calls and the same is true for the fixed sinusoidal FM (fSFM) call (Kanwal et al. 1994). Therefore, these variations cannot be ascribed entirely to individual vocal signatures. Our observations showed that each bat emits QCFl syllables that vary substantially in frequency, modulation, and length between different utterances (Clement and Kanwal 2004). The context can be predicted with 80 % accuracy, based solely on the acoustic parameters of the QCFl call. QCFl calls emitted by different individuals occupy a dissimilar but overlapping space within the multidimensional acoustic space. In short, prosody likely plays an important role in audio-vocal communication in *P. parnellii*. Prosodic variations, as expressed in speech, enrich communication by carrying secondary information about the context/emotion and may have evolved early in mammalian evolution. The neural basis for the representation and identification of this type of acoustic variation in call structure, however, is still unclear.

13.3.3 Territoriality and Aggression

When roosting in caves, many bat species constantly chatter with each other as they go about their social life, particularly during twilight hours, just before they take flight for foraging (Kanwal, personal observation; Wilkinson and Boughman 1998).

Some of the chatter associated with aggression allows bats to decide whether to fight or to withdraw. Fighting ensues in different situations. A bat may swing its forearm and whack another bat, knocking it off its feet or swing like a pendulum. For affiliative interactions, a dominant male bat may approach a hanging female and enfold her body in his wings to prevent other males from "stealing" females in the colony. The dominant male also lets out a harsh, broadband call that is usually sufficient to turn a sneaky male away. The dominant male then produces whistling sounds and makes genital-to-oral contact with the females to reaffirm their bond (Clement et al. 2006).

13.3.4 Whistling and Singing

The whistling sounds produced by several species consist of nearly pure tones with multiple harmonics and are melodious even to our ears suggesting the common evolutionary connection between avian and mammalian species, including humans. Surprisingly, this similarity is even stronger when we examine the various modes of communication that morphologically diverse species have adopted. Two types of sounds that mustached bats emit frequently are the affiliative whistling sounds and the aggressive, harsh broadband sounds. Close observations have shown that touching and "kissing" and sometimes mouth-to-genital contact frequently accompany friendly bat whistles between a male and a female. Bats also hug each other with their folded wings. Kissing usually consists of a brief contact between the mouths of two bats and may be accompanied by a quick lick of the significant other's lips as well (Clement et al. 2006). In contrast, the harsh, broadband sounds are associated with boxing, nipping, and aggressive biting.

Like songbirds and some species of whales and dolphins, a few species of bats sing as they fly or hover in midair and engage in courtship displays. Singing and babbling in some species of bats attest to their specialized audio-vocal ability. Singing is strongly seasonal in nature and probably establishes territory of the foraging bat. As reported in a few other species, false vampire bats, *Megaderma lyra*, engage in singing flight behavior (Leippert 1994). This behavior is displayed only by the dominant males and can occur at any time of the year. It consists of a stereotyped flight pattern that is continuously accompanied by vocalization. This behavior is clearly one aspect of courtship and is directed only at non-lactating females within the group. This behavior was also elicited by the introduction of new females to the colony. The song flight in *Megaderma*, an Old World microchiropteran species, consists of three stages that have been named the introductory, advancing, and final flight (Leippert 1994). Spectrally distinct segments or strophes accompany each of these stages. These bats also interact socially in various contexts and emit different types of calls. One of these behaviors is labeled "grumbling flight" and involves several bats hovering for a few seconds in a head-to-head formation emitting a series of short and steep downward FMs that frequently terminate in a shallow downward glide. The spectral structure of this sound sequence emitted by *Megaderma* is remarkably similar to the checked downward FM (cDFM) sounds emitted by mustached bats. Hovering behavior accompanied by calling is also exhibited during mating by males of the greater sac-winged, the Mexican free-tailed bats, and the short-tailed fruit bat (McCracken and Wilkinson 1999; Voigt et al. 2008). Reports on social interactions during flight are usually based on chance observations, except for regularly observed honk calls, which are emitted when flying bats are about to collide (Barclay et al. 1979; Fenton and Bell 1979; Suthers 1965).

In the sac-winged bat, singing is used to establish roosting territories. This species employs an unusually large vocal repertoire. Males emit simple syllabic tonal calls while interacting with females and other types of calls consisting of composites when actively defending their territories from other males (Behr and Helversen 2004; Behr et al. 2009). Their songs consist of short repeated tones that do not appear to have any obvious context other than advertising the quality of the singing male. These bats do not hang in large colonies. Rather, each bat has a favorite roost in a tree from where the males sing complex songs to attract the females. As with infant babbling in humans, the pups produce renditions of all known adult vocalization types during bouts of vocalizations.

13.3.5 Mother–Infant Interactions

Bats engage in a multitude of social interactions and exhibit parental care. The level of parental care observed in bats rivals that of large and intelligent species, such as elephants and dolphins. Studies with infrared thermal video analysis of Japanese horseshoe bats show that the mother bat flies out of the cave to feed and upon her return enfolds her baby in her wings to facilitate suckling (Matsumura, personal communication). To do this in complete darkness and find one's own young in a tight group of thousands of pups all huddled together and continuously shifting their position is quite a feat.

Young bats have been shown to be more responsive than adults to playback presentations of echolocation pulses. This could result from a generally increased level of curiosity and playfulness observed in the young ones of many mammalian species. Mother–infant interactions have been shown to be important for vocal learning, which can play an important role in the reproductive success of the offspring (Matsumura 1979). Pups of the lesser spear-nosed bat continuously learn from and match the acoustic parameters of their mother's signature calls (Esser and Schmidt 1989). In greater spear-nosed bats as well as in the greater sac-winged bats, mothers are able to discriminate isolation calls of their own pups from those of other pups (Knörnschild and von Helversen 2008; Bohn et al. 2007).

13.4 Emotion and Motivation

Three major factors determine the acoustic structure of sounds emitted by animals. First, the physical structure of the environment (e.g., dense vegetation) determines what type of sounds will propagate furthest. The level and frequency structure of the ambient noise also determines the level and fundamental frequency of the sounds that can be effectively transmitted from the emitter to the receiver. For example, a species of frog in northeast China emits ultrasonic calls to enable communication above the sounds of a flowing stream (Feng et al. 2006).

The second major factor determining the nature of the sounds is the physical structure of the sound-producing organs. Thus, the pitch and amplitude of the sounds depends on the size of the larynx and the tension in the vocal folds of the organism producing them. This tension and corresponding variations in the acoustic structure of the sound depend on the emotional state of the emitter. Thus, the third major factor has to do with the emotional state of the emitter. This determines the probability of which call type will be emitted and the particular variant of a call that is emitted. Interestingly, this emotive state has universal consequences on the nature of variation in the communication sounds across avian and mammalian species. This relationship between the motivational state and the acoustic structure of sound has been formulated as the motivation–structure hypothesis that was first proposed by Morton (1977, 1982). In the next section, we discuss this with particular reference to mustached bats.

13.4.1 Signal Design

Many species of bats produce a rich variety of sounds for social communication, not unlike some of the more vocal primate species, such as the marmoset, macaque, and owl monkey. Perception of signature acoustic properties of communication sounds is closely associated with the evolution of the neural circuits that govern social behavior. In evolutionary terms, changes in the environment do not impact social behavior as they do the various morphological traits, food preference, and feeding behavior because social behavior is primarily concerned with animal-to-animal interactions. During aggressive interactions, humans too produce noisy (harsh) sounds, as in a traditional war cry. Spectrographic analysis of sounds that shows increasing aggression is associated with increase in amplitude and noisiness in speech sounds in humans (Fig. 13.6). Production of such sounds requires an increase in the sound pressure level together with constriction of the vocal tract, and its presence in bats suggests that this mechanism may have been conserved through evolution, well before the emergence of mankind and even of primates.

According to Pfalzer and Kusch (2003), harsh broadband calls in bats are widely used during aggression, although buzzes and trills also fill this role. The authors also report that tonal calls in bats are often used between mothers and pups, whereas complex sounds are frequently used during mate attraction behavior. Both sexes of *Megaderma* use a low-frequency, multi-harmonic “grumble” as an aggressive call, and males use a mix of tonal CF and FM calls in a display for females (Leippert 1994). Male *sac-winged bats* use harsh, broadband calls to threaten other males and direct tonal calls towards females (Davidson and Wilkinson 2004). By our definition, an act or state of appeasement impacts positively on the affiliation between two

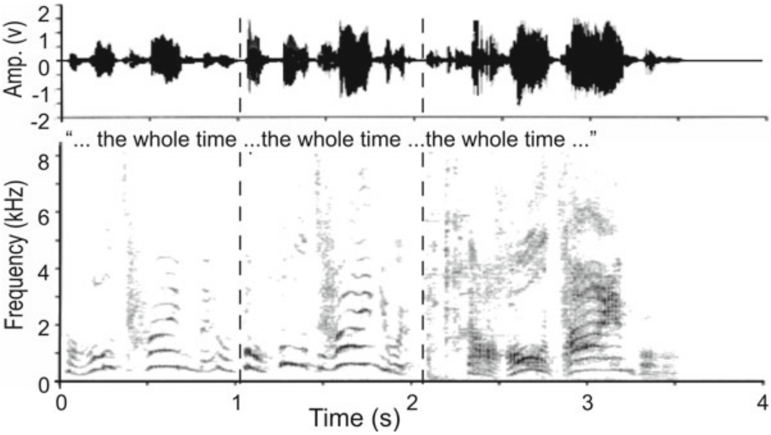

Fig. 13.6 Motivation–structure design of sounds in humans. *Top panel*: Amplitude envelop (*above*) and spectrogram (*below*) of a human voice saying the same words ("the whole time") with increasing levels of anger. Taken from the soundtrack of the movie "Mrs. Doubtfire"

individuals and may be triggered either spontaneously or because of an impending uncertainty. A state of aggression is a possible outcome of a state of defiance and warning. Our data together with the work of Fenton (1985) indicate that bat vocalizations generally follow Morton's (1977) predictions of signal design, such that aggressive sounds are low frequency and noisy, whereas fear-related sounds are high frequency and tonal (Fig. 13.7).

Similar to the sRFM call in mustached bats, *Noctilio leporinus*, *Myotis volans*, and *Myotis lucifugus* produce a "honk" by adding a downward frequency sweep to their echolocation pulse (Barclay et al. 1979; Fenton and Bell 1979; Suthers 1965). *Pteropus poliocephalus* and *Carollia perspicillata* both use a "screech" to avoid collisions (Nelson 1964; Porter 1979). Davidson and Wilkinson (2004) found that a similar call, the screech-inverted V, generally had no contextual association and described the call as a neutral notification "bark" for advertising territorial claims. A syllable with a classic inverted V-shaped structure in the call spectrogram, however, was not observed in mustached bats.

13.4.2 Cognition of Affect

For the perception of affect, it must be processed and represented in particular regions of the brain. The amygdala is a major brain structure that is selectively active during laughing and crying in humans (Sander and Scheich 2001; Sander et al. 2003) and where local field potential activity becomes enhanced during fear conditioning in bats (Ma et al. 2010). It is reciprocally connected with other brain

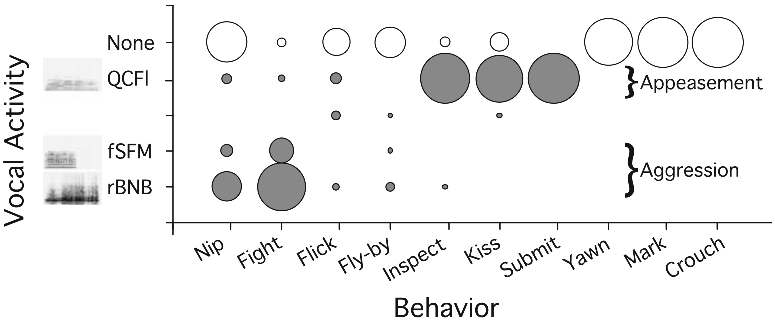

Fig. 13.7 Motivation–structure design of sounds in bats. A *bubble-plot* showing the association of different call types with appeasement and aggression (*filled dark gray circles*). The size of the bubble is proportional to the normalized (percentage of total events) frequency of occurrence of a call type. *Unfilled circles* are proportional to the number of events when a call did not accompany a particular behavior. Adapted from Clement and Kanwal (2012)

structures, such as the hypothalamus and the hippocampus, that are also considered important for processing emotions. Together they form the so-called limbic system. How exactly is affect represented within the limbic system remains unclear.

In humans, speech sounds carry both semantic as well as affective information in the form of prosody/cadence. Each of these two types of information is encoded in the acoustic structure of the same sound and is extracted simultaneously by the two halves of the brain (Zatorre et al. 1992). For many years, this ability was considered unique to humans. Differences in global vs. local processing of FM sounds have been reported in gerbils (Wetzel et al. 2008). In male mustached bats, the left hemisphere responds well to calls and a variety of FMs, whereas the primary auditory cortex (AC) in the right hemisphere is more sharply tuned for echolocation (Kanwal 2012; Washington and Kanwal 2012). The right side is biased to respond to CFs and low-rate (<0.6 kHz/ms) FMs (red-shaded sectors between oblique dashed lines in Fig. 13.8a) that are present within a few call types. The left AC in males responds to a wide variety of FM rates and bandwidths (blue- to purple-shaded sectors in Fig. 13.8a) (Washington and Kanwal 2012).

The amygdala is particularly well known to represent fear memories (LeDoux 2003). Associating an FM sound with a brief and mild pulse of electric current to the leg resulted in enhanced responses to the conditioned sound from neurons in the basolateral amygdala of mustached bats (Ma et al. 2010). After conditioning, enhancement of the response was specific to the parameters of the FM and resulted in increases in heart and respiration rates as well. This provided strong evidence that acoustic parameters within social calls can be associated with aversive consequences and "remembered" by neurons within the amygdala, a brain region that may also mediate immobilization stress in short-tailed fruit bats (Ammersdörfer et al. 2012). The putative brain circuit underlying learning, recall, and expression of auditory fear memories is shown in Fig. 13.8b.

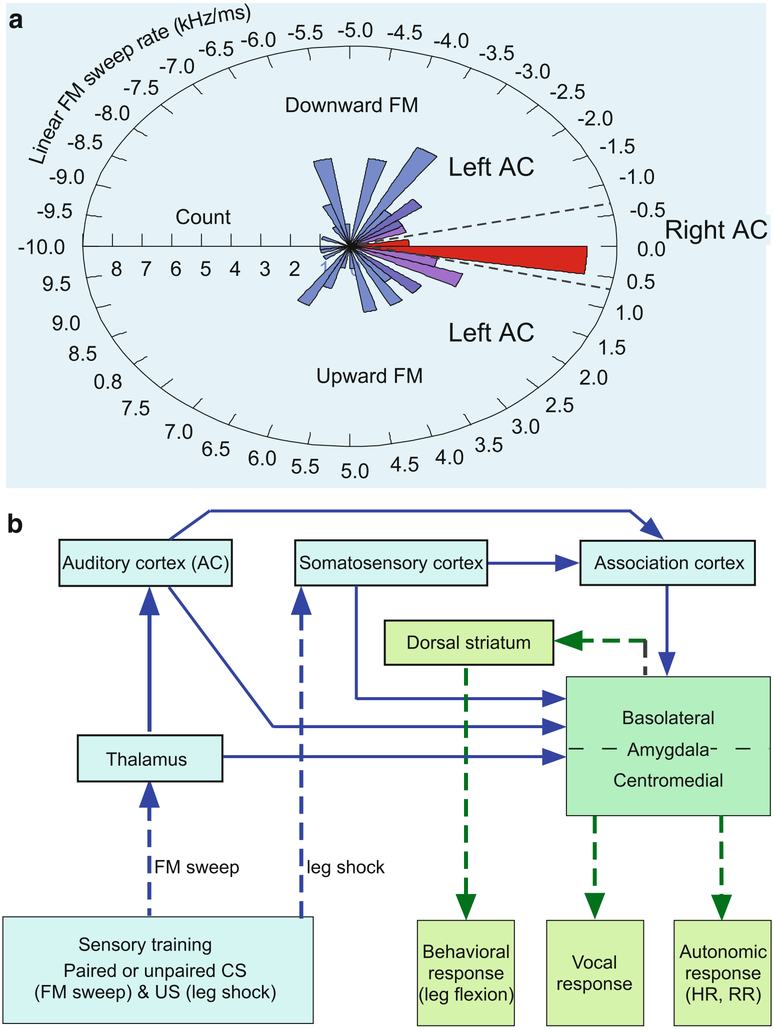

Fig. 13.8 A simplified scheme showing FM representation and decision-making circuits underlying fear-conditioned responses. (**a**) Polar density plot showing the distribution of different FM rates present in the 40–70 kHz frequency range in calls emitted by mustached bats (Zhang and Kanwal, unpublished data). (**b**). Associations between the conditioned stimulus (CS) and unconditioned stimulus (US) are created in the basolateral amygdala (BLA) from auditory and somatosensory inputs. Leg movement is mediated via projections of the BLA to the dorsal striatum (Kelley et al. 1982), whereas heart and respiration rates (HR and RR, respectively) and vocalizations are mediated by parallel pathways from the BLA to *m*otor centers within the brainstem via the centromedial amygdala (Metzner and Radtke-Schuller 1987; Fenzl and Schuller 2005). The BLA has been proposed as a locus for associative plasticity (LeDoux 2003). *Solid lines* indicate direct neuronal projections; *dashed lines* indicate indirect (multilevel) projections. Reciprocal connections between amygdala, AC, and thalamus are not shown. Sensory processing areas and inputs are indicated in *light blue*, motor centers and outputs in *green*, and sensorimotor interface (amygdala) in *bluish green*. Modified from Ma et al. (2010)

13.4.3 Computing Emotion

Studies are underway in a number of species to understand the neuronal basis of emotion in animals (Wilensky et al. 2000; Blair et al. 2005; LeDoux et al. 1985; Panksepp 1998; Rolls 2000). Bat brains contain relatively large amygdalae (Baron et al. 1996). Single neurons in the basolateral amygdala of mustached bats respond to a variety of species-specific calls, particularly to aggressive calls, such as the broadband noise burst (Naumann and Kanwal 2006). Their responses can be long lasting, up to 400 ms after onset of call presentation, especially in response to agonistic call types. These response patterns are similar to the ones observed in the frontal cortex of the same species (Kanwal et al. 2000). These data suggest the presence of mechanisms either for short-term memory and/or integration of multimodal information within the amygdala, which may explain why emotional states last for a relatively long time compared to purely cognitive sensations. In addition, autonomic feedback from the body (heart rate, pain sensations, etc.) also reaches the amygdala, setting up a positive feedback loop that further perpetuates the activity in the amygdala. Affiliative calls, such as the long, quasi CF are not well represented within the amygdala. These data point to two important conclusions. First, non-primate species, such as bats, have structures similar to those of humans for processing emotionally relevant sounds. Second, activity within these brain structures shows that bats, and likely all nonhuman animals, have emotions that are perhaps not as elaborate and complex, but not so different from those experienced by humans.

The evidence in mustached bats of the tight paring of calls with emotionally relevant behaviors, such as fighting and kissing, provides strong evidence that emotions are encoded within sounds (Clement and Kanwal 2012). Furthermore, the role of the amygdala in processing emotional sounds across vastly divergent species, such as bats and humans suggests that, like many sensory driven cognitive functions, emotions too are computed (Randall et al. 2011). Echolocation has been extensively studied at the neurophysiological level over the last 3–4 decades, but neurophysiological studies of the communication of affect have barely begun.

Localized electrical stimulation of the amygdala with a minute electrical current (<10 μA) leads to the emission of social vocalizations as well as echolocation sounds (Ma et al. 2009). These studies in awake bats demonstrate that the receiver as well as the emitter sides of audio-vocal communication is localized and linked to the amygdala. Furthermore, the amygdala has direct projections to other important structures, such as the hypothalamus, which controls relatively long-term physiological states of animals, and the midbrain periaqueductal gray (PAG), which together with vocal motor neurons in the laryngeal branches of the vagus nerve constitutes the final pathway for vocal output. Thus, the amygdala processes and represents the emotional content within social calls as well as initiates vocalizations in response to their perception.

Finally, using computational modeling, we have proposed a mechanism by which neuronal circuits within the amygdala of mustached bat's can compute emotions (Randall et al. 2011). This is the first evidence that emotional valance within sounds is processed in a manner similar to sensory signals. This valence is embedded within and computed from the pattern of frequency modulation as well as the pitch, loudness, and overall acoustic structure of the sounds. From the structure of social calls shown in Fig. 13.4, it is clear that frequency modulations abound, and frequently are the key constituents differentiating one call from another. They also form the basis functions for differential processing of calls within the right versus the left cerebral hemisphere (Washington and Kanwal 2012). These findings open the door to extensive analyses of affect within communicative signals, especially social calls, emitted by virtually all species of animals. Research on bats' behavior and neurophysiology is leading us to a deeper understanding of social and vocal behaviors and how they influence decision making.

13.5 Future Directions of Research

Behavioral adaptations are critical for the survival of any species. Whereas physiology is largely conserved across mammalian species, behavior is directly under the control of modifiable circuits within the brain. Organisms evolve via morphological adaptations and fine-tuning of physiological mechanisms. They evolve and adapt through their behavioral interactions with the environment and socioemotional interactions with conspecifics. Over the last half of the twentieth century, bats have emerged as excellent animal models for understanding ecological and behavioral adaptations in animals. Mustached bats and other species, such as Mexican free-tailed bats and false vampire bats, have exceptionally advanced vocal communication. Therefore, studying these animals has the potential of revealing how speech sounds are represented in the human brain by studying these species. This may seem far-fetched, but several breakthroughs, such as the discovery of combination sensitivity, of multifunctional neurons, of pitch perception, and of hemispheric asymmetry and likely its underlying neural mechanisms, have been made already from research on bats. In fact, a good bit of our understanding of how the human brain processes complex sounds, one of the most important advancements in human evolution, comes from studies of echolocation and call processing in bats as well as nonhuman primates (Kanwal and Rauschecker 2007).

Continued research on bats in the twenty-first century has the potential to build on the findings of a number of neuroethologists to understand the brain's role in guiding social and in particular audio-vocal behavior. This research promises to advance new frontiers, such as decision making, in neuroscience. Advances are expected to come from multisite recording of brain activity from behaving animals, including the ability to track their movements in space using signals from a light and compact global positioning system with an onboard transmitter attached to each animal (Tsoar et al. 2011). Genotyping behavioral traits and testing the effects of gene insertions, such as of the Fox P2 gene, on vocal behavior is another promising direction of research on bats (Li et al. 2007).

13.6 Conclusions

In this chapter, we focused on decision making and on social–vocal behavior in bats. Mustached bats, as well as other species, produce a rich variety of complex sounds for communication. Their calls consist of simple syllables and composites and contain diverse patterns of frequency modulation. The acoustic structure of social calls and their usage within behavioral contexts is consistent with the motivation–structure hypothesis that relates the spectrographic structure to emotive content within sounds. We briefly touched upon what is known about the organization of the brain in bats, particularly as it pertains to audio-vocal communication and the storage of emotive memories and for decision making. Together, the findings reported here challenge the notion that bats and other "primitive" mammalian species express behaviors that are largely innate and reflexive in nature rather than being conscious, insightful, and highly plastic.

Acknowledgments We thank Drs. M. J. Clement and an anonymous reviewer for their constructive comments and suggestions on an earlier version of the manuscript.

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Chapter 14
New Advances in the Study of Group Behavior in Bats

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Abstract The collective behavior of organisms results in spectacular displays that have inspired scientific inquiry for centuries. Recently, rich theoretical work has complemented traditional biological observation to offer fresh insights into the function and mechanisms of animal groups. Although these theoretical studies unquestionably have advanced our understanding of the dynamics of animal groups, further progress will depend on the ability to reconcile numerical methods with empirical data from realistic, field-based studies of existing, as well as new, animal models. Fortunately, the technical challenges associated with collecting such data from large, free-ranging groups are becoming less daunting as a new generation of field-portable technologies becomes increasingly available. By combining novel analytical approaches and better integration of different data modalities, it is now possible to produce stunningly informative representations of the behavior of animal groups. Although the group dynamics of bats are as intriguing and potentially informative as those of other animal groups, until now the difficulty of access has inhibited studying how bats structure and maintain their groups. Here we present some of the first data from large colonies of free-ranging bats. Our initial investigations offer vivid glimpses into the group dynamics of these mammals and underscore the value and importance of this focus for further experimental research in the

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DOI 10.1007/978-1-4614-7397-8_14,

field. Such studies could not only reveal more about functional complexity in nature and further inform our understanding of animal groups but suggest principles by which we organize and might even improve our own society.

14.1 Collective Behavior in Animal Groups

From the swarms of billions of locusts to the schools of fish, flocks of birds, and marching ungulates, examples of collective behavior in large groups of organisms abound in nature and have long fascinated humankind. The synchronous, seemingly purposefully coordinated movement of hundreds of thousands of individuals appears to give these formations a life of their own that transcend the characteristics of the members comprising the overall assembly.

Animal aggregations occur across multiple spatial and temporal scales—from bacteria to elephants and from ones lasting a few seconds to others lasting a lifetime (Allee 1931). Assemblages can form either due to intrinsic mechanisms (self-organize) or in response to an extrinsic stimulus that may lose meaning as group numbers grow. Elements of collective pattern formation have even been observed in inanimate particles suggesting that there are basic physical reasons of such phenomena (Haken 1983; Graham and Wunderlin 1987).

Several explanations are available as to why organisms aggregate. For example, a group provides specific benefits to its members such as better defense against predators, either through a dilution effect (Hamilton 1971), confusion effect, increased vigilance, early detection, or physical protection. Other benefits include better information transfer for locating patchy resources or access to mates. However, groups also have notable costs, for example, easier detection and capture by predators, intraspecific competition, local resource depletion, and disease transmission (Parrish and Edelstein-Keshet 1999).

Beyond the mere accumulation of individuals in the same space and point in time, grouping behavior relates to the coordinated efforts of conspecifics creating the element of cohesion, of acting as one. These interactions scale up, from an individual's behavior to the harmonizing motion of hundreds to millions of conspecifics, and thus entail higher levels of organizational complexity (Fig. 14.1). Whether a mere coincidence or a purposeful outcome of a coordinated effort, why and how these large collectives emerge has been the goal of scientific exploration that spans centuries.

Although studies on the properties of group behavior have been limited mostly to biological observation, new approaches towards scaled laboratory experiments, numerical simulations, and theoretical modeling are changing the field. Theoretical modeling has unquestionably advanced our understanding; however, further progress will be difficult without a reconciliation between laboratory- and field-based research. Of paramount importance is constructing experimental designs capable of capturing the rich information associated with the natural movements of large groups of organisms in the wild.

14.2 Group Behavior in Bats

Where do bats fit in this field and why do we know so little about them in this context? The technological developments that now make it possible and arguably easier to learn more about bat group behavior is the focus of this chapter. Our goal is not to do an extensive review of existing literature, rather to outline the basic elements that now allow us to collect such data. We share information about the methods that we have developed our initial findings and discuss their implications for moving the field forward. Our work is a direct response to the need for empirical data in new models that integrate quantitative description of group behavior with experimental work. We seek to identify the mechanisms used by large groups of conspecifics to synchronize their motions and to understand the adaptive nature of group behavior in bats, with implications for other species. Beyond our mere interest in functional complexity in nature, ultimately, this research could inform not only our understanding of animal groups but also the principles by which we organize our own society and how to improve on some of these organizational challenges.

Although bats engage in group behaviors that are no less impressive than other well-known examples of collective motion, there is little information about why they do it and how they maintain their aggregations over time (Fig. 14.1). Some of the reasons for the limited understanding of collective behavior in bats are our still incomplete understanding of group behavior in general and our inability to adequately observe and describe the behavior of bat groups in the wild. The difficulty of studying the movement of many, small, fast-moving individuals is compounded by the inability to effectively observe and record their movement at night. Yet the tremendous diversity of bats, including their wide distribution across multiple spatial and temporal scales, the richness of their social structure, and the range of morphologies and body sizes, makes bats excellent candidates for such considerations.

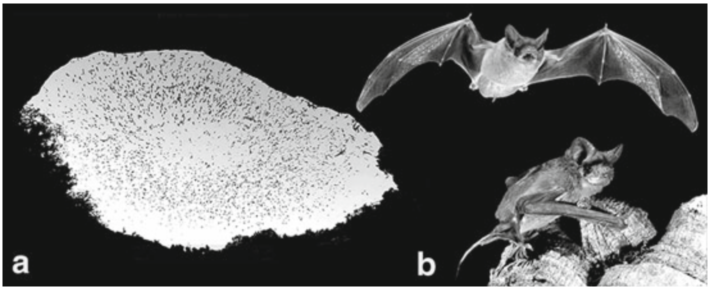

Fig. 14.1 (**a**) The evening emergence of the Brazilian free-tailed bat *Tadarida brasiliensis* (**b**) offers a stunning example of group behavior in bats

14.3 Methods and Techniques to Study Group Behavior of Bats in the Field

Several newly developed or recently updated technologies make deployment of advanced equipment in the field easier and thus have revolutionized access to a new generation of field data for the study of group behavior. Thermal imaging, high-speed videography, long-range laser scanning, and multichannel sound recording have all significantly improved and are more accessible. Although representing different data modalities, each technology can be integrated with the rest for the reconstruction of movement in three or more dimensions. The advantage of thermal imaging allows one to observe organisms in complete darkness without artificial lights. High-speed video makes use of high temporal resolution to permit detailed analysis of fast events. Long-range laser scanning allows accurate, remote surveys of complex physical environments (Fig. 14.2). High-speed sound systems detect, record, and analyze vocalizations of bats revealing much about their ecology and behavior.

Although single detectors (e.g., thermal or high-speed cameras, scanners, microphones) are indispensible for the observation of animal behavior, the true potential of these technologies emerges when several instruments are combined into integrated arrays, allowing the three-dimensional study of animal movement. In principle, two cameras (but three microphones) are sufficient to reconstruct the 3D position of objects in space; however, when the tracking of multiple objects is necessary, three and even four units are the practical lower limit.

The requirements for positioning, synchronization, and spatial calibration of 3D camera arrays in the field are the same as in the lab (reviewed in Holderied and Jones 2009; Dumont and Swartz 2009). The additional complexity arises from the scale of these installations. Synchronization of different camera units is possible by using internal hardware methods (e.g., gen locking, signal generation, various IRIG protocols) or using a discrete external event visible to all cameras. For example, when synchronizing thermal cameras, a piezoelectric lighter provides a brief thermal event that the cameras can record for later reference. Appropriate synchronization is also needed when working with different data modalities. For example, in traditional cameras, the video and audio streams are synchronized internally but most research-grade high-speed and thermal cameras do not have sound-recording capacity. Clashing together two objects (metal poles or similar) in the FOVs of the cameras while recording on the audio channel will present a discrete event in both the cameras and audio track. Aligning the video sequence with the audio channel using a nonlinear editor allows for temporal synchronization of the two streams.

The spatial calibration of large-scale, field-deployed arrays is rather complicated. Well-established indoor methods using the Direct Linear Transformation approach (Abdel-Aziz and Karara 1971; Tsai 1987; Hedrick 2008) require large-scale calibration objects with known dimension that are difficult to build in the field. Another more recently developed method uses a free-moving, small-scale calibration “wand” (Svoboda et al. 2002; Hedrick et al. 2012). The recorded movement of the wand is used to reconstruct the camera array. The availability of such algorithms, however,

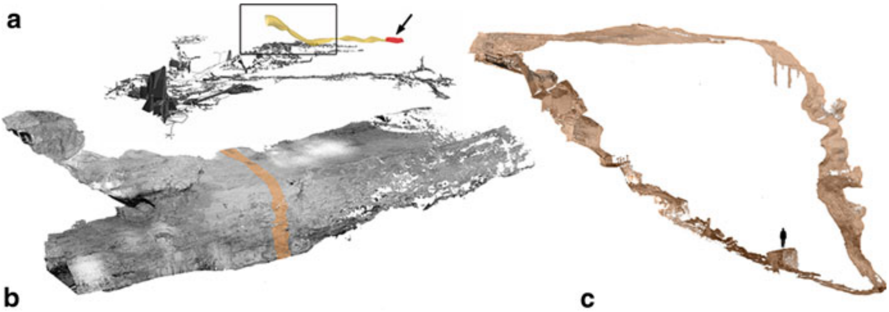

Fig. 14.2 (**a**) Three-dimensional representation of the known extent of Carlsbad Cavern using traditional left-right-up-down surveying (LRUD). *Black arrow* points to the location of the bat roost. *Shaded section* indicates the portion of the cave used by the bats to emerge and return. *Rectangle* indicates the area of the cave that was surveyed with long-range, laser scanning. (**b**) A point-cloud model of a 200-m-long portion of Carlsbad Cavern that was the site of the research presented here. The model was generated from 17 separate laser scans. *Dark band* on the model indicates where a cross section of the corridor was taken. (**c**) A cross section of the main corridor at the location where data in this publication were collected. *A 1.85-m human figure* in the *lower portion of the panel* is provided for scale

is still relatively limited, although research is ongoing and open-source solutions will soon be available (Hartley and Zisserman 2003, T. Hedrick personal communication). Another calibration alternative uses laser scanners.

14.3.1 Thermal Imaging

Recent work has described extensively the application of thermal imaging and computer vision analysis for the observation and census of large colonies of bats (Betke et al. 2007, 2008; Hristov et al. 2008, 2010) (Fig. 14.3a). The ability to see in the dark alone makes thermal cameras particularly useful to study bats. Nevertheless, several features make them especially challenging to use under field conditions as part of 3D arrays. Smaller, portable cameras with non-cooled sensors are convenient for setting up in the field; however, they record smaller images limiting angular resolution and making reconstruction of individual movement in densely packed groups difficult. Additionally, these cameras record at slower frame rates that are often inadequate for fast-moving bats. The cameras also have limited data storage capacity and lack the interface to synchronize multiple units and thus require custom solutions that complicate post-processing of the data.

Larger, high-end models with cooled sensors record in higher resolutions (1024 ×1024) at up to 125 fps. In addition these cameras can be connected to high-performance hard drives permitting continuous recording for extended periods. Such systems, however, are expensive to purchase, maintain, and operate and are bulky and complicated to set up under field conditions where they require line power to support large computer stations for data storage and camera control. Therefore, thermal cameras should only be used when other options are not possible (i.e., dark environments).

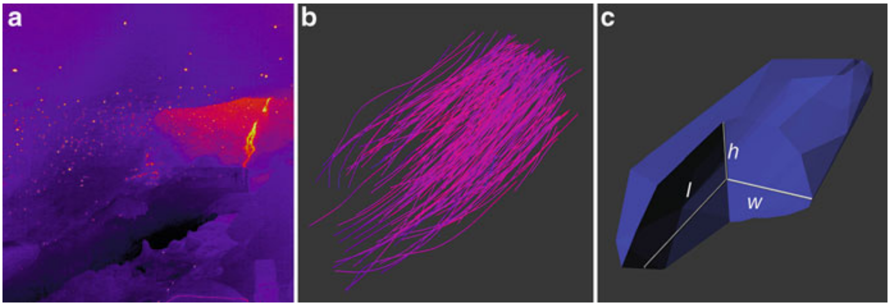

Fig. 14.3 (**a**) Representative image from an array of thermal cameras used to reconstruct the flight pattern of a group of emerging free-tailed bats (*T. brasiliensis*) inside Carlsbad Cavern, New Mexico, USA. (**b**) The reconstructed, three-dimensional flight trajectories for a group of 250 bats, flying through the main corridor of Carlsbad Cavern. (**c**) Volumetric representation of the group of bats in (**b**) showing overall group shape

14.3.2 High-Speed Video

The salient characteristic of high-speed videography is the ability to record hundreds to thousands of images per second, allowing viewers to observe detailed motions not visible to the human eye. Biologists use high-speed cameras to observe the three-dimensional characteristics of animal movement, biomechanics of terrestrial and aerial locomotion, fluid mechanics, etc. using arrays of two or more units to provide three-dimensional (3D) reconstructions. Several recent reviews provide excellent accounts of the new developments and applications of high-speed video data collection in the laboratory (Holderied and Jones 2009; Dumont and Swartz 2009). The benefits of high-speed videography, however, are relatively unexplored in the field.

Several characteristics of high-speed cameras make them challenging for use under field conditions. For example, until very recently, such cameras were complicated scientific instruments; they lacked built-in displays, required connection to computers for operation, demanded high light levels, were bulky and tedious to set up outdoors, and had high power and data management requirements. The technology, however, continues to develop and improve at a fast pace; modern cameras are quite portable, boast sensors with high light sensitivity, and have advanced solutions for data storage, transfer, and backup. Our current system of high-speed cameras consists of four identical units, capable of recording 1280 × 800 pixel images at rates as high as 3250 fps. Higher frame rates are possible by reducing the resolution of the recorded image. Each camera can function on its own without a connection to a computer or in combination with other cameras permitting flexible deployment.

An innovative solution for the storage of data is a new technology that pairs a solid-state memory with high-speed cameras that provides a first-step storage for high-speed data. One such example is the Cinemag® technology developed by Vision Research, Inc. that utilizes a large capacity solid-state device to permit the transfer of data at very high rates, reducing lengthy data transfers. For example, transfer of 34 GB takes only 45 s versus 17 min over GigE connection. In addition to traditional high-speed cameras, a new generation of cameras using GigE network protocol is available. Within the limits of their bandwidth, these cameras provide either high-resolution images at low frame rates (usually 60–125 fps) or trade image size for higher frame rates (generally up to 350 fps). These are more compact than traditional high-speed cameras, and with the development of new data transfer protocols like USB 3.0 and Thunderbolt®, the tools at the disposal of the field biologists are increasing.

14.3.3 3D Laser Scanning

Organisms do not exist in isolation but instead are immersed and interact with objects in their environment at different scales. Obviously, one cannot understand group behavior without understanding the organism's natural environment. In the laboratory, where flat, horizontal floors and vertical walls provide convenient frames of reference, a general description of the experimental setup such as recording equipment, stimulus placement, or target orientation is sufficient to quantify the environment. In the field, however, reference frames are difficult to establish, and quantitative description of the environment is challenging. Currently, detailed representations of the environment are very limited or lacking entirely. When we change the focus of our investigations from the individual to the scale of the group, however, features of the environment become increasingly important since they affect not only the individual but also the entire group.

Long-range laser scanning is an emergent technology that uses laser light as a noncontact method to accurately survey complex environments. Sometimes referred to as LIDAR, 3D scanning, or time-of-flight range finding, the technology is fast gaining popularity in a number of disciplines and applications. Here we use the term 3D laser scanner as the most general form of the technology but refer specifically to a low-power, laser-based device that uses a noncontact method of collecting distance measurements.

3D laser scanners use a vertically revolving mirror in combination with a horizontally panning base to steer a laser beam and estimate the distance between features in the physical environment and the device. Hundreds of thousands of laser readings can be acquired in a single second resulting in a dense "point-cloud" that accurately represents the 3D environment in virtual space (Fig. 14.2). The point-cloud can be used as is for direct distance, area, or volume measurements, or the data can be converted to other formats for use in CAD programs or the generation of polygonal or parametric NURBS models. Since the 3D laser scanner uses a line of sight, a single scan is rarely sufficient to capture all the information. Multiple scans from different perspectives resolve these limitations but require alignment or registration of multiple files, although automated post-processing of the data is becoming more efficient and increasingly automatic.

The most prominent feature of 3D laser scanning is the high accuracy of the survey and relatively fast data collection. The technology was originally developed for engineering applications but has more recently been adopted in research applications such as computer science, mechanical engineering, landscape design, architecture, heritage preservation, and some ecological work to estimate tree height or vegetation percent cover (Van Pelt et al. 2004), or even colony census (Azmy et al. 2012). The application of laser scanning to study group behavior holds great promise. In our work we use a pair of FARO Focus 3D® laser scanners to quantify the 3D environment, including cave dimensions and topography. We also use them to calibrate and survey large-scale camera and microphone arrays to virtually represent and simulate movement of organisms and their groups.

14.3.4 Multichannel Sound Recording

One of the unique characteristics of microchiropteran bats is their reliance on biosonar to orient and navigate in the dark; thus, much insight can be gained from studying their acoustic behavior. There is a long history of utilizing high-speed sound recordings to study acoustic behavior, foraging habits, species identification, and habitat use in bats (reviewed in Brigham et al. 2004; Parsons and Szewczak 2009). More recently, multichannel sound systems have informed a better understanding of sonar beam characteristics in the laboratory (Ghose and Moss 2003) and 3D position of echolocating bats in the field (Holderied et al. 2005). Several features of acoustic 3D reconstruction make the approach particularly useful for field applications. Omnidirectional microphones can sample large volumes, because they generally have broader directionality compared to optical devices. Sound-recording equipment also does not require additional lighting and is generally more portable than image-based systems. The method, however, has notable limitations as well—reconstruction is only possible when bats echolocate and the number of individuals that can be tracked is low.

Many of the limitations of the acoustic approach to 3D tracking, however, can be overcome by integrating acoustic information with other modalities. For example, combining multichannel sound recording with visual data can compensate for periods when a bat is not echolocating or a call is not strong enough to be detected by all microphones. This approach is particularly useful in the context of group formations where multichannel recording alone cannot resolve and assign the calls of different bats to the correct individual and position within the group. When paired with video-based information about the precise location of bats, it is possible to study the relationship between relative positions in the group and call characteristics. Furthermore, inferences can be made about the relative use of echolocation by bats when the group or environment changes. How does grouping affect the acoustic behavior of individuals? Do they emit more or higher intensity calls, echolocate less (Gillam et al. 2010), change their call design (Gillam et al. 2007), or rely on other sensory modalities to form their groups (Sterbing-D'Angelo et al. 2011)?

The seemingly disparate technologies described above can integrate seamlessly into a single, multimodal representation of the environment and behavior of

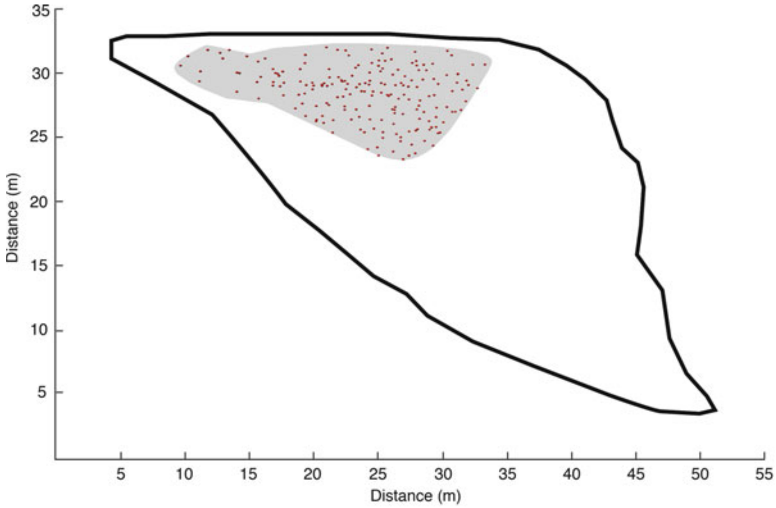

Fig. 14.4 A cross-sectional contour of Carlsbad Cavern main corridor indicating a representative position and spread of the column of bats flying through it

grouping organisms. We can now record the free movement of bats in their natural setting during the day and at night, at high temporal resolution and with minimal disturbance to the animals. Furthermore, we can capture details of their movement, spatial arrangement, acoustic behavior, and three-dimensional environment and give a stunning representation of their fascinating behavior (Figs. 14.3 and 14.4).

14.4 Analytical Methods

The techniques described to this point for gathering empirical data of group behavior in the wild can generate a plethora of multidimensional data that is as complex to work with as it is exciting and useful. Until recently, most numerical data on groupings were generated via mathematical simulation; therefore, new strategies are needed to guide the analyses. An excellent introduction to the topic is provided by the STARFLAG project with a two-part publication on the empirical and analytical methods for studying three-dimensional animal groups (Ballerini et al. 2008a, b; Cavagna et al. 2008a, b). Bat emergence groups are different from the discrete groups of fish and birds in that there is no formal beginning and end to their formations; rather they are continuous aggregations that rarely undergo dramatic shifts in their direction of travel. As a consequence, group measurements are not representative of the entire column, but instead, to a subset of bats within the calibrated space of the cameras (Fig. 14.3). For example, the measure of group length, the dimension along the velocity vector, does not reflect the distance measure of the entire column but the length of the formation containing the subset of individuals. The notion of a discrete group of interest, therefore, is appropriate in columns of emerging bats if defined from the perspective of an individual and its interactions with nearby group members. In our work, we refer to the continuous emergence as the "column" and the cohort of sampled bats as the "group."

14.4.1 Flight Trajectory Parameters

From the 3D positional data generated for each bat over a given period, the most common translational parameters include the bat's velocity, curvature, and acceleration. Velocity ($\mathbf{V}$), displacement over time, can be decomposed into its magnitude and direction, or speed (s) and unit tangent vector ($\mathbf{T}$)—a vector that points in the direction of movement and has a magnitude of 1, where $\mathbf{V}=s\mathbf{T}$. Curvature ($\boldsymbol{\kappa}$) describes the rate at which $\mathbf{V}$ changes direction along the flight path. For example, for motion in which $\mathbf{V}$ does not change direction (i.e., a straight line), $\kappa=0$. However, movement with constant, nonzero speed and curvature is a circle, with a radius equal to $1/\kappa$. The unit normal vector ($\mathbf{N}$) defines the direction of curvature and is perpendicular to $\mathbf{T}$ (see Crenshaw et al. 2000). Translational acceleration ($\mathbf{A}$), commonly referred to as simply "acceleration," is composed of the rate of change in (1) the magnitude of velocity, or speed, parallel to $\mathbf{T}$, which we refer to as the tangential acceleration ($\mathbf{A}_{\mathrm{Tang}}=s'\mathbf{T}$), and (2) the direction of velocity, parallel to $\mathbf{N}$, commonly known as centripetal acceleration ($\mathbf{A}_{\mathrm{Cent}}=\kappa s^2\mathbf{N}$), such that $\mathbf{A}=s'\mathbf{T}+\kappa s^2\mathbf{N}$. Note that curvature is the rate of directional change as a function of the distance traveled, while centripetal acceleration is a function of time. These parameters can be calculated at any time point within the recorded interval, using either numerical differentiation or by fitting the 3D data to a function (e.g., spline functions), independently for each individual.

Once the flight parameters for each individual have been calculated, the same measures can be determined for the sampled group by calculating the mean values of the individuals, to include the average position or centroid, the average speed and direction, and accelerations. Additional group parameters include the dimensions: length ($\boldsymbol{l}$), width ($\boldsymbol{w}$), and height ($\boldsymbol{h}$), as well as cross-sectional area, volume, and density (Fig. 14.3c). The parameters above provide simple measures that are useful in defining the properties of the group at single points in time and identifying features that change or remain consistent throughout the time interval, as the group moves away from the colony roost. Additionally, these parameters provide the means for comparing distinct group cohorts to determine how group characteristics and individual flight behavior differ with changes in social and environmental conditions.

Whereas these parameters provide important measures of overall group properties and dynamics, they give little information about individual positions within the column and the interaction among group members. To investigate these higher-level questions, new methods must be developed for identifying and tracking a bat's position within the column and between their surrounding neighbors. One possibility is to calculate the distances between all bat pairings, i.e., interindividual distances. For each individual, we can determine the rank order, by distance, of all the other group members and identify the "*n*th"-nearest neighbor of interest. However, while interindividual distances are useful in determining the proximity between individuals, they provide insufficient detail about the spatial arrangements of individuals and how they change over time. Answering these questions requires transforming the 3D Cartesian position of each bat (i.e., their *XYZ*-coordinates) into meaningful representations that reflect either their own position within the column or the position of other bats relative to its own flight trajectory. Examples of these include the perpendicular distance of a given bat and/or the angles of a vector, relative to a defined plane. Additionally, a vector can be decomposed into its parallel and perpendicular components, using defined reference planes and lines that represent the reference frame of the hierarchal level of interest.

14.4.2 Scales of Analysis–Reference Frames

The three most useful reference frames that we have used in our analysis of group movement are "Global," "Group," and "Individual." The definition of each representation depends on whether the orientation of the frame changes over time and between individuals within the group. Three elements are needed when defining a three-dimensional (3D) reference frame or coordinate system: (1) the point of origin; (2) the plane of interest, defined by its normal vector, perpendicular to the surface of the plane (not to be confused with, although related to, the normal vector defined previously); and (3) a reference vector within the plane and also perpendicular to the normal. For a more detailed description of coordinate systems, planes, and vectors, see Kreyszig (1991).

The "Global" system is temporally constant and universal (i.e., applied equally to each individual). In this framework, an arbitrarily selected "fixed" point acts as the origin of the system; the horizontal plane is defined by the vertical gravitational vector, the normal; and a second "fixed" vector, for example, the *X*-axis, provides the reference vector within the horizontal plane. In our work, the cameras are calibrated such that the positional outputs for the individuals, i.e., their *XYZ*-coordinates, are aligned to a "Global" reference frame (Fig. 14.5a).

The "Group" reference frame is temporally dynamic but remains universal among individuals at each time point. In this framework, the group centroid acts as the origin, the group flight trajectory acts as the normal to define the plane of interest, and the component of the gravitational vector perpendicular to the flight trajectory defines the reference vector within the plane. Obviously, the origin and vectors of this framework will change from one time point to the next as the column moves, but this system describes each bat's position within the column, independent of changes in position and orientation of the column, which would not be possible when using the "Global" system (Fig. 14.5b).

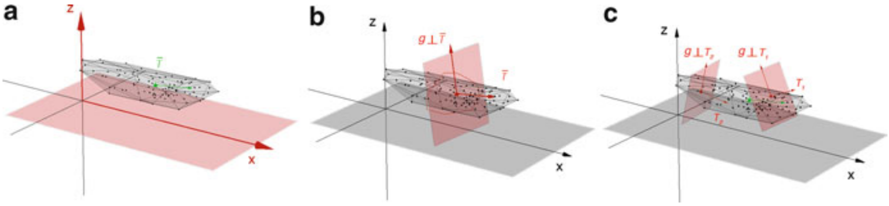

Fig. 14.5 Example reference frames for describing group motion. (**a**) Global reference is defined by the *XYZ*-coordinate output of the calibrated camera system. The calibration is arranged so that the *Z*-axis, parallel to gravity, is the normal to the horizontal plane and the *X*-axis defines the horizontal reference vector. (**b**) Group Reference. The average position of group members defines the origin (*pentagram*), the average tangent of the group ($\overline{T}$) defines the normal of the group reference plane, and the component of gravity perpendicular to the average tangent ($g \perp \overline{T}$) defines the reference vector within the plane. (**c**) Individual Reference. The position of each bat defines the origin for its own reference system. Each tangent (T_1 and T_2) defines the normal of their respective reference plane, and the component of gravity perpendicular to each tangent ($g \perp \mathrm{T}_1$ and $g \perp \mathrm{T}_2$, respectively) defines the reference vector within each plane

Although some elements of collective behavior can be understood at the level of the group, others require an individual-centric approach to the analysis. The "Individual" system is both temporally dynamic and independent, changing from one individual to the next. Here, a reference frame is applied to each bat, independent from the group and other bats. The origin is defined by the bat's current position, its tangent defines the normal to the plane of interest, and the component of the gravitational vector perpendicular to **T** provides the reference vector within the plane. Thus, it is possible to track the position of other group members relative to the current position and flight trajectory of each individual (Fig. 14.5c). Which of these systems is used depends on the questions at hand. If describing the movement of the column over time, then the "Global" system is ideal, but if describing the position and movement of individuals within the column is of interest, the "Group" system is more useful. The "Individual" system appropriately describes how individuals perceive and interact with other group members relative to their own flight trajectory.

14.4.3 Angular Distribution

An important measure of the three-dimensional arrangement of individuals in a group is their angular distribution with respect to different frames of reference. For any of the three frameworks described above, the 3D Cartesian positions of group members can be transformed into either cylindrical or spherical coordinates.

Spherical representations are ideal for when positions of objects, or individuals, have the *potential* to occur in any direction at any distance (e.g., flock of birds or adjacent bats in a column). A visual illustration of the angular distribution can be constructed using an elliptical (Mollweide) projection on a unitary sphere

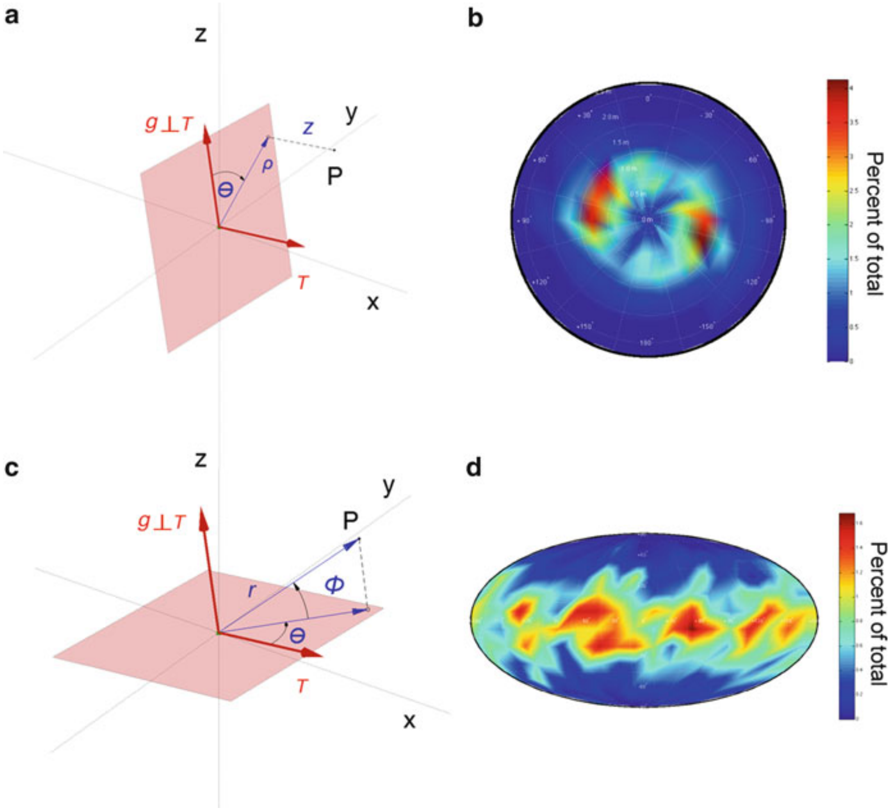

Fig. 14.6 Elliptical representations of positional distributions. (**a**) Converting the *XYZ* Cartesian coordinates of point (*P*) into cylindrical coordinates—radial coordinate (ρ), angular coordinate (θ), and vertical coordinate (z). (**b**) Polar projection of the distribution of bats within a column. *Radial grid* represents the angular coordinate, in degrees. The *ring grids* represent the radial coordinate, in meters. (**c**) Converting *XYZ* Cartesian coordinates of a point (*P*) into spherical coordinates—radial coordinate (r), azimuthal coordinate (θ), and elevational coordinate (ϕ). (**d**) Spherical (Mollweide) projection of the distribution of the five nearest neighbors. *Horizontal axis* represents the azimuthal coordinate, in degrees. *Vertical axis* represents the elevational coordinate, in degrees

(Fig. 14.6c, d) wherein the angular position of the nearest neighbor from the perspective of each members is plotted to give a cumulative, first-person perspective of the spatial arrangement (Ballerini et al. 2008a, b, Cavagna et al. 2008b). The benefit of this representation is that in most animal groups there is a nonuniform, anisotropic spatial structure with important implications for understanding the mechanisms of grouping. For example, in flocks of European starlings, the arrangement of individuals in the group is highly anisotropic within the proximity of the first 6 or 7 neighbors but becomes increasingly isotropic beyond that range, suggesting that group members rely on the number and positions of nearby conspecifics rather than the absolute distance among them (Ballerini et al. 2008a).

Cylindrical coordinates are best suited for describing objects that are cylindrically organized like a column of bats, where the goal is to describe the position of a group member relative to the center vector of the aggregation (Fig. 14.6a, b). It can determine if individuals belong to the group's interior or the periphery and the effect of relative positions on their collective behavior.

14.4.4 Spatial Structure of the Group

In addition to nearest-neighbor distance and angular distribution representations, several algorithms from computational geometry can be used for describing the spatial arrangement of individuals in groups by dividing the cluster into discrete regions. These algorithms specify how points in space are connected and how the resulting regions around them are defined. Two of the most common examples with the richest tradition in behavioral ecology are the Delaunay triangulation and Voronoi tessellation methods.

The mathematical concepts and algorithms are beyond the scope of this chapter, but the overall goal of these dual structures is to divide the 3D space occupied by a set of discrete points, i.e., the position of individual group members. From the Voronoi tessellation, we can find all the points in space whose distance from an individual is less than or equal to the distance to any other individual. This relationship can be visualized as a convex polyhedron unique to, and surrounding, the individual point, known as a Voronoi cell. As an integral part of the Voronoi tessellation algorithm, Delaunay triangulation identifies the surrounding neighbors (three or more) that determine the shape and volume of the Voronoi cells for each individual. Together, these two techniques define the volume of the Voronoi cell surrounding each individual, determine where that cell fits within the spatial arrangement of the group, and identify and track the neighbors most likely to influence their spatial boundaries. The Voronoi cells of an individual can be described either as a closed polyhedron with a defined volume or an open, incomplete polyhedron with an infinite volume. This difference is determined by whether the individual is "surrounded" by a sufficient number of neighbors, as would be the case for individuals within the interior of the group. An open Voronoi cell occurs among the peripheral individuals who have at least one exposed side (Fig. 14.7).

The advantage of these methods over traditional, linear representations of spatial arrangement is that they relate to functional properties of the group. These could be regions of influence in order to maintain the cohesion of the cluster or zones of danger that relate to perceived vulnerability to predators (Hamilton 1971; Viscido et al. 2001). For example, spacing among individuals could be based on either metric distance or topological arrangement. In the first case, focus members of the group maintain a fixed range from surrounding individuals. In the second, a focus individual maintains a fixed number of proximal neighbors even if the distance and thus density change over time. The Selfish Herd hypothesis (Hamilton 1971; Vine 1971) suggests that predators choose to attack individuals that have compromised

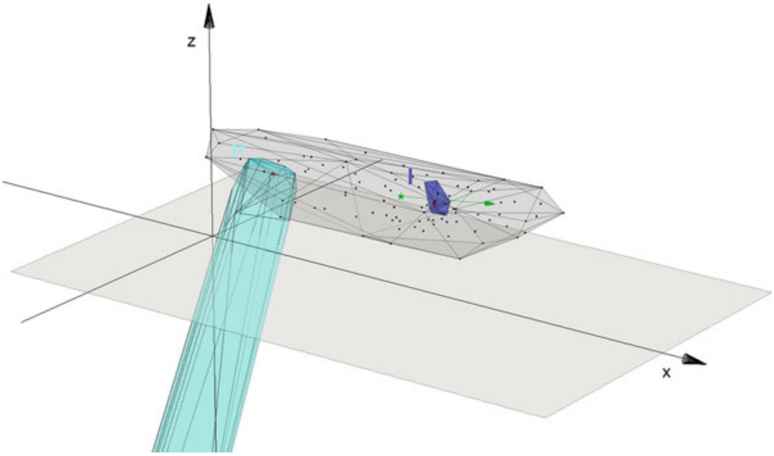

Fig. 14.7 Voronoi space definitions within a group of emerging bats. For an individual *I*, located in the interior of the group, the convex polyhedron "cell" obtained by Voronoi tessellation is contained within the volume of space defined by the group. For an individual *P* located near the periphery of the group, the Voronoi cell extends far beyond the volume of the group

positions in the group. Description of the spatial arrangement of the group, therefore, provides functional characterization of these positions and generates testable hypothesis about function and significance.

14.5 Examples and Initial Findings from a Model Bat

To illustrate the above-described conceptual framework and methodologies for studying group behavior of bats in the wild, here we present our initial findings from the investigation of collective behavior in a model bat—the Brazilian free-tailed bat *Tadarida brasiliensis* (Wilkins 1989) (Fig. 14.1b).

This species accumulates in enormous colonies and exhibits a variety of behaviors of interest at the level of the group—clustering, spiraling, emerging, and returning in densely packed assemblages. In North America, *T. brasiliensis* overwinter in central and northern Mexico but migrate to south central USA in spring where they form breeding colonies in the hundreds of thousands to several million individuals (Betke et al. 2008; Hristov et al. 2010).

The nightly emergences of free-tailed bats are the most visible aspect of their collective behavior. The emergence starts with a few individuals dashing out of the roost, but the rate increases quickly to several thousand bats per minute, flying in close proximity to each other in a massive exodus. To a bystander, the collective motion appears complex, even chaotic, but the emergence aggregations are not a passive convergence of individuals in space and time. Instead, the tightly packed flight formation stays together for several kilometers as bats slowly gain altitude before dispersing to forage individually. During most of the season, the bats emerge near dusk; however, in late June and most of July, during the period when adult females nurse their young, emergences in broad daylight are common and may continue into the night. The natural variation in emergence rate and ambient light provides an excellent opportunity to study the effect of group size and environmental conditions on the collective behavior in this species. In addition, emerging free-tailed bats are frequently predated upon by diurnal (hawks) and nocturnal (owls) raptors, providing an opportunity to study the mechanisms as well as the potential adaptive values of their collective behaviors.

In one of the first quantitative descriptions of group behavior of bats in the wild and one of very few to document the natural movement of individual group members during flight (Holderied et al. 2005; Ballerini et al. 2008a, b), we recorded and reconstructed, in 3D, the emergence of Brazilian free-tailed bats from Carlsbad Cavern in New Mexico, USA. We used the unique morphology of the cave to describe the flight of bats in an underground passage as they traversed the distance from their roost to the entrance. Because of the topology and size of this cave, it provided a rare opportunity to collect baseline data on group behavior of bats in an elegantly controlled natural setting. The colony of bats at Carlsbad, on average about 350,000 individuals (Hristov et al. 2010), roosts in a section of the cave 0.6 km away from the main entrance (Fig. 14.2). Unlike other smaller caves, the bats must fly this distance underground through one of the largest corridors in the cavern. Using this natural underground thruway allowed us to study the flight and group behavior without the influence of wind, predators, and ambient light.

We used independent arrays of thermal and high-speed cameras to record multiple emergences from several carefully selected vantage points inside the cave. We used laser scanners to capture the interior dimensions and morphology of the cave and to survey and calibrate the large camera arrays. We reconstructed a 200-m section of the main corridor of the cave, and the cameras sampled ~3,400 m^3 of calibrated space. We recorded multiple evening emergences and selected for this analysis a single emergence that best characterized the range of emergence rates and related group sizes. From this selection we isolated three discrete cohorts of bats (e.g., groups) that represented the highest number of individuals that we can reconstruct reliably with our current capabilities (~250).

We found that the bats maintain group cohesion even when they flew in the relative confines of the cavern. The group did not occupy the entire volume of the corridor, suggesting that the bats were not limited by the available space, rather that the group structure and dynamic were the result of inherent influences (Fig. 14.4). The overall cross section of the flight formation was 81.6 ± 7.84 m^2 and the "defined" volume of the group was 652 ± 24 m^3. As a result, the average density of bats in the group was 0.4 bats/m^3. The dominant orientation of the column was aligned with the longitudinal dimensions of the cave, and the column moved parallel to the horizontal plane. The vertical (***h***) dimension of the flight formation was notably smaller than the transversely horizontal (***w***), giving the overall flight formation a distinctly small ***h/w*** ratio of 0.43 ± 0.18 and thus a horizontally stretched shape (Fig. 14.4).

This characteristic has been observed in other flying animals and appears to be due to limitations in flight performance. It is easier for individuals flying closely together to maneuver laterally than vertically where producing lift might be more costly. This is particularly pronounced in birds and bats with high aspect ratio and high wing-loading morphologies like *T. brasiliensis* that are adapted for fast, relatively non-maneuverable flight.

The angular distribution of individuals in the column changed when different ranked distances of the neighbors were considered. At long distances, the pattern indicated a strong concentration of bats in front and behind the focal individual, a configuration that is consistent with the overall cylindrical shape of the column (Fig. 14.6b). When the *n*th-nearest neighbor was considered (for $n=1$–5), the configuration was distinctly different with strong presence of individuals in a narrow band in front and to the sides of the focal bat and well-defined lack of bats above and below (Fig. 14.6d). These patterns in angular distribution are unique and differ from those of other animal groups (Ballerini et al. 2008a).

We detected no relationship between the relative position of bats in the column and the magnitude of flight speed, translational acceleration, or curvature (Fig. 14.8a, b). The average flight speed of bats in the group was 8.7 ± 1.4 m/s, and the average curvature of individual flight trajectories was 0.19 ± 0.06 rad/m. In addition, while the average speed of bats was relatively high, the difference in relative flight speed of individuals compared to the group mean was quite small—on average 0.41 ± 0.11 m/s.

Nevertheless, several important differences are apparent in the pattern of flight and interactions between bats in the interior of the group and those closer to the periphery. For central bats, the relative proportion of the velocity vector (**rV**) and the translational acceleration vector (**rA**), determined by their component perpendicular to the central axis, indicate that some bats flew in the average direction of the group while others did not. For individuals in the periphery, a larger proportion had larger values of **rV** and **rA**, indicating that bats changed their direction of flight relative to the average movement of the group (Fig. 14.8c). Indeed, further investigation of the signed values of **rV** and **rA** indicates that bats in the interior of the column move towards and away from the center of the group (have positive and negative values), whereas bats in the periphery displayed mostly positive values. It appears, therefore, that individuals in the periphery tend to move towards the middle of the group whereas those in the interior simply maneuvered around each other (Fig. 14.8d).

These observations suggest a possible mechanism for maintaining the organization of the group. Individuals in the interior are likely unaware of their proximity to the periphery, and their goal is to maintain their current position while avoiding collisions. This likely produced the small but alternating change of direction indicated by their trajectory curvature and signed values for **rV** and **rA**. For peripheral individuals, the objective is to return to the interior of the column, the direction of correction clearly indicated by the asymmetric arrangement of bats around them. Such a mechanism is likely to produce a “sloppy” organization of the group with a high latency of response to changes in direction and relatively high turnover of individuals between the interior and exterior of the formation. Indeed, this prediction is

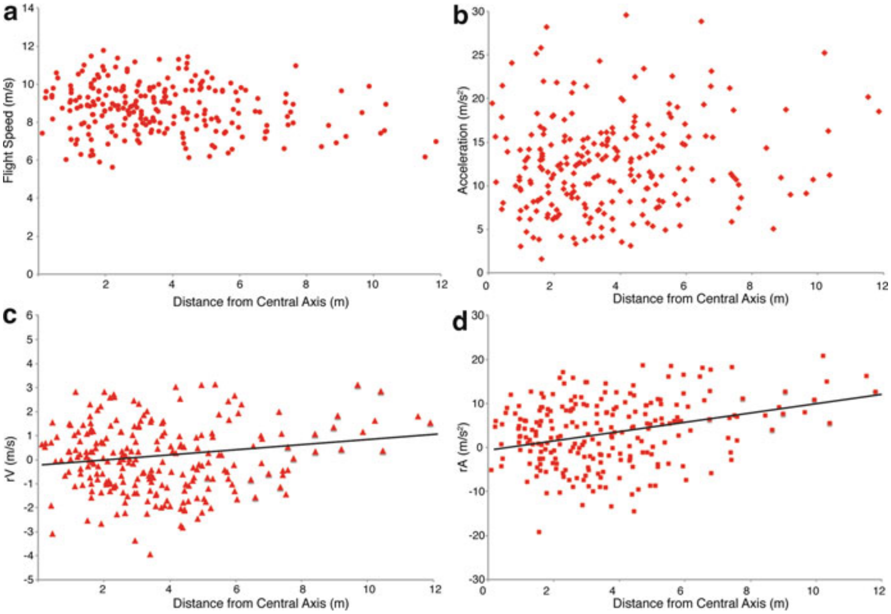

Fig. 14.8 Relationships between flight parameters and relative position of bats in the group. There is no relationship ($p > 0.5$) between the flight speed and trajectory curvature and position in the group (**a**, **b**), respectively. The relative proportion of translational acceleration rA (**c**) and signed values of *A* (**d**) change as a function of relative position in the group, suggesting that bats in the periphery gravitate towards the center ($p < 0.05$, see text)

supported by the analysis of individual trajectories and by natural observation of emerging bats at the cave entrance. Bat groups lack the impressively synchronous patterns displayed by schools of fish and flocks of birds; instead, it is the scale of the emergence, the number of individuals in these groups, that is the most impressive aspect of their collective behavior.

One finding, in particular, stands out the most from these early analyses—the surprisingly small nearest-neighbor distance and overall dense distribution of bats in the group. In our sample, the minimum nearest-neighbor distance was barely 0.06 ± 0.018 m and the average nearest-neighbor distance was 0.17 ± 0.07 m. Calculating the spacing among individuals has been an elusive measure for large aggregations. Theoretical work posits that two opposing forces maintain group cohesion—attraction towards the interior at a finite distance and repulsion among group members at some minimum threshold. A general assumption in these models is that members will not collide and repulsion at some minimum distance is one of the most robust rules of collective grouping behavior. Consequently, the limits imposed on such algorithms are often set beyond the distance of physical contact (reviewed in Couzin et al. 2002; Kunz and Hemelrijk 2003). Particularly notable in our results, therefore, is that the minimum and average nearest-neighbor distances are well below the 0.3-m wingspan of free-tailed bats, implying that contrary to theoretical predictions, collisions do occur among individuals. We have since confirmed that collisions are widespread in groups larger than 50 individuals with a strong relationship to relative position within the group. We detected significantly more collisions in the central portion of the emergence columns vs. the periphery.

Clearly, the collision of individuals with either stationary objects in their flight path or among bats moving at largely different speeds could be potentially dangerous and should be avoided. In rare cases we have observed bats with broken wings or torn flight membranes which can have fatal consequences. However, as shown by our measures of small differences in relative flight speed and curvature, the flight environment of the emergence column is not as complex as perceived from the perspective of a stationary observer. The seemingly abrupt dashes of bats from side to side and past each other are merely subtle maneuvers amplified by our different frame of reference. From the perspective of the bats, their movements are controlled, evasive maneuvers around other group members and most collisions are without negative consequences.

14.6 Conclusion

The technologies, methods for analysis, and data presented here demonstrate that it is now possible to accurately and reliably reconstruct the properties and individual behavior in large groups of free-ranging bats. Our preliminary work has offered a stunning glimpse into remarkable group dynamics and holds great promise for understanding other chiropteran and animal groups. These initial findings indicate the importance of natural observation to validate theoretical models and the need to incorporate realistic empirical data in the formulation of new testable hypotheses. Beyond the baseline condition presented here, it is interesting and timely to consider what is the effect of group size on the individual behavior and overall group dynamic. How do external factors like site topology, ambient light level, wind, or the presence of predators affect the behavior and structure of the group? What mechanisms and assembly rules for grouping are used to maintain their aggregations and what sensory modalities do bats rely on? Are the physical and social interactions in the group competitive or cooperative? Finally, what is simply a pattern and what a function in the collective group?

The answers to these questions will come from the continued marriage of theoretical work with careful natural observations and further investigation of rich model systems like the Brazilian free-tailed bat. For example, experimental disruption of the organization of the group with obstacles presented in the flight path of the column could help to understand how the group responds and what mechanisms are used to realign the group. Furthermore, careful manipulation of the physical dimensions and acoustic properties of the obstacles is likely to lead to better understanding of the organizational mechanisms, traffic rules, and sensory modalities that bats use. Do bats rely on local frames of reference or global ones? Do they use one sensory modality and if so, which one, or multiple ones? In addition to the extremely developed sonar capabilities, there is increasing evidence that bats rely on sight to orient at scales beyond the range of their echolocation (Holland et al. 2010; Mistry 1990). Furthermore, new evidence indicates that wing sensors have profound effect on flight capability (Sterbing-D'Angelo et al. 2011). The possibility that bats could use this sensory modality in the context of the group is particularly intriguing. These results would indicate diverse and complex behavior with interesting insight for the evolution of grouping and the use of existing adaptations for new purposes.

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Chapter 15
Recent Advances in Bat Migration Research

Jennifer J. Krauel and Gary F. McCracken

Abstract In this chapter we discuss the state of research on bat migration and compare some of these patterns to those of birds. We begin with an overview of the literature on migration, apply migration theory, and discuss case studies on four bat species on which the greatest knowledge and understanding of migratory patterns exists. We also discuss what is known of motivating factors for bat migration and where research needs are apparent.

15.1 About Migration

Migration is the movement of animals that are following seasonal availability of resources and strategies involve an astonishing variety of schemes. Birds and mammals differ from insects in migration: individual birds and mammals can do many round trips, usually reproducing only once per trip (but see Rohwer et al. 2009), whereas insects typically undergo multiple generations per migratory cycle (Drake and Gatehouse 1995). Dingle (1996) asserted that migrating animals display some or all of the following characteristics: (1) traveling longer distances and in relatively straighter lines than during foraging, (2) displaying special predeparture or post-arrival behaviors such as hyperphagia, (3) storing energy to use during or after the trip, and (4) not displaying normal responses to stimuli such as pheromones or prey. Migration differs from dispersal which is generally unidirectional.

Understanding animal migration is important. Migratory animals include wild birds that span political borders and spread zoonotic disease that can affect humans

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_15,

(see Chap. 16) and agricultural insect pests such as the corn earworm (*Helicoverpa zea*) which cause billions of dollars of economic damage. Migratory bats help suppress migratory pests (Lee and McCracken 2005). The migratory lesser long-nosed bat (*Leptonycteris curasoae*, Fleming 2004) and the straw-colored fruit bat (*Eidolon helvum*, Richter and Cumming 2008) are important pollinators and seed dispersers following seasonal blooms of flowers and fruit over thousands of kilometers and international borders. Moving animals transfer energy and nutrients among ecosystems (Wikelski et al. 2007). Migratory animals also face habitat fragmentation and climate change, which are likely to be disruptive (Sherwin et al. 2012; Wilcove and Wikelski 2008). In addition, increasing deployment of wind turbines to counter climate change kills thousands of migratory, and other, bats (see Chap. 20).

15.2 Overview of Migration in Bats

There are several reviews on bat migration (Cryan and Veilleux 2007; Fenton and Thomas 1985; Fleming and Eby 2003; Griffin 1970; Hutterer et al. 2005; Popa-Lisseanu and Voigt 2009) showing at least 87 species in 10 families for which all or part of a population migrates regionally or longer-distance (Table 15.1). Most migration research concerns birds (Faaborg et al. 2010) that have some similarity with bats (Dingle 1996), but there are significant differences in scale and behavior. Bat migration is much less common than in birds (Brigham et al. 2012; Woods and Brigham 2004), covers shorter distances and possibly shorter stopover intervals (Fleming and Eby 2003), and involves foraging while migrating.

Bats migrate to follow roosts and/or food (Fleming and Eby 2003). In temperate areas, some bats move regionally (100–500 km each way) to and from hibernacula (Rodrigues and Palmeirim 2008). Long-distance (~>1,000 km) migrants in temperate areas often forgo hibernation by overwintering in milder climates offering food (Fleming and Eby 2003), although some European bats undergo long migrations to hibernacula (Hutterer et al. 2005). In tropical or subtropical areas, long-distance migrants follow transient fruit or nectar resources (Fleming and Eby 2003). Whether insect-eating bats migrate in response to seasonally available prey remains unknown as these patterns are obscured by the lack of information on seasonal insect availability beyond agricultural pests. Although regional migrants are assumed to move in search of roost sites (Griffin 1945, 1970; Tuttle 1976), many latitudinal migrants may be pursuing food, with roosts being a secondary factor.

Migratory behavior appears to have independent, multiple evolutions, especially in the western hemisphere (Bisson et al. 2009; Popa-Lisseanu and Voigt 2009). The origin of most bat species and the greatest diversity occurs in the tropics where more stable resource bases occur; thus, temperate species migration is not likely an ancestral trait (Fleming and Eby 2003). Although migratory behaviors in bats are probably less diverse than in birds, some patterns exist. For example, there is sex-based migration (Ibanez et al. 2009), with females moving farther north in the spring in North America (Cryan 2003, but see Kurta 2010) as well as in Europe and Australia

Table 15.1 Bats known to migrate or suspected of migration with longest documented one-way migration distance (km)

Family	Species if known	Distance	References
Molossidae	*Eumops perotis*		Medellin (2003)
	Eumops underwoodi		Medellin (2003)
	Mops condylurus		McGuire and Ratcliffe (2011)
	Nyctinimops macrotus	L	Medellin (2003)
	Otomops madagascariensis		CMS (2012)
	Otomops martiensseni		CMS (2012)
	Tadarida brasiliensis	1,800	Cockrum (1969)
	Tadarida insignis		Funakoshi and Yamamoto (2001)
	Tadarida latouchei		CMS (2012)
	Tadarida condylura		O'Shea and Vaughan (1980)
	Tadarida bemmelini		O'Shea and Vaughan (1980)
	Tadarida pumila		O'Shea and Vaughan (1980)
	Platymops setiger		O'Shea and Vaughan (1980)
Phyllostomatidae	*Anoura geoffroyi*		Medellin (2003)
	Carollia perspicillata	<200	Fleming (1988)
	Choeronycteris mexicana	L	Valiente-Banuet et al. (1996)
	Leptonycteris curasoae	>1,000	Cockrum (1991)
	Leptonycteris nivalis	>1,000	Moreno-Valdez et al. (2004)
	Leptonycteris sanborni		Cockrum (1991)
	Platalina genovensium		CMS (2012)
	Sturnira lilium		Mello et al. (2008)
Vespertilionidae	*Antrozous pallidus*	R	Medellin (2003)
	Barbastella barbastellus	290	Hutterer et al. (2005)
	Eptesicus fuscus		Medellin (2003)
	Eptesicus nilssonii	450	Hutterer et al. (2005)
	Eptesicus serotinus	330	Hutterer et al. (2005)
	Ia io		McGuire and Ratcliffe (2011)
	Lasionycteris noctivagans	U	Cryan (2003)
	Lasiurus borealis/blossevillii	U	Cryan (2003)
	Lasiurus cinereus	U	Cryan (2003)
	Lasiurus ega		Medellin (2003)
	Lasiurus intermedius		Medellin (2003)
	Lasiurus seminolus	U	Perry et al. (2010)
	Lasiurus xanthinus		Medellin (2003)
	Myotis auriculus		Medellin (2003)
	Myotis blythii	488	Hutterer et al. (2005)
	Myotis brandtii	618	Hutterer et al. (2005)
	Myotis californicus		Medellin (2003)
	Myotis capaccinii	140	Hutterer et al. (2005)
	Myotis chiloensis		Fleming and Eby (2003)
	Myotis ciliolabrum		Medellin (2003)
	Myotis dasycneme	350	Hutterer et al. (2005)
	Myotis daubentonii	304	Hutterer et al. (2005)
	Myotis evotis		Medellin (2003)

(continued)

Table 15.1 (continued)

Family	Species if known	Distance	References
	Myotis griescens	>500	Tuttle (1976)
	Myotis lucifugus	>500	Fleming and Eby (2003)
	Myotis myotis	436	Hutterer et al. (2005)
	Myotis mystacinus	240	Hutterer et al. (2005)
	Myotis nattereri	327	Hutterer et al. (2005)
	Myotis sodalis	500	Fleming and Eby (2003)
	Myotis thysanodes		Medellin (2003)
	Myotis tricolor		O'Shea and Vaughan (1980)
	Myotis velifer		Medellin (2003)
	Myotis volans		Medellin (2003)
	Myotis yumanensis		Medellin (2003)
	Nyctalus lasiopterus	U	Hutterer et al. (2005)
	Nyctalus leiseri	1,568	Hutterer et al. (2005)
	Nyctalus noctula	1,546	Hutterer et al. (2005)
	Perimyotis subflavus	R	Fleming and Eby (2003)
	Pipistrellus nathusii	1,905	Hutterer et al. (2005)
	Pipistrellus pygmaeus	U	Hutterer et al. (2005)
	Pipistrellus pipistrellus	1,123	Hutterer et al. (2005)
	Scotoecus hindei		O'Shea and Vaughan (1980)
	Scotophilus nigrita		O'Shea and Vaughan (1980)
	Vespertilio murinus	1,787	Hutterer et al. (2005)
Miniopteridae	*Miniopterus australis*		McGuire and Ratcliffe (2011)
	Miniopterus inflatus		McGuire and Ratcliffe (2011)
	Miniopterus natalensis		Miller-Butterworth et al. (2003)
	Miniopterus schreibersi	833	Hutterer et al. (2005)
Emballonuridae	*Emballonura monticola*		CMS (2012)
	Emballonura semicaudata		CMS (2012)
	Taphozous mauritianus		O'Shea and Vaughan (1980)
	Taphozous melanopogon	200	Gopalakrishna (1986)
Pteropodidae	*Eidolon helvum*	1,500	Thomas (1983)
	Epomorphorus wahlbergi		McGuire and Ratcliffe (2011)
	Myonycteris torquata	750	Thomas (1983)
	Nanonycteris veldkampii	750	Thomas (1983)
	Pteropus alecto	U	Breed et al. (2010)
	Pteropus poliocephalus	978	Ratcliffe (1932)
	Pteropus scapulatus		Ratcliffe (1932)
	Pteropus vampyrus		Epstein et al. (2009)
Hipposideridae	*Hipposideros commersoni*		O'Shea and Vaughan (1980)
	Hipposideros lankadiva	475	Gopalakrishna (1986)
	Triaenops persicus		O'Shea and Vaughan (1980)
Rhinolophidae	*Rhinolophus landeri*		O'Shea and Vaughan (1980)
	Rhinolophus hildebrandti		CMS (2012)
Megadermatidae	*Cardioderma cor*		O'Shea and Vaughan (1980)
Rhinopomatidae	*Rhinopoma microphyllum*	900	Gopalakrishna (1986)

L long-distance migrant, *R* regional migrant, *U* maximum distance unknown, blank indicates no distance information available

(Fleming and Eby 2003). Bats also migrate in groups, sometimes even mixed-species groups (Cryan and Veilleux 2007; Fleming and Eby 2003). Bat migration sometimes coincides with mating behavior (Cryan and Veilleux 2007). Migratory bats typically share similar morphologies like high wing aspect ratios and high wing loading (Norberg and Rayner 1987) facilitating high-speed, long-distance flight. However, behavioral and morphological characteristics likely facilitated the evolution of migration, rather than being a causal agent (Fleming and Eby 2003).

Bisson et al. (2009) suggest that migration evolved independently in several vespertilionid lineages. Many cave-roosting *Myotis* are regional migrants but none move long distances. In contrast, many species of *Lasiurus* are long-distance migrants, but none appear to be regional migrants. Thus, Bisson et al. (2009) conclude that long-distance migration is less likely to have evolved in cave- than in tree-roosting bats, in contrast to how migration evolved in birds, starting with short- to long-distance migrations. However, Berthold (1999) posits that the evolution of migration in birds is ancestral and likely driven by coexistence of both migratory and nonmigratory genes in the same population, expressed depending on a threshold variable. Thus, ecology, morphology, or perhaps physiology drives the evolution of migration, and this could explain the probable independent evolution in multiple lineages of bats.

Tracking bats during seasonal movements is especially difficult, and what we know comes from large-scale banding efforts conducted in the first half of the twentieth century (Cockrum 1969; Glass 1982; Griffin 1945; Hutterer et al. 2005; Steffens et al. 2007; Tuttle 1976) wherein more than a million bats in Europe and North America were captured at roosts and banded, with recovered bands showing clues to seasonal movements. Individual Brazilian free-tailed bats (*Tadarida brasiliensis*) banded in caves in southwestern USA show some individuals moved 1,800 km into Mexico, at rates of about 50 km/day (Cockrum 1969; Glass 1982). In Europe, banding started in the 1940s in seven countries and continues today in 35 countries. Data are available for 47 European species of bats and consolidation of records is underway, but even partial results show a striking pattern of movement for many long-distance migrants between northeastern and southwestern Europe (Hutterer et al. 2005). Banding of bats in North America and several European countries has decreased after massive banding-related mortality, often associated with inexperienced banders (Ellison 2008, but see Rodrigues and Palmeirim 2008). Although banding can be harmful to bats, tracking individual bats is crucial to understanding bat migration, and improvements in banding technology would greatly assist in this effort.

15.3 Applying Migration Theory to Bats

There is considerable theoretical and integrative research on migration (Berthold 1999; Dingle 2006), but almost all treatments ignore bats (e.g., Akesson and Hedenstrom 2007; Chapman et al. 2011; Dingle 2006; Hein et al. 2012, but see Hedenstrom 2009) because knowledge remains patchy and often information such as which species migrate, population sizes of those species, and their migratory routes remains unknown. Although population sizes of North American migratory tree bats are considered not measurable (Carter et al. 2003), we cannot clearly estimate the magnitude of any threat without population estimates. Applications of molecular data offer estimates of current and historical population sizes and should be explored (Russell et al. 2011). Also, current tracking technology is limited (Holland and Wikelski 2009). Thus, fundamental research must still be addressed before migration theory can be applied to bats.

Migration appears to be a plastic behavior. In birds, substantial genetic variation for migration propensity, distance, and direction exists even in partial or nonmigratory populations (Pulido 2007, 2011), and many bird species change their migration patterns or stop migration altogether (Sutherland 1998). For example, house finches (*Carpodacus mexicanus*) introduced to eastern North America from a nonmigratory population quickly adopted migration (Able and Belthoff 1998). Populations of blackcaps (*Sylvia atricapilla*) that previously migrated from Europe to the Mediterranean and Africa now overwinter in the British Isles (Pulido 2007). Partial migration, where migrant and nonmigrant conspecifics share a common site during one period of their annual cycle, is common (Chapman et al. 2011; Berthold 1999). Many bat species are partial migrants (Fleming and Eby 2003). In some species (North American tree bats), males and females appear to migrate differently (Cryan 2003), and in others (e.g., Brazilian free-tailed bats), only part of the population migrates (Laval 1973). Moussy et al. (2012) review the effect of migration and dispersal on genetic structure of populations.

Migratory birds have smaller brains than nonmigrants (Sol et al. 2005). McGuire and Ratcliffe (2011) show a similar pattern with bats and suggest that transporting smaller, less massive brains supports an energy trade-off hypothesis. Alternatively, larger brains in nonmigrants may reflect selection for behavioral flexibility and the need to find food in seasonally variable habitats and this might also explain why migratory birds are less successful invaders (Sol and Lefebvre 2000). Although brain size is smaller, the hippocampus, important to spatial memory in many birds and mammals (Moser 2011), is proportionately larger in migratory birds, but not in migratory bats (McGuire and Ratcliffe 2011). Avoiding seasonal variation in resources by hibernating may result in less selection for behavioral flexibility in nonmigratory bats.

Most birds fuel their migration primarily through fat stores (Gwinner 1990) as do many bats (McGuire et al. 2009), and the processes of acquiring and using fat stores, both for migration and hibernation, are a focus of current research. In many mammals increased fat increases leptin production, resulting in appetite inhibition and increased metabolic rate (Florant and Healy 2012). In little brown bats, body mass increased before migration and hibernation, but leptin levels were low and even dissociated with fat deposition (Kronfeld-Schor et al. 2000; Townsend et al. 2008) which might explain the ability of bats to continue adding fat. Decreased adiponectin has been linked to obesity in rodents; however, adiponectin levels decreased in tissues of fattening bats but not in circulation, indicating that seasonal weight gains in bats differ from pathological weight gain (Townsend et al. 2008).

That bats use stored fat for migration has been suggested (Fleming and Eby 2003; O'Shea 1976; Tuttle 1976), but it is difficult to distinguish between fat used for migration as opposed to hibernation (McGuire and Guglielmo 2009), and these two uses may be fundamentally different. Most mammals fuel high-intensity exercise primarily through protein and carbohydrates, and the ability to use stored fat for extended migration is not well documented (McGuire and Guglielmo 2009). When actively foraging, bats use energy from harvested insects rather than stored fat (Voigt et al. 2010), and there is growing evidence that bats forage while migrating (Reimer et al. 2010; Valdez and Cryan 2009). A study of fat storage in hoary bats (*Lasiurus cinereus*), not known to hibernate, found differences in body fat percentage in those captured during migration versus nonmigration periods and increased enzymes indicative of stored fat conversion during migration (McGuire et al. 2013). Further, females had larger fat stores and optimized intercellular fatty acid transport structures which may be related to spring migration during pregnancy (McGuire et al. 2013). Pregnant female hoary bats are less likely than males to use torpor (Cryan and Wolf 2003) and travel greater distances than males (Cryan 2003). McGuire et al. (2013) also reported lower body weights in migrating than nonmigrating hoary bats as well as reduced size of digestive organs, thereby reducing the weight carried during migration. Reduced digestive organs suggest that foraging during migration is opportunistic rather than required. The combination of increased fat reserves and the use of daily torpor distinguishes bat and bird migration strategies. Foraging by bats may act to "top off" fuel reserves, whereas birds "empty their tanks" and completely refuel during migratory stopovers (McGuire 2012). Another intriguing difference is an increase in lung capacity in bats during migration, not observed in birds (McGuire et al. 2013).

The time available for feeding is the main limiting factor in bird migration distance (Kvist and Lindstrom 2000), resulting in a metabolic ceiling for storing fat despite the ability of passerine birds to migrate at night and feed by day. Hedenstrom (2009) using a migratory model showed that because most bats neither feed nor migrate diurnally, time and fueling are more constrained in bats than birds. He proposed selection pressure between autumn's longer nights for feeding and transport and lower food abundance as winter approaches, resulting in an optimal migration period (Fig. 15.1a). This model assumes that bats do not forage during migration, which may be invalid. In addition, bats generally do not spend entire nights foraging (Shiel et al. 1999). For example, length of night was not related to foraging time for female hoary bats, even after parturition when energy needs were high (Barclay 1989). Finally, Hedenstrom (2009) did not consider the fluctuating availability of migratory insects, an important influence on an optimal migration date (Lee and McCracken 2005; Rydell et al. 2010). We present an alternate model (Fig. 15.1b) using fluxes in insect availability and corresponding changes in foraging times; some level of body condition (mass or lipid levels) would predict optimum departure time.

Birds and other animals use a variety of sensory cues in orientation and navigation (Akesson and Hedenstrom 2007) and often adhere to map and compass theory, i.e., determining position with respect to the goal (map) and determining goal

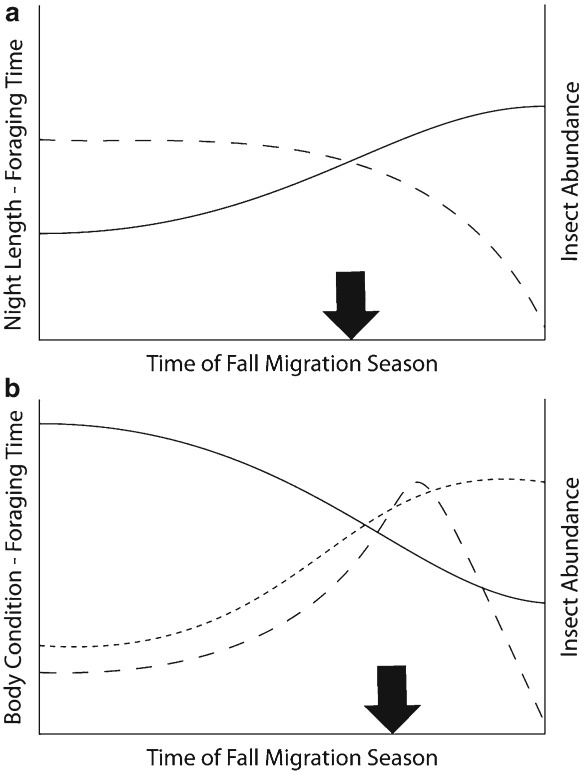

Fig. 15.1 (**a**), after Hedenstrom (2009), the *solid line* represents night length (available foraging time) as a surrogate for body condition. *Dashed line* denotes insect abundance; *arrow* indicates optimal time for maximum migration speed; (**b**) represents an alternative model where insect abundance is lower and rises with fluxes of migrating insects. Line designations as in (**a**). Departure time to permit optimum migration speed is a function of body condition is represented by the dotted line in (**b**), which tracks insect abundance

direction (compass). Map theory has been applied to adult avian migrants, reptiles, amphibians, and fish, and compass orientation is common in arthropods and juvenile birds (Holland 2007). Bats have good spatial memory (Holland et al. 2005) and Tsoar et al. (2011) showed that Egyptian fruit bats (*Rousettus aegyptiacus*) use multiple visual landmarks for navigation, but long-distance homing involves other mechanisms. Like many animals, bats can sense Earth's magnetic field (Holland et al. 2006) which can provide two types of directional information: inclination (direction toward or away from the equator) and polarity (north/south direction). Birds are thought to respond only to inclination (Beason 2005). The preference of the Chinese noctule (*Nyctalus plancyi*) to roost in the north end of their cage suggested a response to magnetic polarity (Wang et al. 2007). Bats calibrate the magnetic field using sunset (Holland et al. 2008) but unlike birds, do not appear to use polarized light for calibration (Holland et al. 2010). Bats that emerged long after sunset did not use a star compass calibrated by the geomagnetic field in place of sunset calibration (Holland et al. 2010) and thus responded differently than birds.

Many animals migrate using specific routes (Dingle 1996) and so apparently do bats. Lesser long-nosed bats follow a specific and relatively narrow path through a resource gradient (Fleming 2004; Morales-Garza et al. 2007), but most migration routes are probably broader and more diffuse. Tree bats in North America follow regular routes and are netted in specific locations at specific times, but these observations are anecdotal (Cryan and Veilleux 2007; Valdez and Cryan 2009). Tree bats appear to track closer to roost sites along mountain ranges (Baerwald and Barclay 2009) and avoid crossing open prairies. Other insectivorous bats follow linear landscape features to specific departure points before crossing open ocean (Ahlen et al. 2009), and it is not uncommon for migrating bats to follow riparian zones and shorelines (Barclay 1984; Serra-Cobo et al. 1998; Furmankiewicz and Kucharska 2009).

Many migratory birds use stopover sites (Berthold and Terrill 1991; Hedenstrom 2008), and there is evidence for this in bats (Cryan and Brown 2007; Dzal et al. 2009; Taylor et al. 2011), but if they remain for extended periods or are simply foraging along the way remains generally unknown. The definition of a migratory stopover is scale- and species-specific (Taylor et al. 2011). Cryan and Brown (2007) reviewed records of hoary bats "stopping over" on the Farallon islands during fall migration and found them present for 1–35 days during the fall, but could not quantify how long any individual stayed. Silver-haired bats *Lasionycteris noctivagans* stopped for several days during spring migration at a lake in Manitoba, Canada (Barclay et al. 1988). Dzal et al. (2009) found evidence that silver-haired bats and hoary bats use Long Point, Ontario, Canada, as a migration flyway, and that *L. noctivagans* stopped over during August. Taylor et al. (2011) and McGuire et al. (2011) radiotracked 30 *L. noctivagans* at Long Point in fall 2009 and observed seven "stopover flights" and 23 distinct migratory departures. However, most stayed only one night except when weather conditions forced delay, and most had sufficient fat reserves to complete migration without additional foraging (McGuire et al. 2011). *Myotis lucifugus* are the most common species at Long Point, and Dzal et al. (2009) found that the genetic diversity during fall migration was higher than at swarming areas, suggesting that bats assemble there from diverse areas before crossing the lake. In Texas, *Tadarida brasiliensis* show a fall, and to a lesser extent spring, spike in their use of urban roosts, indicating possible migration stopovers (Scales and Wilkins 2007). Populations of *T. brasiliensis* vary at Carlsbad Cave, New Mexico, including a large temporary increase in mid-October (Altenbach et al. 1979; Hristov et al. 2010). Cockrum (1969) anecdotally reports hundreds of thousands of these bats arriving overnight at caves during migration and then departing days later, consistent with our own observations in fall at Frio Cave, Texas. Many of these caves apparently function as maternity roosts as well as migratory stopovers.

Weather influences migration for most flying animals (Shamoun-Baranes et al. 2010) including birds (Able 1973; Liechti 2006; Nisbet and Drury 1968). Most nocturnal migrant birds fly in tailwinds or light winds rather than strong or headwinds (Richardson 1990). Migratory bats are also more active in light winds (Arnett et al. 2008; Horn et al. 2008). Hoary bats were more likely to land on an island stopover during low wind periods, low moon illumination, higher cloud cover, and to a lesser extent low barometric pressure (Cryan and Brown 2007). However, a separate study documented this species flying often in unfavorable winds (Baerwald and Barclay 2011). Silver-haired bats were less likely to leave a stopover and continue migration during rain (McGuire et al. 2011). The timing of bird migration is related to cold fronts, especially during strong cold fronts in North America with falling pressure and temperature (Able 1973; Richardson 1990). Bats also migrate with cold fronts (Cryan and Brown 2007). Birds with high wing loading and aspect ratio are less affected by weather variables during spring migration (Saino et al. 2010) as presumably should bats having similar wing form. Migratory insects normally fly at much slower speeds than birds and bats, but during migration insects achieve similar speeds by moving with favorable wind conditions (Alerstam et al. 2011). There is evidence that bats take advantage of concurrent migration patterns to forage on migrating insects (Lee and McCracken 2005) and even birds (Ibanez et al. 2001; Popa-Lisseanu et al. 2007).

15.4 Four Examples of Bat Migration

Baker (1978) distinguished between facultative and obligate migrants. Facultative migrants are sensitive to local cues like resource availability and may not migrate without them. Obligate migrants are less sensitive to cues and most individuals migrate even if resources remain locally available. The hoary bat is an example of a tree-roosting long-distance migrant, with a primarily north–south migration route, is not known to hibernate, and is a likely obligate migrants. The second example, Schreiber's bent-winged bats, is another probable obligate migrant and an example of regional migrants that move seasonally between maternity and hibernacula caves. These widespread old-world bats do not appear negatively affected by banding (Rodrigues and Palmeirim 2008), and we summarize information connecting behavior, population structure, and movement. Third, Brazilian free-tailed bats are facultative migrants with North American populations that vary widely in migratory behavior, distance, sexual bias, and direction. Finally, we include here a long-distance tropical migrator, straw-colored fruit bat and compare its migration to long-distance movements of other tropical fruit bats.

15.4.1 Long-Distance Migrant Tree Bats: Hoary Bats

A sense of urgency for research on long-distance migration by tree-roosting bats is motivated due to the large numbers of these bats killed by wind turbines (see Chap. 20).

In the spring, female hoary bats move through New Mexico about a month earlier than males, and they apparently travel in groups, fly below the canopy along streams, and forage during migration (Valdez and Cryan 2009). Females are usually pregnant during spring migration and less likely to use torpor (Cryan and Wolf 2003). Both in spring (Valdez and Cryan 2009) and fall (Reimer et al. 2010), the diet of hoary bats consists primarily of moths. Based on carcasses found at wind energy facilities, male hoary bats passed through Alberta, Canada, in late July, followed by females and young in early mid-August (Baerwald and Barclay 2011), and in the fall, hoary bats were recorded more often by acoustic detectors set at 30 m, than at or 67 m above ground level (Baerwald and Barclay 2011). Hoary bats may fly at higher altitudes in fall than in spring (Johnson et al. 2011; Valdez and Cryan 2009), although it is possible that bats are flying too high for detection in spring. At the Canadian wind facility, hoary bat activity was best predicted by falling barometric pressure, and this was reflected in fatality rates (Baerwald and Barclay 2011).

A comparison of acoustic detection sites on a north/south gradient across the Eastern USA reveals a pattern of hoary and silver-haired bats moving north in the spring and south in the fall (Johnson et al. 2011), although this could reflect foraging activity variations due to insect population fluxes. In Hawaii, hoary bats apparently perform a seasonal altitudinal migration, with both sexes moving to lowlands during breeding season and then returning to highlands for the remainder of the year (Menard 2001). Seasonal altitudinal migration is also suggested for hoary bats in the Galapagos Islands (McCracken et al. 1997).

15.4.2 Regional Migrant Cave Bats: Schreiber's Bats

Many cave-roosting bats tend to move shorter distances, 500 km or less, and with less of a standard compass orientation than tree bats and in the spring, many species radiate from common hibernacula in a star-shaped pattern (Hutterer et al. 2005).

The cave-roosting regional migrant Schreiber's bent-winged bat (*Miniopterus schreibersii*) is one of the most widespread species, in Europe, Africa, and Australia. Genetic structure occurs between subpopulations in southeastern Europe (Bilgin et al. 2008) and Australia (Cardinal and Christidis 1999) and existence of the closely related *Miniopterus natalensis* in South Africa (Miller-Butterworth et al. 2005).

The combination of strong philopatry and extensive banding has resulted in details of seasonal movements. In Portugal (1987–2005), 36,000 bats were banded and tracked (Ramos Pereira et al. 2009; Rodrigues and Palmeirim 2008, Rodrigues et al. 2010) providing evidence that females stage at spring roosts until just before parturition when they move to maternity roosts in caves. Following weaning, they move to other caves where they spend autumn and sometimes winter. Males leave hibernacula later and change roosts during maternity season, and roost temperature was more likely to influence migration destination than insect availability, as inferred from temperatures at foraging areas (Rodrigues and Palmeirim 2008).

While many migratory bat species show little evidence of population structure (Petit and Mayer 2000; Russell et al. 2005), Schreiber's bat is an interesting exception and extensive banding data enable us to compare population structural and observed behavior to predictive models (Ruedi and McCracken 2009). For example, strong patterns of structure in mitochondrial DNA (mtDNA) are considered to indicate strong female philopatry to breeding sites, and more diffuse patterns in males indicate sex-biased movement by males during breeding seasons. In this scenario, nuclear DNA (nDNA) patterns would not show structure. In the Portuguese Schreiber's bat colonies, while both males and females visit different maternity caves, all females raise pups only in the cave in which they were born (Rodrigues et al. 2010). Mating occurs at hibernacula shared among the colony's maternity roosts (Rodrigues and Palmeirim 2008). As a result of this strict philopatry to maternity caves, all gene flow is male-induced during regional migrations (Rodrigues et al. 2010). The strong patterns in mtDNA also appear, at a weaker level, in nDNA, reflecting the strong regional philopatry observed in both males and females (Ramos Pereira et al. 2009).

In a study of the closely related South African Schreiber's long-fingered bat (*M. natalensis*), Miller-Butterworth et al. (2003) found similar population structure even though migration varied between subpopulations. However, neither migration distance nor zoogeographic barriers prevented gene flow between colonies, so structure was due to philopatry or other differences. In one subpopulation, morphological differences mirrored the genetic distinctions, with the northern colony (intermediate migrants) showing higher wing aspect ratios than other colonies (shorter-distance migrants) (Miller-Butterworth et al. 2003).

15.4.3 Long-Distance, Facultative, and Partial Migrants

Migratory patterns of Brazilian free-tailed bats *(Tadarida brasiliensis)* are more difficult to define than for the tree and cave bats described above. In contrast to the highly structured populations of *M. schreibersii*, populations of Brazilian free-tailed bats are panmictic, showing no genetic structure (Russell et al. 2005), but bats in different geographic areas demonstrate different migratory behaviors. In the southeastern USA, they appear to be sedentary and to use torpor in winter (Cockrum 1969; Laval 1973), whereas on the west coast, bats probably migrate but not necessarily long distances or in a north–south direction (Krutzsch 1955). In mid-continent a large segment of the population are long-distance migrants, traveling up to 1,900 km between Mexico and the USA (Cockrum 1969; Glass 1982); however, many males may remain in Mexico year-round (Davis et al. 1962; Glass 1982), while other males move north where they mate in the spring in Texas (Reichard et al. 2009). Reports of large maternity colonies in Mexico (Lopez-Gonzalez and Best 2006) and of bats overwintering in the USA (Geluso 2008; Scales and Wilkins 2007) further confuse the picture.

Bats overwintering at Carlsbad Cave, New Mexico, included individuals of both sexes and varying ages, and evidence indicates active feeding in winter except during high winds (Geluso 2008). Banding of large numbers in the 1950s and 1960s (Cockrum 1969; Glass 1982; Villa and Cockrum 1962) showed migration between caves in the southwestern United States and Mexico. Bats leaving nursery caves in Oklahoma range into Texas and Mexico during the fall, traveling as far as 1,840 km, and do not return until the spring (Glass 1982). The maximum documented migration rate was 32 km/day (Villa and Cockrum 1962). Cold weather appears an important factor spurring fall bat movement (Constantine 1967; Svoboda and Choate 1987). Brazilian free-tailed bats change roosts frequently before and after the maternity season with population sizes fluctuating greatly in caves in Kansas during spring (Twente 1956), and Davis Cave in South Central Texas is a staging area in spring for bats going north to Oklahoma caves (Short et al. 1960). In the fall, populations shift from caves to nearby bridges (Horn and Kunz 2008).

The considerable variation in migration strategies found in Brazilian free-tailed bats may be analogous to Blackcaps (*Sylvia atricapilla*) found across Europe and Africa which show wide variations in migratory strategies with very little population structure (Perez-Tris et al. 2004).

15.4.4 Migration in Old-World Fruit Bats

Even less is known about migration of old-world fruit bats than for microchiropterans, and there are undoubtedly many migratory pteropodids as yet unstudied (Fleming and Eby 2003). Most documented migration by fruit bats is regionally restricted, nondirectional, and tracks ephemeral food resources (Fleming and Eby 2003), except for the straw-colored fruit bat (*Eidolon helvum*). At least one population of these large bats leaves their African savanna habitat during the dry season and moves 2,518 km (Richter and Cumming 2008) to take advantage of large fruiting events (Richter and Cumming 2006). Between five and ten million *E. helvum* spend October through December at Kasanka National Park, Zambia, arriving as fruit ripens and leaving when fruiting slows (Richter and Cumming 2006). It is unclear whether this foraging pressure is sustainable, as areas are becoming degraded, resulting in higher tree mortality and fire risk (Byng et al. 2010). Richter and Cumming (2008) tracked four male *E. helvum* with satellite transmitters from Kasanka back to the Democratic Republic of Congo and showed that bats moved an average of 90 km/day and traveled 2,518 km in 149 days. Thomas (1983) suggests this movement is to avoid competition for fruit resources.

Other examples of wide-ranging movements of pteropodids are less obviously migratory. A number of *Pteropus* species have been studied as reservoirs for viral pathogens; at least three *Pteropus* species are known to harbor Hendra or Nipah viruses. Radiotracked *P. vampyrus* traveled across political borders in Southeast Asia (Epstein et al. 2009), including Thailand, Sumatra, Malaysia, and Indonesia, flying up to 363.4 km. One bat covered 130 km in 2 h while foraging (Epstein et al. 2009), and in another case, a *P. alecto* traveled over 3,000 km (Breed et al. 2010).

15.5 Future Directions

Our knowledge of migration in bats has been limited (Griffin 1970), but as new technologies are applied, knowledge will increase (Bridge et al. 2011; Cryan and Diehl 2009; Holland and Wikelski 2009).

Satellite tracking offers long-distance monitoring of individuals (Wikelski et al. 2007), but current transmitters are too large for most bats (Aldridge and Brigham 1988). However, exciting results are coming in from tracking large bats (Richter and Cumming 2008; Tidemann and Nelson 2004). Smith et al. (2011) found that optimal tracking design for *Pteropus* was species- and ecology-specific with solar powered transmitters only working for bats that roost higher in canopy, allowing for recharging during the day.

Stable isotopes can infer movements of individual bats over long distances because stable hydrogen isotopes (δD) are absorbed into tissues and fur from water. By matching isotope values from tissue samples with known values from water sources across a landscape, one can determine at what location those tissues formed. Stable hydrogen isotope (δD) values from the hair of bats (captures or museum collections) identified movement patterns of North American tree bats (Britzke et al. 2009; Cryan et al. 2004). Fraser et al. (2012) showed that contrary to previous assumptions, male *Perimyotis subflavus* migrated in north–south directions like hoary or silver-haired bats, rather than as regional migrants. Despite the usefulness of stable isotopes, there are important limitations; quantifying underlying hydrogen isotope gradients in water supplies is needed to provide more meaningful spatial resolution. In addition, understanding molt patterns is critical because species vary in annual molt cycles thereby affecting δD values (Fraser et al. 2010), although using δD values from hair from sedentary bat species avoids the molt-timing problem (Popa-Lisseanu et al. 2012). In that study, when combined with δ^{13}C and δ^{15}N values, δD values predicted locations of known bat samples significantly more accurately.

Advances in radar technologies allow tracking the movements of individuals and groups of individuals and possibly to distinguish among taxa (Ahlen et al. 2009; Chilson et al. 2011; Gauthreaux et al. 2008; Horn and Kunz 2008, but see Kunz et al. 2007). Bruderer and Popa-Lisseanu (2005) compared video and radar data to differentiate small, medium, and large bats from migrating birds, although distinguishing among similar-sized bats (e.g., *Nyctalus noctula* and *Eptesicus serotinus*) required analysis of additional behavioral or ecological features. Their data also showed that during migration, flight speeds for *N. noctula* and *E. serotinus* were higher than expected. Study-specific radar installations are not necessarily required to apply this technology to broader questions of bat migration. The newly developed National Mosaic and Multi-Sensor Quantitative Precipitation Estimation system (NMQ) Web portal offers public access to NEXRAD historical data that will enable tracking of migrants across North America (Chilson et al. 2011), but much work needs to be done to standardize the data sets for biological use.

We believe the following will be at the forefront of future migration research on bats: (1) placing bats in the theoretical context of migration; (2) understanding the physiology of energy storage and use during migration; (3) exploring links between seasonal and spatial changes in food availability, the timing of migration events, and the use of stopover locations in bats; (4) documenting long-distance movements of individual bats across international boundaries and establishing connectivity of their populations through use of satellite technology; and (5) learning more about individual and group movements and assessing population sizes and trajectories using information from radar networks. The continued growth of wind power and its impact on bat populations as well as effects of climate change on the movements of bats, their prey, and the pathogens that they vector all suggest that migration in bats will be an increasingly important focus of research.

Acknowledgments The authors thank Paul Cryan, Erin Baerwald, and Liam McGuire for discussions and invaluable feedback that greatly improved the quality of this chapter.

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Chapter 16
Speciation Dynamics of the Fruit-Eating Bats (Genus *Artibeus*): With Evidence of Ecological Divergence in Central American Populations

Peter A. Larsen, María R. Marchán-Rivadeneira, and Robert J. Baker

Abstract An increasing number of studies have identified complex diversification patterns of Neotropical faunal groups. One example of such complexity is found in bats of the widely distributed and locally abundant Neotropical genus *Artibeus*, wherein both allopatric and hybrid speciation events have been hypothesized. However, conflicting hypotheses regarding the timescale of diversification for *Artibeus* exist, and therefore, temporal inferences of the speciation events within the genus remain in doubt. We examine hypotheses regarding the chronology of diversification events within *Artibeus*. Our results indicate the most parsimonious time of origin for the genus was during the late Miocene to early Pliocene, with multiple speciation events during the early Pleistocene. Considering this evolutionary timescale, we revisit a century-old systematic debate regarding the status of Central American populations known as *Artibeus lituratus intermedius*. We present nuclear genetic data that indicate *intermedius* is distinct from *lituratus* and hypothesize that this distinction was ecologically driven, likely involved sympatry and reinforcement, and occurred during the late Pleistocene or early Holocene. Collectively, the data from *Artibeus* indicate that multiple speciation processes

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_16,

underlie extant levels of diversity within the genus. Our analyses provide further evidence for complex origins of the Neotropical fauna and contribute to a greater understanding of the natural processes underlying the origin of species.

16.1 Introduction

A growing number of studies are utilizing genome-scanning techniques such as amplified fragment length polymorphisms (AFLPs) or restriction-site-associated DNA sequencing (RADseq) to examine genome-wide signatures of natural selection (Davey and Blaxter 2011; Rice et al. 2011). Such studies have the potential to identify regions of the genome experiencing divergent selection and can elucidate speciation processes in natural settings. However, exploring the historical processes underlying the origin of species within non-model organisms requires data from a number of sources (Coyne and Orr 2004). For example, it is important to have a well-supported phylogeny of all extant taxa and an understanding of the timing of lineage formation within the group. It is within this framework that we explore the evolutionary history of one of the most successful radiations of the New World leaf-nosed bats (family Phyllostomidae), fruit-eating bats of the genus *Artibeus*.

Artibeus includes approximately 12 species that, collectively, are distributed throughout the Neotropics (Larsen et al. 2010a). Several species are endemic to subtropical and tropical forests in Central America (e.g., *Artibeus inopinatus* and *Artibeus hirsutus*) and South America (e.g., *Artibeus amplus*, *Artibeus fimbriatus*, *Artibeus fraterculus*) (Appendix A). Such biogeographic patterns of endemism can be used to evaluate hypotheses regarding the evolutionary history of the genus (see Rheindt et al. 2009), therefore making *Artibeus* an ideal group in which to study speciation. Although numerous systematic studies of *Artibeus* have been conducted, few have discussed the timing of diversification events within the genus (Larsen et al. 2010b; Redondo et al. 2008), and none have formally examined alternative hypotheses for the time of origin of *Artibeus* (Baker et al. 2012; Redondo et al. 2008). Redondo et al. (2008), using molecular calibrations from Teeling et al. (2005), hypothesized a time of origin for *Artibeus* at approximately 13.2 million years ago (mya). Alternatively, Baker et al. (2012), using molecular calibrations from Jones et al. (2005), hypothesized a much more recent time of origin for the genus (~6.6 mya). A closer examination of the hypotheses regarding the time of origin of *Artibeus* is warranted because complex patterns of diversification are hypothesized to have contributed to present species diversity within the genus (Larsen et al. 2010a, b).

Here, we evaluate alternative hypotheses regarding the time of origin for *Artibeus* using molecular clock analyses of mitochondrial datasets from previous studies (Hoofer et al. 2008; Larsen et al. 2007; Redondo et al. 2008). To identify the most parsimonious reconstruction of the evolutionary history of the genus, we (i) compared the resulting chronograms to time estimates of major paleogeological events in the Neotropics [e.g., closure of the Panamanian Isthmus (Collins et al. 1996;

Keigwin 1982; Kirby et al. 2008) and extensive uplifting of the Andes Mountains (Gregory-Wodzicki 2000)] and (ii) compared relative DNA mutation rates across each dataset with those published from Chiroptera (Bininda-Emonds 2007). In light of the results of these analyses, we explore the timescale of diversification within *Artibeus* and discuss the evolutionary history of the genus. Furthermore, an understanding of the tempo of diversification events within *Artibeus* allows us to revisit one of the most contentious systematic debates found within the relevant literature on the genus (Davis 1984; Guerrero et al. 2008; Lim et al. 2004; Marchán-Rivadeneira et al. 2012; Redondo et al. 2008; Simmons 2005). This debate centers on the status of Central American populations known as *Artibeus lituratus intermedius* and whether or not *intermedius* warrants taxonomic recognition (see Simmons 2005). We examine the population structure and phylogeographic history of the *A. lituratus* complex (including *intermedius*) by performing phylogenetic, spatial, and demographic analyses on both nuclear AFLP and mitochondrial DNA data. Our results provide further evidence for the complex origins of the Neotropical fauna and contribute to a greater understanding of the natural processes underlying the origin of species.

16.2 Materials and Methods

16.2.1 Divergence Time Estimates

Analyses of the timescale of diversification of *Artibeus* consisted of relaxed and strict molecular clock methods using two mitochondrial DNA sequence datasets. One dataset consisted of 16S ribosomal and cytochrome-*b* gene DNA sequence data from ten species of *Artibeus* (2,804 bp; Hoofer et al. 2008), and the second consisted of cytochrome-*b* sequence data from all known extant species of *Artibeus* (12 species, 1,140 bp; Hoofer et al. 2008; Larsen et al. 2007; Larsen et al. 2010b; Redondo et al. 2008). GenBank accession numbers for all specimens examined are listed in Appendix B. *Enchisthenes hartii, Ectophylla alba*, and *Dermanura* (*watsoni*, *rosenbergii*, *tolteca*, and *phaeotis*) were used as out-groups in all analyses (Baker et al. 2003; Van Den Bussche et al. 1998). We performed a Likelihood Ratio Test (LRT; Felsenstein 1981) to test the hypothesis of a strict global clock for both datasets. All molecular clock analyses were performed using BEAST version 1.5 software (Drummond and Rambaut 2007). The GTR+Γ+I model of nucleotide substitution best fits both the 16S and cytochrome-*b* datasets (Modeltest version 3.7; Posada and Crandall 1998) and was used in all BEAST analyses.

We used the multi-locus dataset to test alternative hypotheses regarding the origin of *Artibeus* as proposed by Redondo et al. (2008) and Baker et al. (2012). Redondo et al. (2008) estimated the split between *Artibeus* and *Dermanura* to have occurred approximately 13.2 mya (17.9–8.8), with *Artibeus* diversifying 11.7 mya (16.5–7.5) and *Dermanura* 12.2 mya (16.9–7.9). Alternatively, Baker et al. (2012)

hypothesized the time to the most recent common ancestor (TMRCA) for Mesostenodermatini (sensu Baker et al. 2003) at approximately 11.51 ± 2 mya and the TMRCA between *Artibeus* and *Dermanura* ~6.62 ± 1 mya. Dates proposed by both studies were used as secondary calibration points with normal distribution priors (Ho 2007) in separate BEAST analyses. Parameters for estimating node dates followed previously published methods (Larsen et al. 2010b). The cytochrome-*b* only dataset for all species of *Artibeus* was then analyzed using the priors from the most parsimonious reconstruction, following the methods outlined above.

16.2.2 Amplified Fragment Length Polymorphisms

Our methodologies for generating and phylogenetically analyzing AFLPs from specimens of *Artibeus* followed those previously published (Larsen et al. 2010a, b). AFLPs from 47 individuals of seven species of *Artibeus* (*A. aequatorialis* [$n=7$], *A. fraterculus* [$n=6$], *A. inopinatus* [$n=2$], *A. jamaicensis* [$n=7$], *A. lituratus* [$n=15$], *A. obscurus* [$n=2$], *A. planirostris* [$n=8$]; Appendix B) were included for analyses of intrageneric variation. AFLPs were generated from nine individuals of *A. lituratus intermedius* (Appendix B). Genotyping and scoring of AFLPs followed previously published methods (Larsen et al. 2010a, b).

Scoring and subsequent analyses were performed on two separate AFLP datasets. Dataset 1 consisted of seven species of *Artibeus* and nine individuals of *A. lituratus intermedius* ($n=56$) and Dataset 2 consisted of *A. aequatorialis* (outgroup), *A. lituratus*, and *A. lituratus intermedius* ($n=31$). To examine intrageneric variation, Dataset 1 was analyzed using MrBayes, version 3.1 (Huelsenbeck and Ronquist 2001), PAUP* version 4.0b10 (Swofford 2002), and GenAlEx version 6.3 software packages. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed to examine the significance of genetic variation among and within species. Genetic distances were generated in GenAlEx using the Nei–Li model. Statistical significance of structure within Dataset 1 was measured by bootstrapping (500 iterations) and by Bayesian posterior probabilities. Bootstrap values greater than 0.75 and Bayesian posterior probabilities greater than 0.95 were considered significant. A principal coordinates analysis (PCoA) was used to identify structure among individuals in Dataset 2.

16.2.3 Detection of Outlier AFLP Loci

We approached the debate over the specific/subspecific status of *intermedius* by screening 386 AFLPs from Honduran populations of *intermedius* and *lituratus* (*palmarum*) for potential outlier loci. Identification of the presence/absence of outlier AFLP loci was performed following the methods of Beaumont and Nichols (1996) and Beaumont and Balding (2004) using the software package DFDIST (Beaumont and Nichols 1996). Outlier loci were identified as those having F_{ST} values significantly higher than neutral loci. Empirical F_{ST} values were calculated for each AFLP locus, and an empirical distribution was created by discarding 30 % of the highest and lowest F_{ST} values. This distribution was the trimmed mean F_{ST} and was used to provide an estimate of neutral loci (i.e., unaffected by outlier loci). A null F_{ST} distribution, with a similar trimmed mean, was then generated based on 50,000 simulated loci, and upper and lower confidence intervals were identified using this null distribution. Loci located outside of the 95th quantile of the null F_{ST} distribution were identified as outlier loci. Significance levels were set at 95 % for all analyses. Using these methodologies, we performed two comparisons (1) Central American *lituratus* vs. South American *lituratus* and (2) Central American *intermedius* vs. Central American *lituratus*.

16.2.4 Population Structure of **A. lituratus**

We examined the population structure and demographic history of *A. lituratus* by analyzing genetic variation within a sample of 119 complete cytochrome-*b* gene sequences (distinct haplotypes) gathered from GenBank which were generated from individuals collected throughout the Neotropical distribution of the species (Appendix B). Cytochrome-*b* gene sequences from an additional ten specimens were generated following the methods of Larsen et al. (2007) (Appendix B; GenBank accession numbers HQ702535–HQ702544).

Estimates of nucleotide (π) diversity and tests of neutrality [Tajima's D (Tajima 1989) and Fu and Li's D^* and F^* (Fu and Li 1993)] were performed using DNASP version 5.0 software (Librado and Rozas 2009). The demographic history of *A. lituratus* was further investigated by conducting a mismatch distribution using Arlequin version 3.5 software and a Bayesian skyline plot (BSP) using BEAST version 1.5 software (Drummond and Rambaut 2007) following the methods of Lack et al. (2010). For the mismatch distribution, empirical results were compared with a sudden expansion model (Rogers and Harpending 1992), which predicts a unimodal mismatch distribution. We used the BSP to estimate effective population size through time, thus providing a graphical representation of the demographic history of *A. lituratus*. A mean rate of 0.020 substitutions per site per million years (estimated from cytochrome-*b* analyses presented below) was used, and our analysis consisted of two runs of 30 million generations. The log and tree files from these runs were combined and were examined for stability, sufficient mixing, etc. (Drummond et al. 2002). All parameters were checked using TRACER v1.4 software http://beast.bio.ed.ac.uk/Tracer.

In order to evaluate the correlation between genetic and geographic distances within *A. lituratus*, we truncated the dataset to 39 individuals (see Appendix B) collected from distinct localities and performed a Mantel test using Alleles in Space (AIS) software version 1.0 (Miller 2005). Latitudinal and longitudinal positioning of individuals lacking precise collecting coordinates from voucher information was determined using Google Earth version 5.2 (http://earth.google.com). Potential barriers to gene flow were examined by implementing Monmonier's algorithm (Monmonier 1973) in AIS software.

16.3 Results

16.3.1 Timescale of Diversification

The LRT significantly rejected a global molecular clock for the 16S dataset ($\Delta L = 33.03$; $P < 0.01$); however, it did not for the cytochrome-*b* only dataset ($\Delta L = 16.01$; $P = 0.52$). Therefore, we employed a relaxed uncorrelated lognormal molecular clock with unlinked substitution models for the multi-locus dataset and a strict clock for the cytochrome-*b* only dataset. Using priors from Redondo et al. (2008), the mean rate of evolution for the multi-locus dataset was 0.0069 substitutions per site per million years (95 % highest posterior density [HPD]: 0.0048, 0.0092), and the Yule birth rate was 0.099 (95 % HPD: 0.047, 0.159). TMRCA for extant species of *Artibeus* was approximately 11.0 mya (95 % HPD: 7.8, 14.3) with the majority of speciation events occurring during the late Miocene to mid-Pliocene (Fig. 16.1a). Alternatively, using the priors from Baker et al. (2012), the mean rate of evolution was 0.0154 subs/site/million years (95 % HPD: 0.0125, 0.0186) and

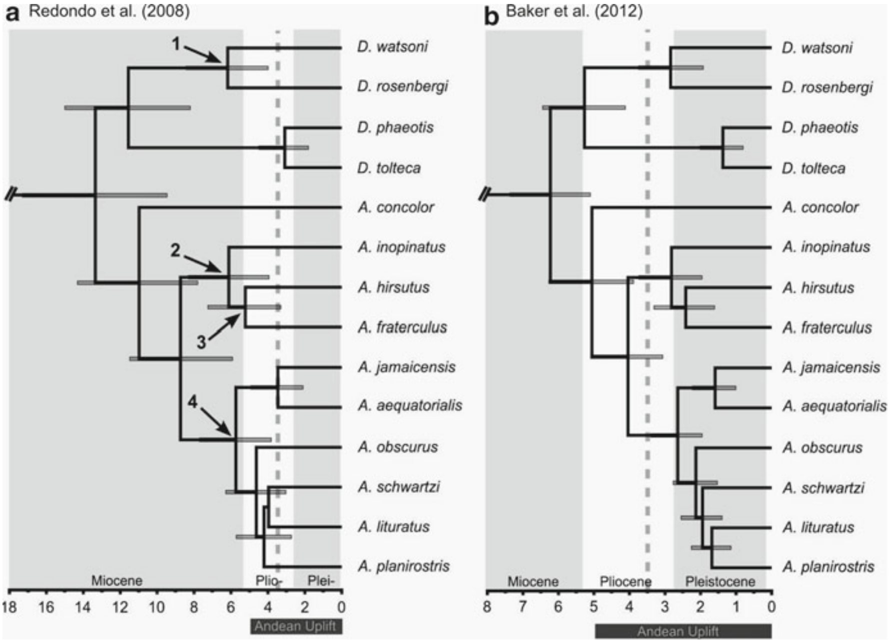


Fig. 16.1 Alternative hypotheses for the timescale of diversification of *Artibeus* based on 16S and cytochrome-*b* data from Hoofer et al. (2008). Dashed line identifies closure of the Panamanian Isthmus (~3.5 mya). Dark shaded bar identifies periods of extensive uplifting of the Andes Mountains (5 mya to present). **a**: Chronogram using the priors of Redondo et al. (2008). *Numbered arrows* identify inconsistencies with respect to major paleogeographic events (see Sect. 16.4). **b**: Chronogram using the priors of Baker et al. (2012)

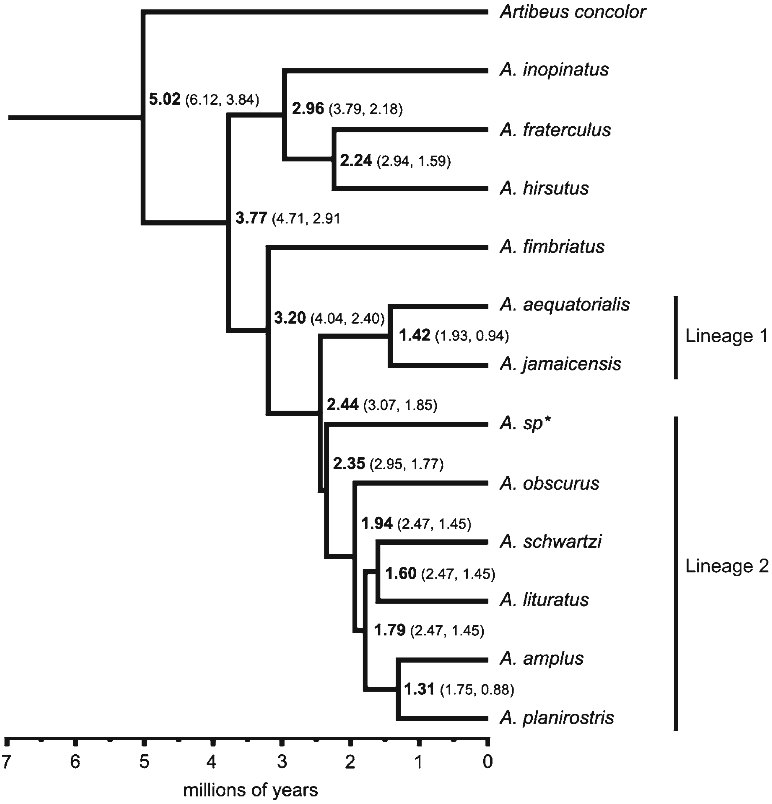


Fig. 16.2 Most parsimonious chronogram of all known extant species of *Artibeus* based on cytochrome-*b* DNA sequences. Geological time estimates for each node are shown in bold followed by upper and lower limits (95 % HPD) in parentheses. Lineage 1 identifies species that diverged west of the Andes Mountains and in Central America. Lineage 2 indentifies species that diversified east of the Andes Mountains. Although contemporary populations of *A. schwartzi* are the result of reticulate evolution (see Larsen et al. 2010a), the mitochondrial genome typical of *A. schwartzi* likely evolved in a now extinct species that originated east of the Andes Mountains (Lineage 2). *Asterisk* identifies unrecognized species-level variation in South American populations referable to *A. obscurus* (Redondo et al. 2008)

the Yule birth rate was 0.216 (95 % HPD: 0.110, 0.328). TMRCA for extant species of *Artibeus* was approximately 5.1 mya (95 % HPD: 3.8, 6.3) with the majority of speciation events occurring during the late Pliocene to mid-Pleistocene (Fig. 16.1b). Based on the results of the LRT, a strict molecular clock was employed to infer divergence times (using the priors of Baker et al. 2012) for all known extant species of *Artibeus* with the cytochrome-*b* only dataset. The mean rate of evolution (estimated from the data) was 0.020 substitutions per site per million years (95 % HPD: 0.015, 0.025), and the Yule birth rate was 0.232 (95 % HPD: 0.127, 0.345). The most parsimonious chronogram is based on congruence with geological events and molecular rates of evolution (Fig. 16.2).

16.3.2 AFLP Variation

Six primer pairs (*ECO* RI + three bases; *ASE* I + three bases) used for selective amplification produced 414 scorable bands (Dataset 1; see Sect. 16.2.2). Of the 414 scored bands, 70 were monomorphically present in all specimens examined and 344 were polymorphic (with 243 being parsimony informative). Average interspecific Nei–Li genetic distances among species were similar to those previously reported (Larsen et al. 2010a), and the value across all species examined was 3.4 %, ranging from 4.5 % (*A. inopinatus* vs. *A. fraterculus*) to 2.7 % (*A. aequatorialis* vs. *A. planirostris*). The genetic distance value between *A. l. intermedius* and *A. lituratus* was 3.0 %. All phylogenetic analyses revealed eight statistically supported clades, corresponding to the seven species used in our analyses, plus an additional clade corresponding to populations of *A. lituratus intermedius* in Central America (Fig. 16.3). Additionally, the AMOVA indicated significant genetic structure (PhiPT = 0.40; $P < 0.001$) within the eight clades, with 40 % of the total genetic diversity residing among species (including *intermedius*) and 60 % within species. AFLP Dataset 2 (see Sect. 16.2.2) consisted of 386 scored bands. Of these, 36 were diagnostic (either fixed or present or absent) of *A. aequatorialis*, 26 of *A. lituratus intermedius*, and 73 of *A. lituratus* (excluding *intermedius*). The PCoA resulted in three distinct clusters corresponding to the three taxa examined (Fig. 16.4). Forty-five percent of the total variation was explained by PC 1, 24 % by PC2, and 9 % by PC3.

Nine loci were identified outside of the 0.95 quantile for the analyses between Honduran populations of *A. lituratus intermedius* and *A. lituratus palmarum* (Fig. 16.5). Of these, five loci and two loci were outside of the 0.97 and 0.99 quantiles, respectively. Alternatively, zero outlying loci were identified between Central and South American populations of *A. lituratus* (i.e., excluding *intermedius*; Fig. 16.5a).

16.3.3 Demographic History of A. lituratus

Summary statistics observed within our sample of cytochrome-*b* sequences (1,140 bp) from *A. lituratus* ($n = 129$) were number of variable sites (S) = 205; nucleotide diversity (π) = 0.01; Tajima's D (D) = −1.87*; Fu and Li's D (D') = −4.44**; and Fu and Li's F (F') = −3.94** (* = $P < 0.05$; ** = $P < 0.095$). The mismatch distribution resulted in a relatively unimodal distribution that is indicative of a historical range expansion ($\pi = 17.09$, $\theta_0 = 1.58$, $\theta_1 = 58.98$; Fig. 16.6). Similarly, the BSP showed a recent expansion by *A. lituratus* corresponding to approximately 75 kya (Fig. 16.7). TMRCA for the 129 haplotypes was estimated at ~850 kya (HPD 554 kya–1.15 mya). Results of the Mantel test indicated a lack of correlation between genetic and geographic distances ($r = 0.07$; $P = 0.08$). A single potential barrier to gene flow located along northern South America and the southern Lesser Antilles, corresponding with the Trinidad and St. Vincent specimens, was identified by Monmonier's algorithm.

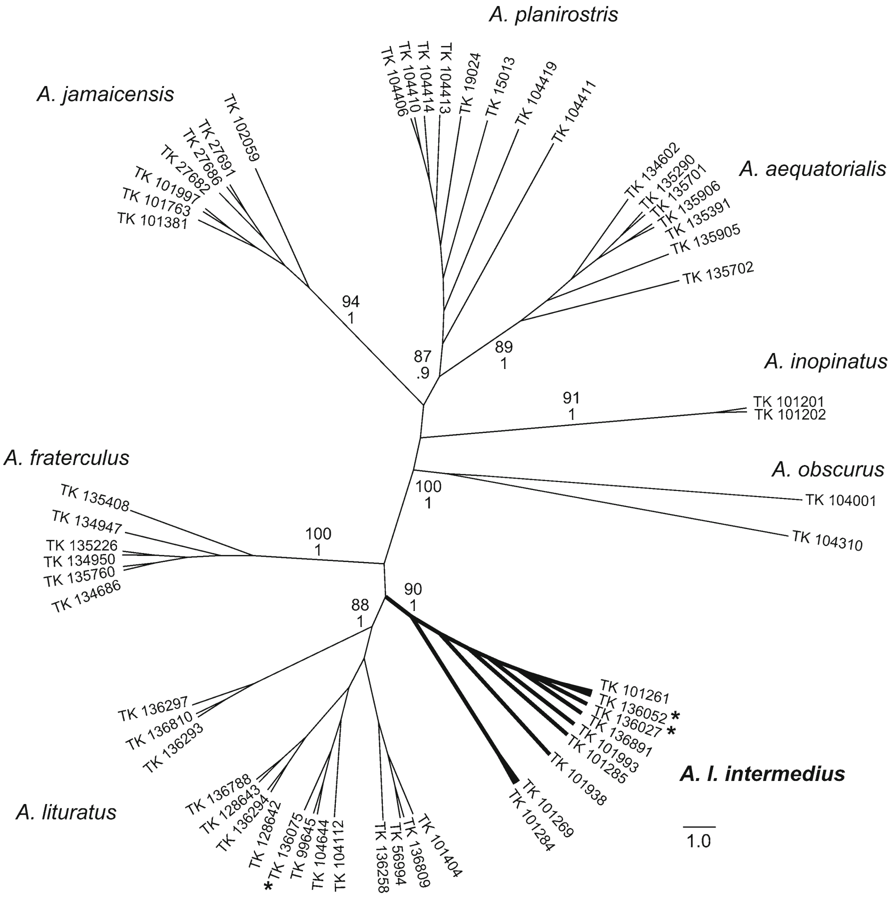
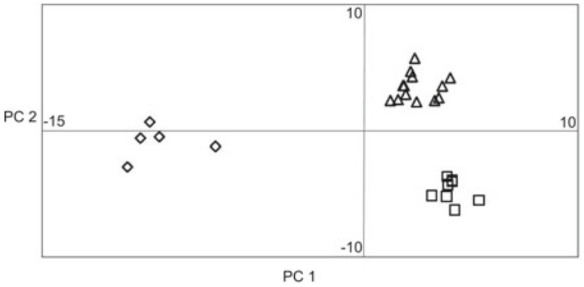


Fig. 16.3 Unrooted neighbor-joining phylogram based on 414 AFLP bands from six species of *Artibeus* plus *A. l. intermedius* (*bold clade*). *Numbers* along branches indicate bootstrap support values (*top* score; percent of 500 iterations) and Bayesian posterior probabilities (*bottom* score). *Asterisks* identify sympatric specimens collected from the Parque Nacional Azul, Comayagua, Honduras

Fig. 16.4 Principal coordinates analysis of 386 AFLPs from *A. aequatorialis* (*diamonds*), *A. lituratus* (*triangles*), and *A. l. intermedius* (*squares*)



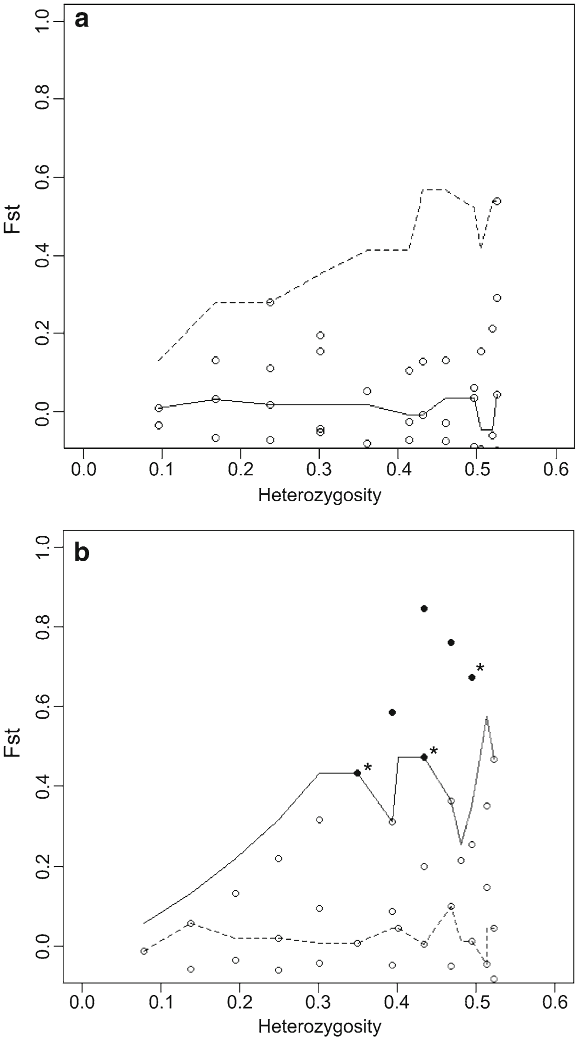


Fig. 16.5 Results of the Dfdist analyses of *Artibeus lituratus*. (**a**): Central American *A. lituratus* (excluding *intermedius*) vs. South American *A. lituratus*. (**b**): Honduran *intermedius* vs. Honduran *A. lituratus*. Each plot shows the empirical distribution of F_{ST} values for AFLP loci (*circles*) with respect to a simulated 95th quantile for neutral evolving loci (*dashed line*). *Black circles* indicate loci with F_{ST} values above the 95th quantile (outlier loci). The solid line represents the mean F_{ST} value over the range of heterozygosity. *Asterisks* identify overlapping loci (i.e., two with similar values)

16.4 Discussion

Our analyses indicate that the most recent common ancestor for extant species of *Artibeus* originated during the late Miocene to early Pliocene, with the majority of subsequent speciation events occurring during the early Pleistocene (Figs. 16.1b and 16.2). Two features of the data support this hypothesis. First, congruence exists among biogeographic distributions of extant taxa (including species of the closely related genus *Dermanura*) and major paleogeographic events (Fig. 16.1b). These consist of (1) origin of *trans*-Panamanian lineage (*A. inopinatus*, *A. hirsutus*, and *A. fraterculus*) coinciding with the closure of the Panamanian land bridge (~3–4 mya; Keigwin 1982; Kirby et al. 2008), (2) divergence between *A. hirsutus* and *A. fraterculus* (~2.24 mya) subsequent to the closure of Panamanian land bridge,

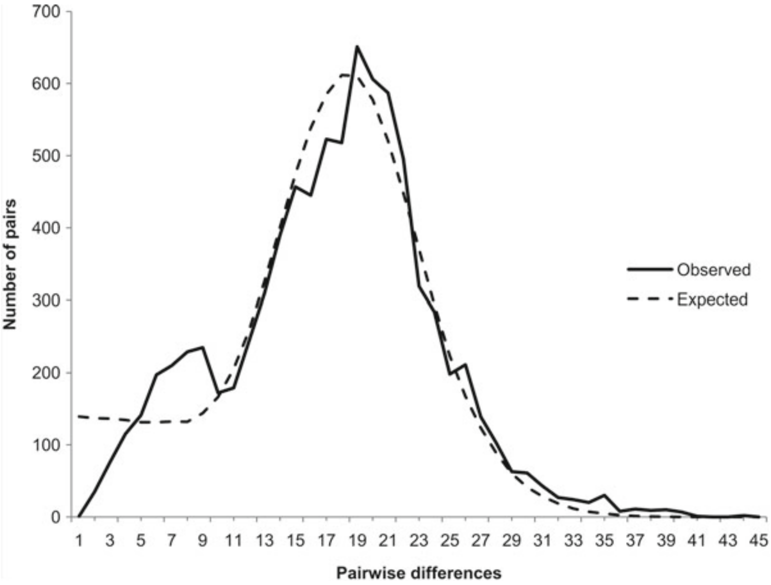


Fig. 16.6 Mismatch distribution based on cytochrome-*b* data from *Artibeus lituratus* ($n = 129$). *Solid line* indicates observed distribution of pairwise differences and dashed line indicates expected distribution of the sudden expansion model

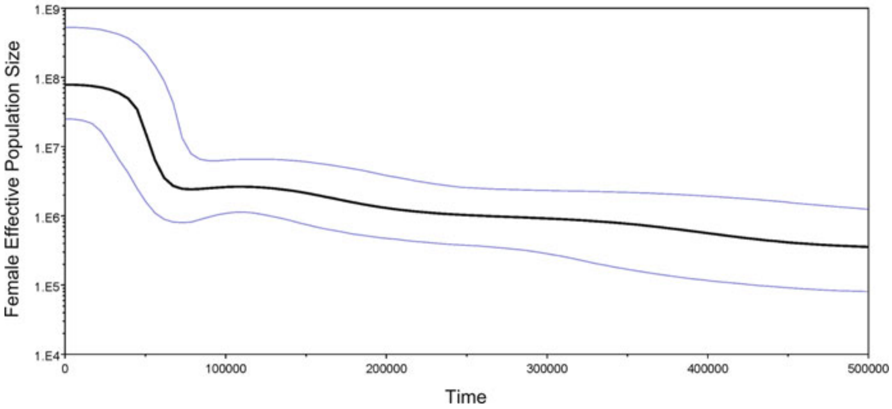


Fig. 16.7 Bayesian skyline plot showing the demographic history of *Artibeus lituratus*. The x-axis is time in years and the y-axis is an estimate of female effective population size. The thick black line is the median estimate for the reconstruction and blue lines are the 95% highest posterior density estimate

and (iii) origin of *D. rosenbergii* (~3.0 mya; endemic to the Chocó) coinciding with the development of the Chocó (Haffer 1967; Gentry 1982). Second, with respect to a late Miocene to early Pliocene origin, the rate of evolution for the cytochrome-*b* gene is compatible with previously established rates of evolution for this gene within Phyllostomidae (Baker et al. 2012).

Several authors have identified biogeographic patterns of diversification within South American populations of *Artibeus* (Larsen et al. 2007; Lim 1997; Lim et al. 2004; Patten 1971; Patterson et al. 1992; Redondo et al. 2008; Van Den Bussche et al. 1998). Patterson et al. (1992) hypothesized that the uplifting of the Andes Mountains during the last 2–5 million years and inundation of the Amazon Basin in the late Pleistocene had a major impact on the diversification of South American species of *Artibeus*. Furthermore, they regarded the *trans*-Andean biota (e.g., *A. fraterculus*) as representative of a historical connection with Middle America. Our analyses reinforce the hypotheses of Patterson et al. (1992) in that (1) our chronogram indicates that the most recent uplifting of the Andes Mountains played an important role in the diversification of South American species of *Artibeus* (Fig. 16.1b) and (2) the most recent common ancestor between the southwestern Ecuadorian endemic *A. fraterculus* and the Middle American endemic *A. hirsutus* existed approximately 2.2 mya, a value younger than the closure of the Panamanian Isthmus (Figs. 16.1b and 16.2).

16.4.1 Biogeographic Patterns in South America

The substantial uplifting of the Andes Mountains during the mid- to late Miocene (~5 mya) greatly altered climate patterns across South America and had a profound effect on the evolution of South American floral and faunal groups (Antonelli et al. 2009; Hughes and Eastwood 2006; Rheindt et al. 2009; Santos et al. 2009). Our results show two main lineages within *Artibeus* that likely originated by vicariance resulting from extensive uplifting of the Andes Mountains (Fig. 16.2). Lineage 1 (Fig. 16.2) includes at least two *trans*-Andean species (*A. aequatorialis* and *A. jamaicensis*), and Lineage 2 (Fig. 16.2) includes at least six *cis*-Andean species [*A. amplus*, *A. lituratus*, *A. obscurus*, *A. planirostris*, *A. schwartzi* (see Larsen et al. 2010b), *A.* sp. (sensu Redondo et al. 2008)].

The evolutionary history of *A. aequatorialis* and *A. jamaicensis* (Lineage 1; Fig. 16.2) has previously been discussed (Larsen et al. 2007, 2010a). Briefly, our results support the hypothesis that the origin of *A. aequatorialis* represents an allopatric speciation event by the Bateson–Dobzhansky–Muller process as a result of isolation west of the Andes Mountains (Larsen et al. 2010a). The sister lineage to *A. aequatorialis*, *A. jamaicensis*, is currently distributed throughout Central America, and the Caribbean and its South American distribution may be restricted to northwestern Colombia (Larsen et al. 2007, 2010a). Our results suggest that the six subspecies currently recognized within *A. jamaicensis* (*jamaicensis*, *parvipes*, *paulus*, *richardsoni*, *triomylus*, *yucatanicus*; Larsen et al. 2007) evolved within the last 1.5 million years. Noticeably, the patterns of *trans*-Andean endemism present in species of *Artibeus*, northward to Central America, are comparable to those in several other nonmammalian taxa (e.g., angiosperms, Prance 1982; birds, Cracraft and Prum 1988; butterflies, Brown 1976).

A greater level of species diversification is observed in the *cis*-Andean lineage (Lineage 2, Fig. 16.2). This diversification occurred mainly during the Pleistocene

(2.5 mya to present) and therefore is compatible with allopatric speciation events resulting from isolated refugia (Haffer 1967). The exception is the extant species of the Caribbean endemic *A. schwartzi*, which appears to represent an example of reticulate evolution resulting from primary contact among multiple lineages in the southern Lesser Antilles (Larsen et al. 2010b). The species within which the mitochondrial genome typical of extant *A. schwartzi* evolved likely originated within the last 1.6 million years (Fig. 16.2) and subsequently invaded the southern Lesser Antilles from northern South America (Larsen et al. 2010b). The remaining species in Lineage 2 evolved sufficient isolating mechanisms to permit sympatry, as evidenced by their current distributions (Appendix A), and have likely evolved effective premating/postmating isolating mechanisms in mainland South America (Larsen et al. 2010b). Thus, the isolation of one of the ancestral species of *A. schwartzi* in the southern Lesser Antilles during the late Pleistocene, in combination with primary contact between Lineage 1 (*A. jamaicensis*) and Lineage 2 (*A. planirostris* and the species in which the mitochondrial genome typical of *A. schwartzi* evolved), has resulted in reticulated evolution, the outcome of which being the extant population of *A. schwartzi* with unique species-level features on the island of Saint Vincent (Appendix A; Larsen et al. 2010b).

One of the most important biogeographic features that has influenced evolutionary patterns within *cis*-Andean species of *Artibeus* (Lineage 2; Fig. 16.2) is the Llanos Savannah in eastern Colombia and Venezuela. The ecological transition between the savannahs, north of the Orinoco River, and the tropical forests, south of the Orinoco, defines the distributional patterns of at least seven species/subspecies of *Artibeus* (*A. amplus*, *A. concolor*, *A. obscurus*, *A. lituratus*, *A. l. palmarum*, *A. planirostris fallax*, *A. p. trinitatus*; Appendix A; Marques-Aguiar 2007). Within *A. planirostris*, there are morphologically distinct subspecies (*A. p. trinitatus* and *A. p. fallax*; Larsen et al. 2007; Lim 1997) that correspond to the regions north (the Llanos Savannah; *trinitatus*) and south (Amazonia; *fallax*) of the Orinoco River. The morphological variation observed between these taxa has been interpreted as species-level variation (Lim 1997), yet the mitochondrial DNA sequence data indicate the presence of a single species (*A. planirostris*), and there is an absence of phylogeographic structuring among *trinitatus* and *fallax* (cytochrome-*b* sequence data Larsen et al. 2007). The significance of the morphological variation exhibited within populations of *A. planirostris* distributed across this ecotone is yet to be determined and warrants a closer examination utilizing a population genomics approach. Notably, a similar pattern is observed in Central American populations of *A. lituratus*.

The current distributions of *A. concolor* and *A. fimbriatus* are associated with tropical rainforests and deciduous forests in northern and southeastern South America, respectively. *Artibeus concolor* (sensu Hoofer et al. 2008; time of origin ~5 mya; Figs. 16.1b and 16.2) is distributed throughout the Guiana Shield and regions of southern Venezuela and northern Brazil (Appendix A: Plate 16.1). When considering the time of origin for *A. concolor*, the current geographic distribution of the species is interesting inasmuch as the species occurs throughout regions east of a vast inland lake/waterway (the Pebas system) that was present in northern South America and Amazonia during the Miocene (Wesselingh and Salo 2006). *A. fimbriatus* is sister to Lineages 1 and 2 (TMRCA ~3.5 mya; Fig. 16.2) and is endemic to the

Atlantic Forest (Marques-Aguiar 2007; Redondo et al. 2008; Appendix A: Plate 16.1). This phylogenetic pattern (i.e., a sister or basal relationship between Atlantic Forest endemics and Amazonian/western South American taxa) is observed in several other chiropteran genera/species, including *Desmodus* (Martins et al. 2007), *Myotis* (Stadelmann et al. 2007), *Platyrrhinus* (Velazco and Patterson 2008), and *Vampyressa* (Porter and Baker 2004). Of these genera, diversification dates have been estimated for *Myotis* (Stadelmann et al. 2007), and the time of origin for the Atlantic Forest endemic *M. ruber* (~4.5–2.5 mya) was similar to our estimate for the time of origin for *A. fimbriatus* (~4.0–2.4 mya). These dates are consistent with previous hypotheses regarding a Pliocene origin for Atlantic Forest endemics (Cabanne et al. 2008; Cortes-Ortiz et al. 2003; Costa 2003).

16.4.2 Central American Diversification

Our chronogram indicates that the origin of the most recent common ancestor for *A. inopinatus*, *A. hirsutus*, and *A. fraterculus* was approximately 3.7–4.7 mya (Figs. 16.1b and 16.2). This result suggests an invasion by the genus into Central America that coincided with the closure of the Panamanian Isthmus. Interestingly, the timing of this diversification pattern is congruent with those observed in *trans-Panamanian* isthmus species of birds (see Smith and Klicka 2010). *A. hirsutus* (distributed in western Mexico) and *A. fraterculus* (distributed in southwestern Ecuador) are sisters, and their most recent common ancestor existed approximately 2.5 mya (Figs. 16.1b and 16.2). When considering the sister relationship of these species to *A. inopinatus*, the data indicate either (1) recolonization of South America by a Central American ancestor within the early to mid-Pleistocene or (2) two separate colonization events of Central America by South American ancestors. *A. fraterculus* is limited in its distribution to the dry tropical forests of southwestern Ecuador and northwestern Peru (Marques-Aguiar 2007) and therefore occurs within a habitat that is similar to those occupied by its closest Central American relatives (Appendix A: Plate 16.1). Similar connections between west Andean and Central American faunal groups have been identified within several phyllostomid bat genera (e.g., *Carollia*, Hoffmann and Baker 2003; *Dermanura*, Solari et al. 2009; *Glossophaga*, Hoffmann and Baker 2001; *Platyrrhinus*, Velazco and Patterson 2008).

16.4.3 Evidence for Ecological Divergence

Ecological speciation occurs when barriers to gene flow evolve between populations as a result of ecologically based selection (Rundle and Nosil 2005). Under this model, natural selection is driven by adaptive divergence due to ecological changes or occupation of novel habitats. An interesting corollary to such ecologically driven speciation events is that genetic isolation between populations can arise rapidly (i.e., within dozens of generations; Hendry et al. 2007) and thus it is unlikely that neutral markers (e.g., mtDNA) would exhibit genetic divergence under the conditions of an ecologically driven speciation event (see Orr and Smith 1998). Collectively, the data presented herein has led us to the hypothesis that the taxon referable to *A. lituratus intermedius* in Central America is the product of a recent ecologically driven speciation event. This hypothesis is supported by (1) a *cis-Andean* origin and recent Neotropical expansion by *A. lituratus* (Figs. 16.2, 16.6, 16.7), (2) evidence of divergent selection in AFLP data (Fig. 16.5), (3) maintenance of morphological and genetic (nuclear) distinction in sympatric populations of *A. l. intermedius* and *A. l. palmarum* (Davis 1984, Fig. 3), and (4) a correlation between environment and morphological phenotypes (Davis, 1984; Marchán-Rivadeneira et al. 2012).

The taxonomic debate surrounding the small (*intermedius*) and large (*palmarum*) phenotypes in Central America has been discussed in detail (Marchán-Rivadeneira et al. 2012; Redondo et al. 2008; Simmons 2005), and several studies (genetic and morphometric) have explored the extent of variation between the two taxa (Guerrero et al. 2008; Lim et al. 2004; Marchán-Rivadeneira et al. 2012; Redondo et al. 2008). The consensus of most of these studies has been that the morphological and/or genetic variation separating *intermedius* and *palmarum* either did not warrant taxonomic recognition or was indicative of intraspecific variation within *A. lituratus*. Interestingly, Davis (1984) and Marchán-Rivadeneira et al. (2012) provided indirect evidence for genetic isolation of *intermedius* by identifying sympatric *intermedius* and *palmarum* morphotypes in Middle America.

Until now, all genetic-based analyses of the variation within *A. lituratus* have been restricted to mitochondrial DNA datasets (Guerrero et al. 2008; Hoofer et al. 2008; Lim et al. 2004; Redondo et al. 2008). These mtDNA datasets have provided valuable insight into the demographic history of *A. lituratus* and collectively indicate a recent expansion by *A. lituratus* throughout the Neotropics (Ditchfield 2000; Redondo et al. 2008). Moreover, the mismatch distribution (Fig. 16.6), Bayesian skyline plot (Fig. 16.7), and tests of neutrality (see Sect. 16.3.3) presented here further support this hypothesis. The observation that all of the most closely related species of *Artibeus* to *A. lituratus* are *cis*-Andean (Lineage 2, Fig. 16.2) indicates that *A. lituratus* originated east of the Andes in South America, and our results indicate that the TMRCA for the cytochrome-*b* haplotypes in our sample is approximately 850,000 years. Thus, Central American populations of *A. lituratus* are likely the result of a more recent colonization by South American *A. lituratus*.

Our AFLP data show two statistically supported sister clades within *A. lituratus* (Fig. 16.3). One clade is entirely comprised of individuals morphologically identified as the subspecies *A. l. lituratus* and *A. l. palmarum*, and the second is entirely comprised of individuals morphologically identified as *A. l. intermedius*. Species identification based upon AFLPs and morphology were 100 % congruent. The Nei–Li genetic distance value separating *intermedius* from *lituratus* is 3 %, a value similar to those separating other species of *Artibeus* (Larsen et al. 2010a). The lack of variation across the nuclear genomes in our sample of *A. lituratus* (excluding *intermedius*), in combination with the results of our intraspecific analyses presented above, provides further evidence of a recent origin/expansion in the Neotropics by *A. lituratus*. However, this fails to explain the unique nuclear genomes of Central

American specimens identified as *intermedius*, especially when considering the peripheral location of *intermedius* with respect to the Neotropical distribution of *A. lituratus*. Moreover, our results provide some evidence that the genetic distinctiveness of *intermedius* and *lituratus* (*palmarum*) is maintained in sympatry (Fig. 16.3). This finding is congruent with previous documentation of several areas of sympatry between the phenotypes of *intermedius* and *lituratus* (*palmarum*), respectively (Davis 1984; Marchán-Rivadeneira et al. 2012).

The documentation of sympatric individuals of *intermedius* and *lituratus* (in combination with structure in both morphological and AFLP data) without evidence of hybridization is compatible with species recognition within the framework of a number of species concepts (see Coyne and Orr 2004). This is because the maintenance of morphologically and genetically defined units in sympatry indicates the presence of an isolating mechanism (pre- or postzygotic), and collectively, the data from *intermedius* and *lituratus* (*palmarum*) suggest the presence of such an isolating mechanism. Thus, our results indicate that *intermedius* should be recognized as a distinct species from *A. lituratus*, reinforcing the hypothesis of Davis (1984). Moreover, our analyses failed to detect outlier loci between Central American (excluding *intermedius*) and South American populations of *A. lituratus* (Fig. 16.5a) but did identify outlier loci between *intermedius* and *lituratus* (Fig. 16.5b). This result would be expected if divergent selection were occurring between *intermedius* and *lituratus*. Because AFLPs represent only a small portion of the genome, our detection of outlier loci between *intermedius* and *lituratus* is noteworthy as it is likely that multiple regions of the genomes of these taxa have differentiated in a relatively short amount of evolutionary time (Butlin 2010).

The AFLP data from *A. intermedius* and *A. lituratus* are inconsistent with a traditional allopatric speciation model. This is because a signature of genetic isolation (Figs. 16.3, 16.4, and 16.5) was identified only in Central American populations between taxa that are correlated with environmental variables (Davis 1984; Marchán-Rivadeneira et al. 2012). There is no evidence of genetic isolation present among the remaining South American subspecies of *A. lituratus* (i.e., *l. lituratus* and *l. palmarum*) or among populations distributed across major geological barriers (i.e., the Andes Mountains). Thus, the most parsimonious explanation for the existence of structure in our nuclear AFLP data, and absence of structure in mtDNA data, from *intermedius* and *lituratus* is that the taxon *intermedius* is the result of intensive directional selection (see Orr and Smith 1998). We hypothesize that genetic isolation between *intermedius* and *lituratus* arose during the late Pleistocene to early Holocene, as paleoecological data indicate the presence of arid conditions (characteristic of the distribution of *intermedius*) in Central America during this time frame (Leyden 1984; Pennington et al. 2000; Piperno and Jones 2003). Additionally, genetic data from the poorly understood subspecies *A. l. koopmani* (sensu Simmons 2005) may shed light on the hypothesis of a recent origin for *A. intermedius*. Originally described as a subspecies of *A. intermedius* (Wilson 1991), the taxon *koopmani* is restricted in its distribution to the Tres Marias Islands off the western coast of central Mexico. Thus far, *koopmani* has not been investigated using genetic data, and therefore, hypotheses regarding its relationship with *A. intermedius* and *A. lituratus* remain untested. Determining the phylogenetic placement of *koopmani* in *Artibeus* is critical to our understanding of the evolutionary history of Central American populations of *A. intermedius* and *A. lituratus*.

16.5 Conclusions

The origin and diversification of the Neotropical fauna and flora has been the subject of many studies (e.g., Cracraft and Prum 1988; Haffer 1969; Hooghiemstra and van der Hammen 1998; Lessa et al. 2003; Moritz et al. 2000; Santos et al. 2009). Although broad-level hypotheses regarding the evolutionary history of the Neotropical fauna have been proposed [e.g., the forest refugia hypothesis (Haffer 1969) and parapatric divergence hypothesis (Endler 1977)], contemporary analyses suggest that an array of processes have contributed to the origin of Neotropical species (Costa 2003; Moritz et al. 2000; Rull 2008; Weir 2006). Examples of evolutionary histories resulting in complex phylogenetic patterns have been identified in multiple taxonomic groups, including bats (*Carollia*; Hoffmann and Baker 2003), butterflies (*Heliconius*; Mallet et al. 2007; Mavárez et al. 2006), flycatchers (*Myiopagis*; Rheindt et al. 2009), snakes (*Leptodeira*; Daza et al. 2009), and poison frogs (*Dendrobates*; Noonan and Gaucher 2006). Collectively, the data from *Artibeus* indicate that several speciation processes (e.g., allopatric, hybrid, and ecological speciation) have contributed to the diversity observed within the genus.

Our results support previous hypotheses regarding the influence that Pliocene and Quaternary Neotropical environments had on diversification events in *Artibeus* (Patterson et al. 1992, Fig. 2). Minimally, this finding indicates that it is likely that the Neotropical chiropteran fauna is not only greatly underestimated but is also the result of multifaceted speciation processes. Further insight into the complex origins of Neotropical chiropteran species will be achieved by examining recently evolved and widely distributed genera (e.g., *Dermanura*, Solari et al. 2009; *Micronycteris*, Porter et al. 2007; and *Platyrrhinus*, Velazco and Patterson 2008), as the mechanisms underlying the origin of genetic divergence in these groups will be less likely to have been eroded by factors such as time (Coyne and Orr 2004). We posit that advanced genome scan studies (i.e., AFLP, RADseq, or other genome-wide analysis) combined with mitochondrial DNA sequence data and morphological-based analyses will identify heretofore unrecognized speciation and hybridization events in multiple taxonomic groups.

Acknowledgments We thank R. D. Bradley for his valuable discussions throughout the stages of our investigation. C. Blair, H. H. Genoways, S. C. Pedersen, C. D. Phillips, C. J. Phillips, R. E. Strauss, and P. M. Velazco provided helpful comments. We are grateful to H. J. Garner, K. McDonald, and J. P. Carrera of the Natural Science Research Laboratory of the Museum of Texas Tech University for assistance with loans of tissue material. This study would not have been possible were it not for the collecting efforts of members of the 2001 and 2004 Sowell Expeditions to Ecuador and Honduras. We thank all researchers who have worked to generate the molecular data used herein. Financial support was provided by J. Sowell, A. Brown, the Texas Tech University Biological Database Program, and the American Society of Mammalogists.

Appendix A

Distribution maps of species of *Artibeus* following the data presented herein and in Webster and Jones (1983), Marques-Aguiar (2007), Genoways et al. (2010), Larsen et al. (2010a), and Larsen et al. (2010b). Species are arranged phylogenetically following Fig. 16.2. Plate 16.1: *A. concolor*, *A. hirsutus*, *A. inopinatus*, *A. fraterculus*, *A. fimbriatus*, *A. aequatorialis*, *A. jamaicensis*, and *A. obscurus*. Plate 16.2: *A. lituratus*, *A. intermedius*, *A. schwartzi*, *A. planirostris*, and *A. amplus*. The distribution of *Artibeus* sp. (sensu Redondo et al. 2008) remains to be determined.

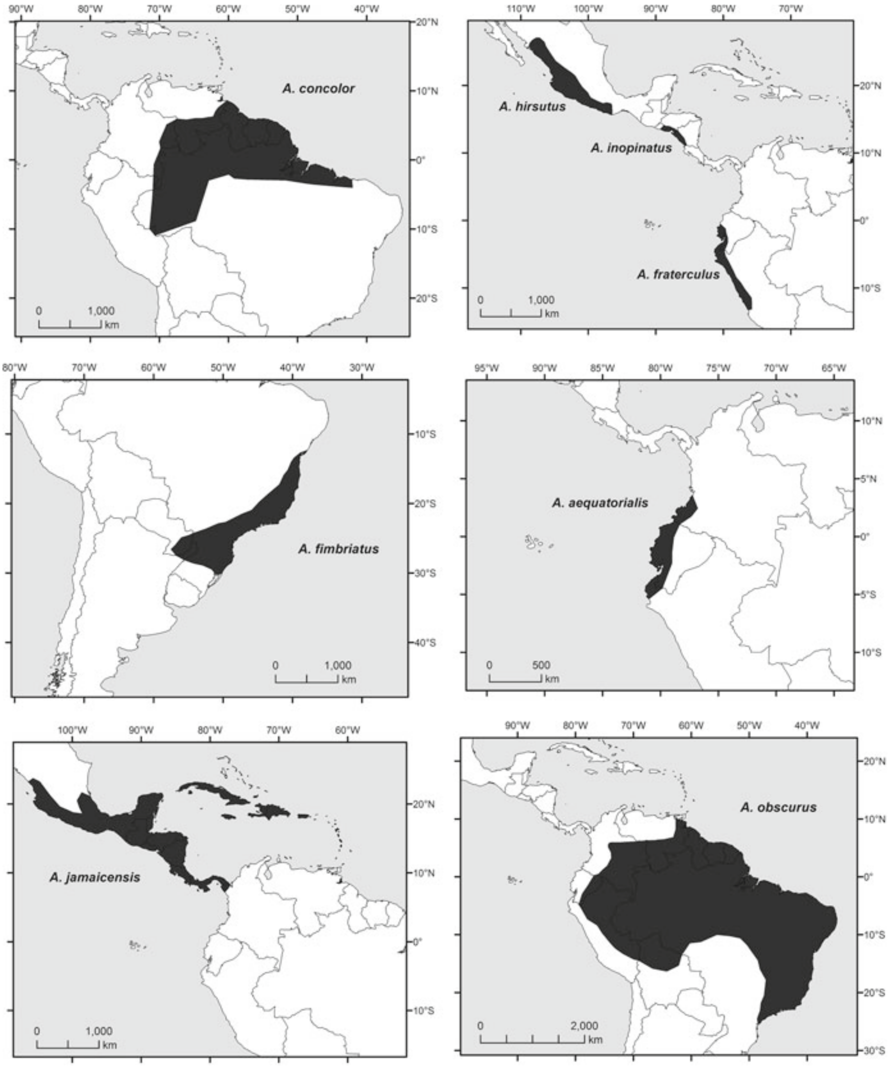


Plate 16.1 Distribution maps of *Artibeus concolor, A. hirsutus, A. inopinatus, A. fraterculus, A. fimbriatus, A. aequatorialis, A. jamaicensis, and A. obscurus*

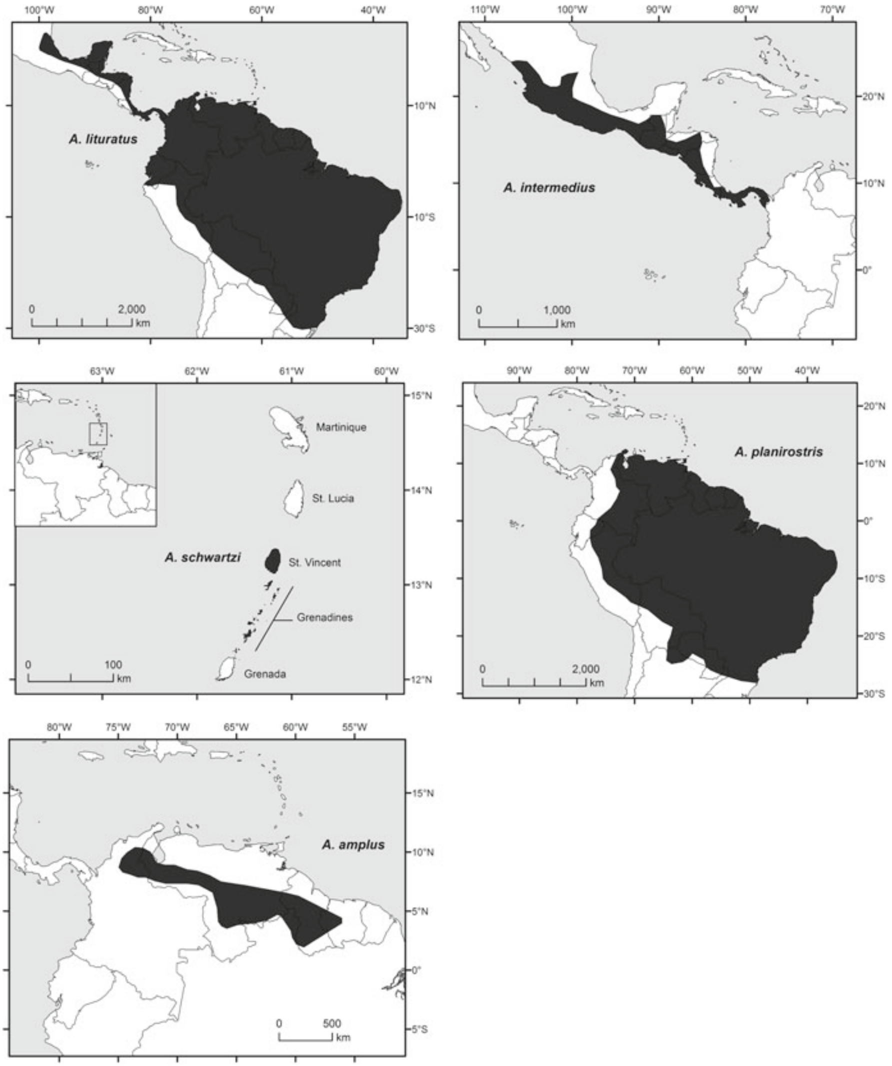


Plate 16.2 Distribution maps of *Artibeus lituratus, A. intermedius, A. schwartzi, A. planirostris, and A. amplus*

Appendix B

Specimens examined. Numbers associated with each specimen are enclosed in parentheses. Cytochrome-*b* sequences from Freygang (2006), Guerrero et al. (2004), Guerrero et al. (2008), Larsen et al. (2007), Larsen et al. (2010b), Redondo et al. (2008), and Van Den Bussche et al. (1998) and 16S ribosomal sequences from Hoofer et al. (2008). Distinct Brazilian cytochrome-*b* haplotypes from Redondo et al. (2008) were used in the mismatch distribution and Bayesian skyline plot analyses (GenBank accession numbers = EU160724–EU160833). TK = Tissue Number: Natural Science Research Laboratory of the Museum of Texas Tech University, Lubbock. GenBank accession numbers reported for all other sequences. * = used in AFLP analysis; † = used in molecular clock analyses, and ‡ = used in cytochrome-*b* haplotype network construction.

A. aequatorialis: Ecuador: El Oro (TK 135391*); Esmeraldas (TK 135701*), (TK 135702*), (TK 135905*), (TK 135906*); Guayas (TK 134602*); Loja (FJ179232†, FJ179186†, TK 135290*). ***A. concolor***: Suriname: Sipaliwini (FJ179223†, FJ179173†). ***A. fraterculus***: Ecuador: El Oro (TK 135408*), (TK 135226*), (TK 135760*); Guayas (DQ869389†, FJ179174†, TK 134686*), (TK 134950*), (TK 134947*). ***A. hirsutus***: Mexico: Michoacan (FJ179226†, FJ179181†). ***A. inopinatus***: Honduras: Valle (FJ179227†, FJ179177†, TK 101201*), (TK 101202*). ***A. intermedius***: Costa Rica: Guanacaste (U66502‡). Honduras: Colon (TK 136293‡), (TK 136891*); Comayagua (TK 101269*), (AY684729, TK 101284*), (AY684730‡, TK 101285*), (TK 101938*), (TK 136052*‡), (TK 136027*); Copan (FJ179230‡, TK 101993*), (FJ179231); Valle (TK 101261*). Mexico: Morelos (AY144338‡), Veracruz (AY144339‡); Panama: Bocas del Toro (AY684737‡). ***A. jamaicensis***: Jamaica, St. Ann's Parish (DQ869480†, FJ179187†, TK 27682*), (TK 27686*), (TK 27691*). Honduras: Atlantida (TK 101763*), (TK 101381*); Copan (TK 101997*); Olancho (TK 102059*). ***A. lituratus***: Brazil: Acre (EU160800‡); Amazonas (EU160761‡); Bahia (EU160725‡); Espirito Santo (EU160740‡), (EU160745‡), (EU160794‡); Maranhao (EU160827‡); Mato Grosso (EU160798‡); Minas Gerais (EU160724‡), (EU160799‡), (EU160814‡), (EU160815‡), (EU160817‡), (EU160822‡); Piaui (EU160829‡), (EU160830‡); Rio de Janeiro (AY684735‡), (EU160729‡), (EU160733‡); Santa Catarina (DQ985483‡), (DQ985485‡); Sao Paulo (AY684736‡), (EU160803‡), (EU160826‡). Ecuador: Esmeraldas (DQ869393‡), (TK 104643‡), (TK 104644*); Pastaza (FJ179233†‡, FJ179194†, TK 104112*), (AY684732‡), (AY684733‡), (TK 104333‡), (TK 104427‡), (TK 104441‡). Grenada: Carriacou (GQ861668‡). Guyana (TK 86512‡). Honduras: Atlantida (TK 101404*); Colon (TK 136258*), (TK 136293‡), (TK 136294*), (TK 136297*), (TK 136788*), (TK 136809*), (TK 136810*); Comayagua (TK 136075*). Paraguay: Dept. Canindeyu (TK 99645*); Dept. San Pedro (TK 56994*). Panama: Bocas del Toro (AY684731‡). St. Vincent and the Grenadines: Union Island (FJ179234‡, TK 128642*), (TK 128643*), (GQ861670‡). Suriname: Brokopondo (AY684740‡). Trinidad and Tobago: Trinidad (U66505‡). ***A. obscurus***: Ecuador: Pastaza (TK 104001*), (TK 104310*). Suriname: Nickerie (FJ179185†); Para (U66506). Guyana (FJ179235†, FJ179184†). ***A. planirostris***: Venezuela: Guarico (DQ869424†, FJ179189†, TK 15013*), Barinas (TK 19024*). Ecuador: Pastaza (TK 104413*), (TK 104414*), (TK 104406*), (TK 104410*), (TK 104411*), (TK 104419*). ***A. schwartzi***: St. Vincent and the Grenadines: St. Vincent (DQ869524†, FJ179193†). ***Dermanura phaeotis***: Mexico: Chiapas (FJ179245†, FJ179218†). ***D. rosenbergi***: Ecuador: Esmeraldas (FJ179258†, FJ179219†). ***D. tolteca***: Honduras: Comayagua (FJ179258†, FJ179216†). ***D. watsoni***: Honduras: Colon (FJ179259†, FJ179205†). ***Ectophylla alba***: Costa Rica: Limon (AY157033†, AY395811†). ***Enchisthenes hartii***: Peru: Cusco (AY395838†); Huanuco (U66517†).

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Part III
Conservation and Education

Chapter 17
Considering Human Development, Socialization, and Enculturation in Educational Intervention Design for Wildlife Conservation: A Case for Bats

Sarah M. Bexell and Rui Xi Feng

Abstract Education is one of the most powerful tools we have to preserve biodiversity, but how we conduct effective educational interventions often proves to be a nuanced endeavor. This chapter will review the negative and positive emotions humans experience throughout their perceptions of and experiences with bats and how those concerned with bat conservation can utilize this knowledge to protect bats. Intervention examples will be shared along with hopes for the future.

17.1 Introduction

In efforts to foster respect for and conservation of wildlife, researchers and conservation practitioners consider the historical and contemporary perceptions of the animals they work with and are passionate about. Both the positive and negative aspects of emotions, attitudes, and understandings have strong influences on how public opinions have been propagated and on how we may appeal for humane behavior toward nonhuman animals, especially those so different from us as to invoke feelings of disdain or fear.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_17,

Because of their nocturnal and elusive nature, unique life history patterns, and misrepresentation in lore and the media, human perceptions of bats are fraught with misunderstandings and sometimes hatred. Few individuals have had the chance to interact with bats, and many who have had the chance found themselves suddenly within an experience that was not particularly positive and in fact was likely frightening. For example, bats entering a human dwelling or humans entering a bat dwelling (e.g., cave) may suddenly become aware of each other by surprise, and unanticipated events drive the adrenalin-filled encounter to the point of fear for both bat and human; or, a caring citizen finding a bat on the ground and picking it up to take to a veterinarian, only later realizing that the bat had rabies dictating the need for rabies treatment; or, more probably, encountering bats simply flying above one's head on a nice summer night or viewing them at a zoo may evoke reactions based upon misrepresentation commonly portrayed in film and/or within human social constructs, which are mostly laden with misconceptions of bat behavior and even more importantly, intent.

Human understandings of bats and their behavior and depictions of them are stigmatized by the media, cultural understandings, and folklore, often inspiring fear and mistrust, not adoration and care. If people were able to see bats for who they are, their impressions about them may change for the better. The truth about bats inspires respect by those who have the chance to get to know them and learn about their ecology, physiology, and social behavior.

The thrust of this chapter is theoretical as well as intervention-based, and we explore the broader potentials of humans as kind peaceable creatures who may be more fulfilled by relationship with wildlife and nature than by exploitation of them. The overall goal of our contribution is to explore ways to positively influence human behavior toward bats.

17.2 Biodiversity Crisis

Today, we are within the sixth mass extinction event on Earth that is being caused for the first time in Earth's long history by the behavior of just one species, *Homo sapiens*. Biodiversity loss may pose the greatest direct threat to human survival because of its destabilizing effects including biogeochemical cycling (Dirzo and Raven 2003; Pimentel and Wilson 1997; Rockstrom et al. 2009) and significantly reducing ecosystem resilience (Rockstrom et al. 2009). From a moral perspective, it is clearly unjust that the needs of one species usurp those of all others.

17.3 Major Thesis: Need for Interventions

In our increasingly degraded world, it is essential that we discover critical intervention strategies to help avoid further massive levels of extinction and biodiversity loss. Education programs need to be designed to help children overcome socially and culturally imposed psychological distancing from nature. In today's technologically driven world, most people tend to forget that our very survival depends on functional ecosystems, and we need to more deeply understand and cherish our connections to the natural world.

17.4 Review of Relevant Theories for Intervention Design and Support

17.4.1 Perceptions of Wildlife in Human Development

Human attitudes predominately determine how we treat animals and environments. For bats, it is unfortunate that the awareness most people have is based in folklore, on the media and movies, and perhaps most negatively on the adverse reactions to mere thoughts of bats by other people which is how most children learn about bats. In order to improve the future for bats, methods for accurate learning need to be understood and embraced by conservationists, teachers, parents, and especially the media.

The scientific literature on the development of emotional dispositions and reactions to animals is rich and if read with the intent toward promoting compassionate behavior toward bats, provides insights and hope for positive change. First we will discuss the science behind development of fear and disgust that often leads to poor attitudes toward animals and their mistreatment. Then we will discuss gateways toward development of positive attitudes and treatment.

17.4.2 The Development of Fear and Disgust

17.4.2.1 Fear

Meltzer et al. (2008) found that the most common child fears reported by parents of children ages 5–16 were those associated with animals, whereas blood/injections and the dark came in second and third. From an evolutionary psychology perspective (Boyer and Bergstrom 2011), fears often serve as an adaptive advantage when they are not pathological. Most likely these reactions are residual protective measures within our brains to keep us safe from harm. Thus, animals that have historically posed the greatest risk to humans [snakes (venom), rats (disease vector), mice (disease), and large predators] insight the greatest trepidation. Tuan (1979) discusses childhood fears of animals and states that "Children readily learn fear of animals through their ability to infer. They are taken to the zoo, where they appear to be enjoying themselves. They notice, however, that in the zoo animals are behind bars: the inference is that animals are dangerous."

Indeed, negative information shared via multiple forms of cultural transmission promotes children's subjective, behavioral, and physiological fear reactions toward unfamiliar animals (Field 2006a, b; Field et al. 2001; Field and Lawson 2003; Field and Schorah 2007; Muris et al. 2003). Children of parents that portray negative information about particular animals showed a significant increase in self-reported fear, whereas transmission of true and positive appreciation clearly resulted in a decrease of children's irrational fears (Muris et al. 2010).

For bats, the most profound and significant barrier to conservation globally is irrational fears (Gunnthorsdottir 2001; Knight 2008; Kunz et al. 2011). However, well-developed environmental education programs can counteract feelings of disgust and fear toward certain animals (Prokop and Tunnicliffe 2008), and children will care for bats even while possessing some fear of them (Kahn et al. 2008). Kals and Ittner (2003) found that fear or disgust about bats did not prohibit an attribution of responsibility to protect bats. These findings are promising because many things in nature provoke fear, but should not inhibit care and conservation. The constructivist theoretical framework (e.g. Piaget 1969; Vygotsky 1978) advises that children actively make sense of their own experiences, integrating different aspects to counteract older knowledge or emotions that may be incorrect or irrational.

Although in early infancy it is not so apparent, possibly because of undeveloped visual acuity, children of all cultures fear of the dark. When given a choice, children by 10 months of age will leave their mother to explore lit rather than unlit areas (Jersild and Holmes 1935). As children grow, their fear of the dark does as well, most likely due to the sense of isolation and disorientation that darkness instills (Tuan 1979). This apparently inherent fear of the dark may additionally be provoked by a startling or unsettling event that occurs under the cloak of night. For example, zoos typically have bats on display in dimly lit rooms to portray their normal behaviors. These areas, of course, are already a scary place for children. Add to this, the seemingly erratic flight of bats flitting around which can be disorienting and very scary to children and parents alike. In some, perhaps many, cases, parents escalate the negative feelings toward bats by acting irrationally.

17.4.2.2 Disgust

Disgust is another strong emotional response to some animals and can be particularly strong if informed by misunderstandings and when animals are physically or behaviorally different from us. Humans may experience disgust when they feel a personal vulnerability from exposure to certain stimuli (Muris et al. 2012), and this promotes avoidance behaviors commonly rooted in the evolutionary psychology associated with biological preparedness (Boyer and Bergstrom 2011; Seligman 1971). Disgust-centered reactions to organisms by adults in the presence of children (sometimes inadvertently, and often nonverbally) taint the perceptions of children, making them more apprehensive and potentially instilling irrational fear and phobias that grow with the child (Bandura 1977; Davey and Marzillier 2009; Muris et al. 2012; Rozin et al. 1999).

For example, in the case of bats, children may hear comments such as they will attack you and get tangled in your hair (injury) or they are dirty animals that carry rabies (disease). Or, seeing their jerky or seemingly erratic movements (unpredictable) might also contribute to feelings of fear and disgust. Thus simple and seemingly trivial comments or unexplained observations can have profound effects on a child's emotional development toward particularly mysterious animals.

17.4.3 Affinity for Animals: Developed or Innate?

Some researchers consider that young children possess an innate affinity and interest in animals (Barrows 1995; Myers 2007). So what causes some children to develop aberrant behaviors that lead to animal abuse? We know that children at a very young age develop their attitudes about and behaviors toward everything in their environment (Myers 2007). Animals provide one of the most provocative sources of interest for most children and, in fact, it appears that children learn much about themselves by observing and attending to nonhuman animals which provide a platform for discovering their relationship to the rest of the animate world (Myers 2007; Shepard 1996).

Psychologists (Myers 2007; Myers and Saunders 2002) are beginning to understand how a sense of connection to animals and, by extension, a connection to a wider ecology should be a product of *normal* human development. Myers (2007) found that children centralize animals in their minds, meaning if animals are present, they are the focus of their attention and interest, and Barrows (1995) suggested that integration with all life's processes during childhood is necessary to cultivate positive human–animal bonds.

It is unequivocal that children, if given the opportunity, develop deep connections with animals (e.g., Freud 1913/1950; Golinkoff and Halperin 1983; Hall and Browne 1904; Isaacs 1930; Kaylor 1909; Kidd and Kidd 1987; Piaget 1969; Ricard and Allard 1993), and the profound importance that animals have on child development occurs cross-culturally (Myers 2007). Not surprisingly, without an opportunity to connect with nonhuman animals during childhood comes an inevitable disconnection from nature (e.g., Gould 1977; Wilson 1984). Shepard (1996) reminds us that the human species emerged from nature as a product of interactions with the living world, and thus we cannot fully understand ourselves without relating to the natural world. Introducing children to the wonderful world of bats has all the mystery and majesty necessary to connect children to the living planet and how the most fascinating animals have evolved and are integral to the survival of all life on Earth.

Starting with Darwin's (1998) seminal work, *The Expression of the Emotions in Man and Animals*, the evolutionary continuity of behaviors and emotions between us and nonhuman animals has been clearly established. Feeling of relatedness and community abounds when we have the opportunity to see that other species also have complex and important lives and that their rich *sentience* is filled by emotions, cares, and interests similar to us (Darwin 1998; de Waal 2009; Fossey 2000; Goodall 2000; Griffin 2001; Moss 2000; Poole 1998; Shanor and Kanwal 2011).

Observing an animal's behavior gives children insight into not only the animal's mind, but also their own. They begin to realize that other species are not things, but instead have thoughts, intelligence, and feelings that give their lives meaning, and usually this is what piques a child's interest, respect, and appreciation (Bexell 2006). Although a long time coming, we now have solid scientific evidence that the mind of many animals shares emotions akin to ourselves, and thus conservation educators can confidently portray many nonhuman animals as sentient beings (for a review, see de Waal 2011) to engender respect, care, and nurturance.

Children have the capacity to determine intentionality between themselves and animals when given time to form safe relationships (Myers 2007). The differences and similarities between human and animal modes of communication can commonly be recognized by children, and animals offer a special realm of interaction and respect, even when intimate contact, such as with bats, is not usually possible.

Young children naturally and willingly empathize with animals and feel compelled to help animals in distress (Melson 2001; Myers 2007; Thompson and Gullone 2003). Child development specialists previously thought that children outgrew their affinity for and connections with animals, but now we recognize children's feelings of fear and/or disgust that develop later in life can become subconscious distancing mechanisms that arise after they learn incorrect information or negative attitudes from others. Children also often develop protective mechanisms when they learn that humans are killing farm animals for food as well as are killed purely for pleasure and human entertainment (i.e., trophy hunting, etc.) or by sick people from natural history museums. Children are forced to cultivate a perspective that animals are things rather than living sentient beings, because one has a harder time treating animals badly when knowing their emotions. Pertinently, children today often become aware that human-caused environmental destruction kills or displaces animals, which again causes unconscious distancing mechanisms to develop to protect their sensitive emotions (Myers 2007).

17.4.3.1 The Role of Social Learning Theory and Modeling

Rachel Carson, the globally recognized founder of the environmental movement, thought that for a child to keep alive, an inborn sense of wonder requires the companionship of an adult who shares and discovers with them the joy, excitement, and mystery of the natural world. Education programs targeted at caregiver adults can help them become rational and positive role models for how children learn about wildlife, especially the misunderstood species such as bats. Thus, although nurturing animals appears to be a universal human nature, appropriate nurturing must be guided by competent role models (Irwin 2003), and it is important to note that most learning occurs on a vicarious basis by observing other people's behavior and the consequences of that behavior (Bandura 1977).

Social learning theory provides the foundation for understanding how social models influence behavior (Carter et al. 2005; Kahn and Cangemi 1979; Melby et al. 1993) and especially environmental education (Horsley 1977; Krasny and Lee 2002). The capacity to learn by observation enables humans (and nonhuman animals) to acquire large, integrated patterns of behavior without having to form them by trial and error. Behavioral change usually begins when someone receives extrinsic incentives for a particular behavior (e.g., observing bats on a summer eve with a cool bat biologist), but to become incorporated into a person's psychological lexicon, intrinsic incentives (e.g., becoming a novice bat expert or citizen scientist) that are reinforced are usually necessary (Bandura 1977). For bats, this might include watching them swoop in the night sky with a parent, listening to their echolocation calls with a community naturalist, or seeing them drink from a pond with a seasoned bat biologist. Having role models whose behaviors differ from the expected can have profoundly positive effects on other people's attitudes (Harris and Evans 1973) especially toward animals such as bats that have traditionally been feared.

17.4.3.2 The Role of Empathy

A key connecting point for biologists to make in the early stages of exposure is the sharing of the deep and broad social and emotional lives of bats as well as their ability to feel pain. Empathy is a strong motivator, and educators can help children feel compassion for bats as individuals by describing the powerful bond between a mother and her pup as well as the bonds that form between newborns in a colony or shared parental care among adults of a colony. That bats form long-term friendships and even visit each other if they end up living in different colonies (Kerth et al. 2011) makes a powerful impression on children and even adults.

Hoffman (2000) showed that our highest morals are founded on empathic feelings we experience, often vicariously, toward others, and although his research focused on human–human bonds, the same theories can be applied to human–nonhuman animal bonding because the basic premise is that awareness of another's distress is a prosocial motivator that correlates positively with helping behavior. Empathic distress diminishes when one acts to help another, but, more importantly, distress will continue at a high level for a much longer period of time if one does not act to help another (Hoffman 2000). Thus, developing empathetic feelings in children around the destructive human behaviors that cause suffering and pain in animals living in nature may prove profitable in motivating them to take a stance against the injustices inflicted on the natural world, bats included. Hoffman (2000) recommends teaching children to look beyond their immediate situation and ask how their actions will affect others, not only now but also in the future, and this exercise can lead to meaningfully positive changes in the attitudes of children toward the natural world.

17.4.3.3 The Role of Playing in Nature

It has been well documented that for children, play in natural settings is very important physically and psychologically (Burriss and Boyd 2005; Hart 1997; Moore

1997; Louv 2005; Nabhan and Trimble 1994), and adult recollections of special places and preferred play areas in childhood usually involve nature (Chawla 1994, 1999; Sebba 1991). Natural areas have been found to satisfy some critical developmental needs of young children, and many scientists fear that without exposure to outdoor play, development will be hindered (Burriss 2005; Kahn 1997, 2002; Lee 2012). Increasingly, children have fewer opportunities to play outdoors, especially in natural or even seminatural areas (Nabhan and Trimble 1994; Louv 2005), and if this does not occur, children never develop a personal bond with the natural world (Louv 2005) and never recognize that all their food, air, and water come from nature. Biophilia, a term coined by Wilson (1984), describes the innate human affinity for the natural world. Wilson describes how a human tendency to focus on life and life-like processes might be based on biological need and integral to development as individuals (Kellert 1993; Wilson 1984).

17.4.3.4 Cross-Cultural Continuity of Concern

Something we feel that holds promise for bat conservation education programs involves understanding how cross-cultural continuity of concern toward animals may develop in children (Hoffman 2000; Myers 2007; Turiel 1983). The development of empathy in young children across cultures is built on feelings toward the suffering or discomfort of other people (Turiel 1983), but research on Chinese and American children shows that empathy for nonhuman animals also exists (Bexell et al. 2005). However, as discussed earlier, as children develop cognitively, they realize there are inconsistencies in what adults say and do to animals and the environment, causing discontinuity due to childhood anxieties (Myers 2007).

17.5 Influence of Culture in Our Relationship with Wildlife and Nature

17.5.1 Chinese Culture and Bats

Paradoxically, the intriguing history and the still present inclusion of bats in cultural mythology and symbolism in China are antithetical to the paucity of present-day knowledge of and irrational fears toward bats. Many bat experts have alluded to the apparent reverence for many species, including bats, in Chinese culture with hope for continued reverence and thus a desire to protect. However, much of the apparent reverence has long since eroded.

China is an ancient country possessing a profound cultural influence from Buddhist doctrine that insists on no killing and the idea that all life should be treated with kindness (Elvin 2004; Song 2004a, b). Also, traditional beliefs lying in neo-Confucianism and Taoism teach compassion for all living things (Tu 1998).

Fig. 17.1 Traditional bat motif carving

Yet this is not the frame of mind of most citizens in China today (Song 2004a, b). Few people in China care about the feelings of animals or possess any concept of animal welfare (Song 2004a) or understand how their personal behaviors impact wildlife conservation. Perhaps even more harmful, Chinese children are taught to fear rather than love many animals. Youth are taught to show sympathy to the weak, and hate the strong, for example, rabbits get sympathy, but tigers or wolves are to be killed. Even today, when children cry, grandparents say "if you don't stop crying, wolves will come to eat you." Common Chinese children's songs describe animals as dangerous or malicious, which may become ingrained as fear and possibly even disdain (Song 2004a), and this disdain may lead to cruelty toward animals, played out on bears (Foley et al. 2011; Ratloff 2005), sharks (Clarke 2004), tigers (Verheij et al. 2010), and countless others. Perhaps luckily for bats, they are rarely if ever noticed, it seems, by modern Chinese even though they blanket the skies of rural and urban China every summer (personal observations of first author, 2006–2012). The fact that bat populations in China are in steady decline (Zhang et al. 2009) shows the unwitting toll of environmental destruction, but also provides great opportunity for conservation effort.

Bats symbolize good luck in traditional Chinese culture, are beautifully depicted on cultural relics (Fig. 17.1), and are discussed in ancient literature and medical books as well as Confucian texts (Ye 2007). The many unique characteristics of bats inspired reverence, including their similarities to both mammals and birds, their nocturnal nature, a belief that they have perfect vision, their longevity, and their abilities to hang upside down and live in caves (Ye 2007).

Because of their mysterious nature, bats became known as "immortals" because they live in high mountain areas among legendary "immortals," such as statuesque Buddhists.

The legend of immortality is based upon animals (or plants) that live mysterious lives and become perpetual beings by, for example, drinking from a particular water source for long time in some mysterious place. Bats were considered this kind of animal and were thought to possess life-spans of approximately 1,000 years (Ye 2007). Their ability to hang upside down is another reason for being revered and considered symbols of luck because the phrase "bats hang upside down" 蝙蝠倒了 (bian fu dao le) sounds similar to福到了(fu dao le) which means "luck comes." The second character in their Chinese name 蝙蝠 (bian fu) has the same pronunciation as the word福 (fu) which means luck.

In the not-so-distant past, bats were commonly carved on doors, chairs, and pillars to invite luck and good fortune, but such elaborate items have become too expensive for common citizens to own. Also, many young people prefer modernly styled furniture, so doors and chairs with carved patterns have disappeared from most daily life in China. Another reason we feel this loss is prevalent is that life in China has become fast-paced in the rapidly developing society, and many do not have time to care about traditional practices. In addition, many other cultural influences have come to China, like American movies, including those that villainize bats and, in some cases policies were developed to disregard traditional practices. Also, young people do not get information about their traditional culture from their parents and grandparents anymore. Practices sometimes deemed unuseful include hanging images of bats on the wall and doors to wish 福 *fu* (good luck), hanging herbs on the doors, and drinking a kind of wine to disperse ghosts and illnesses. However, very recently some experts have made a call for passing on traditional culture, reigniting interest, practice, and reverence and have been successful in bringing back traditional festivals and some traditional practices. This recent change in attitude is an opportunity to revive past reverence and cultural significance of bats.

17.6 Conservation and Humane Education Programs

It is widely accepted that for conservation efforts to succeed, children need to be a primary focus because their morals and attitudes toward the natural world and animals are developing (Carson 1956; Cohen and Horm-Wingerd 1993; UNESCO 1997; Weil 2004; Wilson 1992, 1993, 1994). Young children are naturally curious and still very accepting of new information and ideas, which facilitates inquiry-based learning critical to environmental education (Bredekamp and Copple 1997; Wilson 1992). The ideas and opinions that form commonly turn into lifelong values, ethics, and morals. Young children are fast and eager learners, and the natural world (Burriss 2005) and animals (Myers 2007) provide endless memorable learning opportunities (Chaille and Tian 2005).

Although early years are significant in a child's development (Bredekamp and Copple 1997), many children today are growing up in a way that provides few opportunities to consider the importance and diversity of the natural world (Louv 2005). Commonly, children grow up in a world surrounded by over-consumptive

Fig. 17.2 Children at Number 7 Middle School in Chengdu, China, attending a lecture by bat biologist Dr. Rick A. Adams

automobiles, technology that keeps them isolated and indoors, disposable convenience items, and public media that claim "new" is always better (de Graaf et al. 2001; Louv 2005; Schor 1998). Zelezny (1999) found that environmental education was much more effective among participants who were 18 years old or younger than among adults. Thus, a productive strategy would be to focus predominately on young people as they may continue to advocate for wildlife preservation in the future (Fig. 17.2).

17.6.1 A Tested Program: Summer Camps at Chengdu Research Base of Giant Panda Breeding and Chengdu Zoo

What might interventions to replace irrational fear and disgust toward bats with a conservation ethic look like? To provide ideas toward this goal, we share an intervention created to instill care, respect, and compassion for animals in general during a children's summer camp in China. We designed the intervention around conservation psychology (Clayton and Myers 2009), social learning theory (Bandura 1977), empathy and moral development theory (Hoffman 2000), sociobiology (Wilson 1984), constructivist theory (Vygotsky 1978), and conservation science (Primack 1998). Activities were designed to be sensitive to Chinese culture and included Chinese-specific conservation issues. It was designed to help children form bonds with animals and positively influence their behavior toward nature.

The program consisted of a 5-day-long, overnight camp experience developed for children ages 8–12 and took them along what we call a "continuum of care" (Bexell 2006; Bexell et al. 2009). Students first intimately met small animals (rabbits, guinea pigs, hamsters, parakeets, and tortoises) wherein they were allowed to recognize them as individuals with distinctive personalities and feelings not unlike ourselves. This was followed by less intimate exposure to more exotic species (including giant pandas, red pandas, zebras, golden monkeys, giraffes, and lemurs). Children were then given responsibilities for the care of animals and were actively encouraged to bond with individual animals and think about their emotional state. Our hope was that these intimate experiences would not only instill feeling of care about individual animals, but also extend to a deepening feeling about the environment on which all animals depend, and thus destroying an animal's environment is akin to harming individuals directly. Our hypothesis was that students would be able to make these intimate connections and thereby choose to change their own personal behaviors that harm the environment and wildlife. Near the end of camp, we asked children how our activities that harm the environment might impact the animals they came to know, and we empowered them to talk with their friends and family about their newly found respect for animals and worries about how their environments are treated. Going in, we knew that Chinese culture today generally did not promote positive attitudes toward animals and that consideration of animals' emotions, feelings, and sentience is silly. However, we also realized that with the westernization of China has come an acceptance for the first time of having dogs and cats as pets (versus just working animals) of which feelings of intimacy and love toward nonhuman animals are inherent outcomes, and so the time is right to broaden those feelings to the natural world.

17.6.1.1 Critical Aspects of the Camp

Human–Animal Bond

The camp was designed to foster the human–animal bond in participants as well as provide concrete ways to care for animals and nature (Sobel 1996). Knowledge about animals both in the wild and in captivity was presented in appropriate contexts to elicit emotional feelings from children in a safe environment wherein expressions of love and compassion for animals were acceptable behavior. Our curriculum highlighted those traits that are similar between them and nonhuman animals, but also revealing specialized characteristics of animals to help pique their interest in, and acceptance of, the unique natural history of our diverse world (Hoage 1989). Bats provide a unique and compelling natural history that children, and adults, are naturally drawn to and thus provide an inspiring and stimulating experience that will not be forgotten.

Multiple Points of Contact Between Children and Animals

Another foundational component of our camp program is the provision of *multiple points of contact* with the same animal (Myers 2007; Shepard 1996), in that children need to develop trust whenever they meet a new animal and, of course, this is also true for the animals coming to trust a new human contact. Children acquire trust after observing how an animal reacts to stimuli in different situations, and thus using multiple experiences, a child can see consistency of behavior, and thereby map intentions and understand the basic personality of the animal and therefore feel they can predict the animal's behavior in future interactions (Myers 2007). Thus we provide repeated experiences that allowed an animal to become a familiar acquaintance that establishes a human–animal attunement (Lasher 1998; Myers 2007).

Play in Nature

That children are spending less and less time outside and especially in natural areas where they can explore and learn about the world is becoming a common global condition. Thus, another of the main goals of our camp experience provided both structured natural exploration experiences and also time for free play in natural areas. One of the highlights of the camp for children was constructing and sleeping in tents outside, something none of our participants had ever had the chance to do previously. Not only did this foster connection to nature but also independence and self-confidence in their abilities to care for themselves and peers in a completely new setting and environment with unique challenges.

Evaluating Outcomes of the Camp Experience

In evaluating our camp program, we found statistically significant increases in knowledge about, and positive attitudes toward, animals and the environment. Our observations of children's behavior showed significant change toward a reduction in negative behaviors, such as littering or shouting at animals or making derogatory statements (e.g., "This monkey is stupid."), and increases in the desire to take better care of animals and the environment in the future (Bexell 2006). We found that basing children's outdoor education on the human universals of compassion and morality with solid scientific knowledge of animals and natural systems provided a profound and significant psychological shift in how children view the natural world and their responsibility toward limiting environmental destruction and the loss of biodiversity. Such shifts in human psychology need to occur not just in China but globally, and we feel that getting students out in nature for an extended period of time with immersion into conservation education is paramount and mandatory for any efforts toward the conservation of habitats and species, including bats, if there is any hope of curtailing the sixth mass extinction event on Earth.

17.7 Educational Interventions for Bats

Our use of the word "intervention" herein refers to methods designed to change pathologically destructive human behavior that results in environmental degradation and the massive loss of biodiversity currently under way globally. Such an intervention can be modeled similarly to those designed to combat other maladaptive behaviors that lead to pathological outcomes such as obesity, drug addictions, and hoarding. We now turn specifically to the conservation of bats and how conservation education can be used to undermine engrained attitudes based upon mythology and misunderstandings of the natural history of this group.

17.7.1 Elements of a Program for Bats

The question that must be foremost in the minds of anyone interested in designing an educational intervention for bats is: *What might change maladaptive attitudes and replace them with appreciation, respect, and eventually reverence for bats?* Educating people about how bats are similar to us in behavior, intelligence, and sentience along with the introduction to bats as individuals and their ecological importance and ecological services (e.g., children's literature *Stellaluna* (Cannon 2007) *Max & Bart* (Harris 2013)) (Fig. 17.3) provides a powerful starting point. This can be followed by the amazing aspects of what make bats unique from us and

Fig. 17.3 Cover image of the comic book *Max & Bart* by Cindy Harris that follows the life of a young boy named Max as he meets and learns from his Chiropteran peer, Bart

a special component of our diverse world (e.g., flight, physiology, colony formation, and migration). Taking groups out to actually observe bats on multiple occasions under different settings providing for multiple points of contact and experiential learning to counteract misconceptions and waylay irrational fears. Observations of bats should be narrated by someone who finds bats beautiful, intelligent, and skillful thereby combining verbal, social, and experiential learning activities designed to change the attitudes toward much maligned species to allow people to see theirs were foolish and illogical fears.

17.8 Conclusions

In closing, the rate of loss of wildlife and natural places on Earth necessitates that educators from all walks of life take responsibility for fostering the next generation's attitudes and feelings about the natural world to promote reverence and preservation. In the bat conservation community, we have a lot at stake as bats are losing ground. Herein we provide proven interventions with children that can be modeled for conservation outreach and education anywhere in the world. Our work in China provided unique cultural, but also common, challenges for conservation education and interventions, globally. Without education aimed at changing attitudes across all age groups, the future of our world is dim indeed.

Acknowledgments We would like to thank two anonymous editors and Dr. Rick Adams for excellent advice and editing to improve the quality and clarity of our chapter.

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Chapter 18
Threats to Bats and Educational Challenges

Merlin D. Tuttle

Abstract Like most animals, bats are threatened by habitat loss and degradation. However, they are also uniquely threatened almost universally by humans. In this chapter, I will emphasize the educational issues I believe will be most important to the next generation of bat conservationists. Though threat levels and possible solutions vary widely, the importance of addressing unfounded fear cannot be ignored. Putting disease concerns in perspective has been essential throughout the history of bat conservation efforts and is currently a resurgent issue that threatens the educational progress that has been made in recent decades.

18.1 Introduction

18.2 Early Challenges in North America

Needs for bat conservation were first officially recognized by scientists at a meeting of the American Association for the Advancement of Science (1971) where researchers concluded that bats were rapidly disappearing, ecologically essential, and in need of immediate help (Henshaw 1972). Leading newspapers and magazines published intensely scary stories that often bore no resemblance to reality (Cox 1980; Okie 1979; Remsburg and Remsburg 1977), creating extreme fear of bats (Brass 1994) that became highly profitable for pest and public health industries (Gallager 1977; U.S. Environmental Protection Agency 1980; Strohm 1982; Anonymous 1984).

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_18,

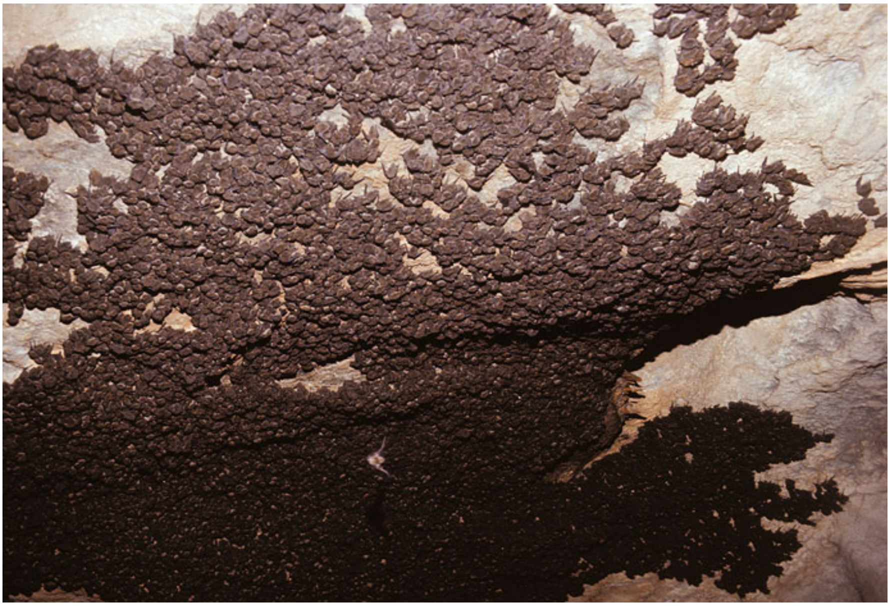

Fig. 18.1 Hibernating gray bats (*Myotis grisescens*) in Hubbards Cave, Tennessee. Protective gates have enabled this once nearly extirpated population to rebuild to more than 500,000

In May 1976, the US Centers for Disease Control and Prevention (CDC) began officially issuing DDT for poisoning bats in buildings that not only needlessly harmed bats but created far greater public health risks than the bats themselves (Barclay et al. 1980; Brass 1994; Kunz et al. 1977; Trimarchi 1978). Denny Constantine, the leading authority on bat rabies, condemned the nationwide drive to create hysteria and the resulting use of exceptionally dangerous chemicals to kill bats. He concluded that "The public health problems posed by bats are relatively insignificant compared to the public health problems usually initiated by those who publicize bats as problematic, typically resulting in an exaggerated, inappropriate response, damaging to the public health" (Tuttle 1988a).

By the time I founded Bat Conservation International (BCI) in 1982, most Americans believed that bats were frequently rabid and would attack people and pets. Bat killing was not limited to those living in buildings (Gillette and Kimbrough 1970; Mohr 1972) but also in natural caves (Fredrickson and Thomas 1965) that housed endangered species (i.e., gray bats) wherein entire colonies were exterminated (Tuttle 1979), resulting in a federal listing as endangered in 1976 (Fig. 18.1).

Help for bats would be impossible without first dispelling widely believed public health myths. Since virtually everyone already "knew" bats were dangerous, our first priority was to present an exceptionally well-documented case to rebut almost universal propaganda. We published a thorough and complete, peer-reviewed paper distributed to US state health departments (Tuttle and Kern 1981). We provided workshops and seminars and collaborated with honest experts who helped gain passage of state legislation in Wisconsin outlawing pesticide use against bats (Wisconsin State Assembly Bill 630). We also shared our experience and information widely through BCI members, empowering them to gain similar progress in additional states; exaggerated claims would be rebutted. Using positive photographic portrayal of bats, we provided pro-bat articles to most of America's leading wildlife magazines and published a layman's handbook *America's Neighborhood Bats* that put risks and benefits in perspective and clearly explained how to solve bat problems or identify honest pest control companies (Tuttle 1988a).

Putting risk into perspective, we noted that rabies from bats kills one or two Americans annually (Johnson et al. 2010), whereas the risk of death from driving 1 mi. in a motor vehicle is greater than the per-year risk of death from rabies in America (Tuttle 1999), and falling vending machines, shaken by irate customers, kill more Americans each year than does bat rabies (Olnhausen and Gannon 2004). Periodically, Americans still experience dire warnings about bats, usually by states with the largest, unjustifiable budgets for rabies control. States that have "passive" rabies prevention programs, wherein people are informed of bite risks, the need for pet vaccinations, and timely reporting of any bites, suffer no more human rabies mortality than states with "active" programs supported by large budgets for surveillance and prevention. In fact, it is extremely unlikely that stricter guidelines or warnings about bats could further reduce this consistently rare problem (Canada Communicable Disease Report 2009; Tuttle 1999).

A 1996 "Bat Rabies Alert" in New York illustrates how counterproductive such campaigns can be. Despite only one bat-transmitted case of human rabies in the state's history, a "bat rabies alert" was declared, and tens of thousands of posters, stickers, and other warning materials were distributed at schools, camps, and fairs, resulting in media stories claiming that bat rabies can be transmitted without contact (Tuttle 1999). New York health officials began vaccinating people who reported seeing bats fly through their yards. Dr. Brendon Brady (Chairman, Board for Finger Lakes Community Care, NY) documented a staggering 398 % jump (46 in 1996 to 183 in 1998) in rabies vaccinations (~$2,000 per person) and wrote, "I am outraged that the Health Department policy is wasting millions to prevent the rarest disease in New York" (Tuttle 2000).

Participants at the 29th Annual North American Symposium on Bat Research (1999) unanimously passed a resolution stating no credible support (Mlot 2000) for the CDC's policy advising postexposure prophylaxis of "persons potentially exposed to bats even where a history of physical contact cannot be elicited" (CDC MMWR 1995). Public health studies from Oregon, Connecticut, and Canada (Cieslak et al. 1998; Serres et al. 2009) independently concluded that CDC's policy had negligible impact in preventing rabies. Oregon found that by following CDC's advice, they would, on average, prevent no more than a single human death per 75 years at a cost of more than $180 million (Cieslak et al. 1998). Canada's National Advisory Committee on Immunization (NACI) abandoned CDC's policy even though bat-transmitted rabies rates are similar to the USA, just 6.7 cases per billion person years (CCDR-NACI 2009).

Worldwide, more than 50,000 humans die of rabies each year (Johnson et al. 2010) with 99 % coming from dogs. And human infection from other lyssaviruses accounted for just five human deaths in Africa, two in Australia, three in Europe, and none in Asia or the Pacific Islands (Johnson et al. 2010).

18.3 Current and Future Challenges

18.3.1 Emerging Diseases

Due to increased reporting of rare but potentially problematic zoonotic diseases, fear of bats is reemerging as a major threat to their conservation. Bats occupy a wide variety of ecological niches, so it is not surprising that diverse viruses have coevolved with them. Most of these bat-borne viruses are probably harmless to humans, but circumstantial evidence suggests that, in addition to rabies, bats may be reservoirs or intermediate hosts for rare but frightening diseases such as Hendra, Nipah, Marburg, and Ebola viruses that can cause lethal encephalitis or hemorrhagic fever in humans (Carrington et al. 2008; Calisher et al. 2008; Wibbelt et al. 2010).

Horseshoe bats (*Rhinolophus*) are hypothesized to be the reservoir for the coronavirus that causes SARS. When a related virus was found in bats, it was presumed to have been transmitted from bats to civets caged in proximity in Asian animal markets. Unfortunately, the hypothesis was presented as fact (Fenton et al. 2006), triggering an onslaught of sensational media warnings of dangerous bats (McKie 2005). Because horseshoe bats are seldom sold in animal markets, the initially hypothesized route of transmission is unlikely. Furthermore, Janies et al. (2008) concluded that though horseshoe bats possibly were involved in the early evolution of SARS, it most recently appears to have spread from humans to civets and pigs.

Coronaviruses have been identified in bats worldwide (Dominguez et al. 2007; Quan et al. 2010; Tong et al. 2009; Watanabe et al. 2010). However, these viruses are also widespread in other animals from birds (Hughes et al. 2009) to whales (Mihindukulasuriya et al. 2008) and normally are benign or cause no more than common colds (Chua et al. 2008; Hun 2011). Most evidence linking viruses to bats is based on discovery of antibodies which does not establish infection or disease by that virus and also ignores what role, if any, bats play in that virus' natural cycle (Calisher et al. 2008).

Disproportionate emphasis on identification of bat reservoirs could prove counterproductive to disease prevention. Leroy et al. (2004) concluded that circulation of Ebola has involved many contamination events among animal species over long periods prior to modern detection and that multiple reservoirs may be involved, including some nonhuman primates, rodents, and shrews. Some experts speculate that filoviruses, such as Ebola and Marburg, may be of plant or arthropod origin with bats and other animals serving only as intermediate hosts (Calisher et al. 2008). Furthermore, Allela et al. (2005) found that up to 32 % of domestic dogs in outbreak areas had Ebola antibodies and could shed the virus without visible symptoms.

Fig. 18.2 Part of a massive colony of straw-colored flying foxes (*Eidolon helvum*) at Kasanka National Park in Zambia that consumes an estimated 6,000 tons of fruit and nectar per night. Their impact on seed dispersal and pollination is enormous, covering a huge area of equatorial Africa during annual migrations

During the 1994 human Ebola outbreak in northeastern Gabon, gorillas, monkeys, and bush pigs were dying and being scavenged for human food. That miners contracted Ebola was assumed due to associating with bats in the mines even though the outbreaks seemed most closely associated with humans scavenging bushmeat (Lahm et al. 2007). In only one instance has bat hunting appeared to be associated with a human case despite the fact that large numbers of straw-colored fruit bats (*Eidolon helvum*) are sold for human food (Leroy et al. 2009). Regardless of whether or not bats are reservoirs for Ebola, direct transmission from them to humans or livestock is exceedingly rare, if it occurs at all (Fig. 18.2).

Other sources of potential confusion are seen in the search for reservoirs of Hendra and Nipah viruses. Hendra has been hypothesized to be transmitted directly from bats to horses when horses eat flying fox placentas (Patterson et al. 2011), though this seems unlikely given that births normally occur in isolated roosting “camps” in forests and that horses are not known to scavenge. Experimental transmission has been demonstrated from cats to horses (Messenger et al. 2003), and barn cats would clearly scavenge placenta, potentially bringing the virus to horses. If true, horses might be protected by isolating them from cats rather than culling an endangered flying fox as proposed by horse breeders (Nolan 2011).

When Nipah virus was discovered in Southeast Asia, wildlife surveillance again focused primarily on bats (Chadha et al. 2006). Contamination of fruits or raw date palm juice by *Pteropus* was widely publicized, though bat biologists were skeptical

(Fenton et al. 2006) and subsequent attempts to isolate Nipah virus failed (Gurley et al. 2007). Human exposure histories varied greatly among outbreaks, suggesting the possibility of multiple sources (Homaira et al. 2010; Montgomery et al. 2008). Johara et al. (2001) reported that 46 % of 92 domestic dogs sampled near infected pig farms tested positive for Nipah antibodies.

We must be concerned about infectious diseases and the roles that wildlife, including bats, may play in their epidemiology. However, when public health is the focus, those who are concerned with conservation must insist on careful consideration of documented facts (Fenton et al. 2006). An editorial in the journal *Nature* cautioned that "The intrinsic conflict between the mandated need to inform the public of potential health hazards and the need of the media for sensational headlines is threatening to compromise the scientific process." The warning was, in part, a response to exaggerated stories about bats (Ashby 1996).

Even if we were to lump all human deaths from Hendra, Nipah, Marburg, Ebola, and SARS viruses together and assume contact with bats to be the sole cause, bats still would be credited with fewer than 3,000 human fatalities globally in the past 40 years, mostly in remote areas where primitive health care is a major contributing factor. In the USA alone, foodborne illness causes an estimated 5,000 human deaths/year (Mead et al. 1999). Balancing public health versus conservation needs can be challenging (Messenger et al. 2003), especially because governments predominantly fund health issues that frighten us most, not necessarily those which pose the greatest threat (Finley 1998).

Lasting progress will require cooperation between virologists and bat biologists. Bats are simply too important to healthy ecosystems and economies for eradication to be considered (Brass 1994; Wibbelt et al. 2010). Furthermore, attempts to eliminate disease through wildlife control typically exacerbate problems while threatening ecosystem balance (Macdonald 1977) (Fig. 18.3).

Sensational coverage of potential risks from bat-borne disease has already created demands for flying fox eradication in Australia (e.g., Nolan 2011) and questioned the wisdom of allowing large colonies of straw-colored fruit bats to congregate in city parks of sub-Saharan Africa (Drexler et al. 2009). Attempts to evict such bats would likely expose them to unsustainable hunting pressures, threatening whole ecosystems that rely on their pollination and seed dispersal (Racey 2004; Chap. 23) (Fig. 18.4).

There is an urgent need to rein in the recent wave of sensational reporting (e.g., Hart 2006; Gilbert 2011). Despite the fact that millions of straw-colored fruit bats have thrived in city parks across Africa, there is no evidence of harm to humans. Nor does such evidence exist in Australia where large flying fox colonies occupy urban areas and rehabilitators care for hundreds of sick, injured, or orphaned flying foxes annually (Selvey et al. 1996).

Certainly, if bats were even remotely as dangerous as has been hypothesized in recent zoonosis literature, major disease outbreaks should have been documented long ago among the millions of people in Asia, Africa, and the Pacific and Indian Ocean Islands who have hunted, sold, or eaten a wide variety of bats throughout human history (Bergmans and Rozendaal 1988; Mickleburgh et al. 2011). Furthermore, since numerous bat populations have been in steady decline for

Fig. 18.3 Flying foxes are primary pollinators for many of Australia's most ecologically and economically important trees. This gray-headed flying fox (*Pteropus poliocephalus*) is pollinating a broad leaf apple tree (*Angophora subvalutina*)

Fig. 18.4 These young bat hunters earn a living shooting straw-colored flying foxes (*Eidolon helvum*) with sling shots in a city park in Abidjan, Ivory Coast, where bat-eating is very popular

decades as a result of overhunting, some being driven to extinction up to 100 years ago (Pierson and Rainey 1992; Racey and Entwistle 2003), it is unlikely that humans are at greater risk today than in the past. Surprisingly, not even guano mining in major, occupied bat caves has been identified as a source of human illness. Clearly, bats have a remarkable safety record.

The story of bats in Austin, Texas, provides an excellent example of how bats and humans can safely coexist when bat conservationists, educators, and public health officials cooperate in presenting a balanced message. In the early 1980s, as 1.5 million Brazilian free-tailed bats (*Tadarida brasiliensis*) began moving into newly created crevices beneath the Congress Avenue Bridge at city center, public health officials warned that they were rabid and dangerous. Sensational news stories made national headlines. People panicked, and the bats were nearly eradicated. BCI met with leadership individuals in the health department, city government, and local news media, gaining their cooperation to present a balanced educational message, not scaring people, but warning them not to handle bats. As a result, the community has benefitted greatly. The bats consume 15 tons of insects nightly and attract 12 million tourist dollars each summer. Furthermore, not one of millions of bat watching visitors has contracted a disease despite extraordinarily close association.

18.3.2 Vampire Control

Latin America is home to three species of vampire bats, one of which, the common vampire (*Desmodus rotundus*), has greatly overpopulated due to the introduction of livestock. Its bites are a primary source of rabies outbreaks that cost the cattle industry millions of dollars annually and kill approximately 50 humans (Acha and Alba 1988; Brass 1994; Schneider et al. 2009).

Not surprisingly, major efforts have been launched to eradicate vampires. Until recently, all bats were killed indiscriminately, threatening ecosystems and economies (Lord 1988; Tuttle 1988b; Brass 1994). Many thousands of bat caves and millions of bats were destroyed (Constantine 1970; Hutson et al. 2001). Such efforts have little or no impact in reducing vampire populations, since these bats typically live in colonies of less than 50 individuals, often unnoticed in wells (Brass 1994; de Oliveira et al. 2009). The most conspicuous bat colonies are highly beneficial but are the first to be eradicated. Even when vampire roosts are accurately identified, roost poisoning threatens entire species of beneficial bats that reclaim roosts after vampires are killed (Aguiar et al. 2010).

Several effective approaches for controlling vampires are now available, but too often nothing is done till after livestock begin to die of rabies making it too late to prevent an outbreak from running its course. Vaccines can provide protection when administered prior to an outbreak, and application of an anticoagulant (often chlorophacinone, diphacinone, or warfarin) mixed with Vaseline, either to wounds where vampires return to feed or to the bodies of vampires captured and released, can be highly effective (Fig. 18.5). Application on bite wounds works best for campesinos with relatively few animals in that it kills only the offending individuals, whereas pasting captured/released vampires works best for larger ranches. Through mutual grooming one pasted bat can kill up to 40 others (Brass 1994; Lord 1988). The latter method should be utilized only by trained teams working in front of advancing rabies outbreaks to create barriers against further spread. Limited

Fig. 18.5 Pasting a common vampire bat (*Desmodus rotundus*) with an anticoagulant poison mixed with Vaseline in Cost Rica. With minimal training, this is an effective tool that can be highly specific in targeting only vampires that are harming livestock

studies have indicated negligible impacts on carnivores and scavengers who may eat poisoned vampires, though potential unintended consequences should be monitored (Brass 1994; Burns and Bullard 1979; Lord 1988). As is, most humans can be protected from vampire bits by screening windows or by mosquito nets, backed up by vaccination when bites occur (Lopez et al. 1992; Peters 2004; Schneider et al. 2009). Unfortunately, education on appropriate controls has lagged far behind scientific knowledge (Brass 1994; Lord 1988), even though education to easily identify vampire bats can be highly effective in solving most problems (Brass 1994). BCI recruited two of Latin America's foremost authorities on vampire control, Rexford Lord of Venezuela and Hugo Sancho of Costa Rica, to produce a 26-min video, titled *Control del Murcielago Vampiro y La Rabia Bovina*, that covered the best available techniques for controlling problem vampires while also showing the importance of protecting beneficial species (Walker 2002). Many vampire control personnel became collaborators in bat conservation.

Unfortunately, many poor campesinos still view all bats as vampires. To help, BCI collaborated with Rodrigo Medellin and associates in Mexico to develop a series of 20 highly entertaining 15-min radio programs, "Aventuras al Vuelo," narrated by a well-known commentator. These aired throughout Mexico and appeared to be highly effective.

Finally, we must keep in mind that wholesale elimination of vampires is neither feasible nor desirable (Lord 1988), and they play an important role in balancing forest animal populations, and rank among the world's most sophisticated mammals, providing numerous opportunities for scientific research (Tuttle 1988a, b).

Vampire saliva contains a veritable treasure trove of novel regulatory molecules, one of which is providing a greatly improved treatment for stroke victims (Medcalf 2012).

18.3.3 Bushmeat

Bats have been hunted and consumed as food throughout human history, especially in tropical and subtropical areas of the Old World. Early harvesting by indigenous people was mostly sustainable, but as human populations expanded, habitat was lost, demand increased, and hunting techniques improved. As early as 100 years ago, some island species began to succumb to extinction as appetites exceeded supply (Pierson and Rainey 1992).

In recent decades, use of firearms and the expansion of commercial hunting have proven especially problematic. Though poorly documented, many populations appear to have been unsustainably exploited, producing alarming declines (e.g. Epstein et al. 2009). Mickleburgh et al. (2011) documented a wide variety of both microchiropteran and megachiropteran bats being used, with the largest species in greatest demand, especially those that form conspicuous colonies in trees or caves. Flying foxes (mostly *Pteropus, Acerodon,* and *Eidolon*) are particularly prized and are highly vulnerable at roosts and when they aggregate at fruiting and flowering trees to feed. Unfortunately, they are most frequently killed while rearing young making losses especially impactful. Smaller fruit and nectar bats that form large cave-dwelling colonies (mostly *Rousettus* and *Eonycteris*) are clubbed at roosts during emergencies or are caught in fish nets or glue traps, and entire colonies may be killed quickly (Craven 1988).

Except for the relatively large *Hipposideros commersoni* and *Cheiromeles torquatus*, most microchiropteran bats are not sold commercially. However, in parts of Southeast Asia, all cave-dwelling species may be hunted (Racey and Entwistle 2003), resulting in severe population declines and biological irrelevancy (Fujita and Tuttle 1991; McConkey and Drake 2006; Mickleburgh et al. 2011). Flying fox hunting is often deeply embedded in cultural tradition (Pierson and Rainey 1992), making legislation nearly impossible to enforce unless supported by local communities. In Madagascar, Rahaingodrahety et al. (2008) noted that hunting laws were widely ignored and suggested that locally managed harvests of foraging bats might be a viable option.

When commercial hunting threatened the survival of *Pteropus samoensis* in American Samoa, I met the hunters as a fellow hunter interested in bats, even accompanying them on several hunts to better understand their reasons for hunting (Fig. 18.6). After learning they shared my concerns regarding the dramatic decline and that they were not hunting from economic necessity, paid big dividends. Teamed with botanist Paul Cox and private funders, Verne and Marion Read, we were able to enlist the hunters' enthusiastic collaboration in gaining hunting regulations and eventually a national park to protect the bats' habitat. Though we hated seeing these bats shot, protecting sufficient numbers for future generations to hunt was better than trying to force cessation of all hunting and almost certainly failing.

Fig. 18.6 Samoan flying fox (*Pteropus samoensis*) riding mid-day thermals above rain forest

Where private land owners control access to bat caves, simple education that allays fear and promotes understanding of bat values can be highly effective. Working with owners of caves occupied by endangered gray bats (*Myotis grisescens*), I will never forget one who asked me to kill all the bats. I picked up a handful of potato beetle wings the bats had discarded beneath their roost and, on exiting, asked if he knew what kind of insect they were from. I had noticed he was growing potatoes and corn nearby. Of course, he recognized some of his worst crop pests and was surprised that bats ate them. When he asked how many insects his colony ate, I estimated about 100 lb nightly but pointed out that they didn't just eat potato beetles. They likely also consumed corn-ear worm moths and mosquitoes. From then on, he was an ardent bat protector!

More recently, responding to an urgent call to BCI for help from Norma Monfort, whose family owned an important bat cave in the Philippines that housed 1.8 million *Rousettus geoffroyi*, we found that several nearby caves had suffered 95–100 % losses due to overhunting (Fig. 18.7).

The government wanted her land for agricultural use, thereby destroying the last major bat colony. Over the next 10 days, we documented area decline, prepared and presented a PowerPoint program for community leaders, and through photographs showed that this one colony could pollinate millions of durian flowers per night. Durian-loving participants, including the mayor, immediately signed a petition requesting critical wildlife habitat status protecting the bats. Norma later established an interpretive center near the cave that attracts thousands of visitors.

Community-level education is extremely important, especially where strong leaders or owners control roosting resources. When I visited Thailand in 1982,

Fig. 18.7 Norma Monfort educates visitors to her Philippine cave regarding the importance of its 1.8 million Geoffroy's rousette fruit bats (*Rousettus geoffroyi*) as vital pollinators of area durian crops. Without such knowledge, her cave would have been destroyed by government mandate

nearly all accessible bat caves had been decimated by overhunting, and formerly huge populations were persisting with only dozens or hundreds of individuals. The only large colony that showed stability was in a small, vulnerable cave but was protected by a guard, hired by its owner to ensure continued guano production.

Khao Chang Pran Cave, about 100 km west of Bangkok, was owned by Buddhist monks who supported their monastery by selling guano as fertilizer. The monks explained that guano sales had fallen by 50 % over 5 years. I found that commercial bushmeat hunters were concealing large nets near one of the entrances, capturing more than 10,000 *Rousettu*s and *Eonycteris* per month, selling them to restaurants (Fig. 18.8). Moreover, thousands of *Tadarida plicata* (Fig. 18.9) and other small, insectivorous species were being killed making the impact clearly unsustainable. I suggested hiring a game warden and by 1990 annual guano sales had risen from $12,017 to $88,660 USD. A thriving, bat-viewing tourist trade had also developed (Tuttle 1990). By 2002, annual sales had risen to US $135,000, and the bats were consuming at least 4 tons of rice crop pests nightly (Leelapaibul et al. 2005). Local benefits undoubtedly included increased durian and petai production and reforestation supporting heavy charcoal demands. The immediate area was declared a no-hunting preserve with heavy fines for violatory.

In Myanmar, many large bat caves are protected from overhunting by local communities who rely on guano extraction (Bates 2003). In New Guinea and Nigeria, agreements permitted overhunted populations of *Dobsonia moluccensis* and *E. helvum* to recover following locally initiated limits on harvesting (Mickleburgh et al. 2011). In the case of *Dobsonia*, an important nursery cave was protected from

Fig. 18.8 Thai bat hunters preparing a night's catch of fruit bats (*Rousettus* and *Eonycteris*) for sale to local restaurants. Throughout the Old World tropics hundreds of thousands of pteropodid bats are caught and sold for human consumption annually without apparent harm to hunters or consumers, though many bat populations have been decimated

Fig. 18.9 Game warden hired to protect the bats of Khao Chang Pran Cave, in Thailand, watches millions of wrinkle-lipped bats (*Tadarida plicata*) emerge

hunting, and for *Eidolon*, hunting was permitted at a large tree-roosting colony but was confined to specific times and seasons. Initiation of restrictions need not be delayed till after detailed studies are conducted, and publicity of successes has far-reaching impacts for education and conservation.

18.3.4 Crop Damage

Fruit growers frequently report crop losses due to flying foxes. In the 1920s, complaints were so numerous in Australia that the governments of Queensland and New South Wales hired a distinguished British biologist, Dr. Francis Ratcliffe, to spend 2 years with a goal of finding a "wholesale method of destruction." He concluded, "The assumption that the flying fox is a menace to the commercial fruit industry is quite definitely false, and cannot be cited as a valid reason for expenditure of public money on its control." He also reported "a good deal of exaggeration," wherein fabrication and embellishment were common (Ratcliffe 1931).

Nearly 70 years later, I visited Jim Trappel, who had grown peaches and nectarines in New South Wales for 30 years. He initially referred to flying foxes as "black devils" that could wipe out a crop in just a few nights. However, he later admitted that he and his fellow growers had greatly exaggerated losses and that flying foxes seldom harmed his crops. He had suffered significant losses just three times in 30 years, wherein 80, 50, and 30 % of his crop was lost. Those were years when gum trees, the bats' preferred local food, had failed to flower. By comparison, he estimated that cute rosella birds (*Platycercus eximius*), which he liked, were damaging roughly 10 % of his unprotected fruits annually. Averaged over time, these birds cost him nearly twice as much as flying foxes, but when bat problems occurred, they posed greater short-term hardships.

To solve both problems, Trappel invested in netting to cover entire orchards, and he enthusiastically reported that the netting had additionally protected his crops against hail damage and led to earlier fruit ripening and higher market prices. With this excellent return on investment, he became a netting distributor. A government-commissioned study confirmed Trappel's basic assessment, both of damage and remedies (Fleming and Robinson 1987), and the government now offers low-interest loans to assist with exclusion netting. Some of the greatest opportunities to further the conservation of bats begin when we genuinely investigate real or perceived nuisance problems, and although many claims are based entirely on misperceptions, these should not be denied without careful investigation.

One of the most harmful, yet widespread and firmly held misperceptions, is that flying foxes harm durian crops by preventing fruit set. Durian trees (*Durio zibethinus*) produce large numbers of flowers that are highly reliant on bats (Fig. 18.10) for pollination (Bumrungsri et al. 2009) after which flowers drop petals, presumably helping hungry bats locate receptive blossoms. When farmers see large numbers of bats triggering a steady rain of flower parts, they assume the bats are destroying flowers.

Fig. 18.10 A dawn bat (*Eonycteris spelea*) pollinating durian (*Durio zibethinus*). Several pteropodid bat species are the exclusive pollinators of this king of Southeast Asian fruits

Even after I convinced them that bats are essential pollinators, some still insisted that they must be harming their crops by sometimes knocking off whole flowers. At that, I point out that the weight of too many large fruits could break branches and that by preventing some fruit development, those remaining have improved quality.

While conducting bat research along the Mombasa Coast of Kenya, I repeatedly heard that bats were a scourge to mango (*Mangifera indica*) and other fruit crops (Tuttle 1984). I examined thousands of mangos during harvest, interviewed growers, and conducted controlled experiments of fruit ripeness preferences of bats. Although many mangos were eaten nightly in orchards, none of them were commercially harvestable nor would captive bats of six species consume harvestable fruit even when deprived of other food (Fig. 18.11). Akberkhan Khan, the largest mango exporter in Kenya at the time, explained that mangos ripe enough to attract bats are too ripe for harvest, and by removing those mangos, bats reduce breeding opportunities for fruit fly and fungal pests. Some growers often mistook bush baby and monkey tooth marks in harvestable fruit for bats. In Malaysia and Indonesia, growers of rambutan (*Nephelium lappaceum*), langsat (*Lansium domesticum*), and water chestnut (*Eugenia aquea*) reported that bats threatened crops only for the last few nights prior to harvest and that most problems could be avoided by using bright lights or fires (Fujita and Tuttle 1991).

Fig. 18.11 An Egyptian fruit bat (*Rousettus aegyptiacus*) eating a ripe mango, missed during harvest. Removal of such fruits aids growers in reducing harmful fruit flies and fungi

When growers and government officials lack knowledge, serious harm can be inflicted. In 1958, Israeli government officials began fumigating caves used by *Rousettus aegyptiacus* using ethylene dibromide and later lindane, in response to fruit grower complaints. Entire cave ecosystems were lost as were approximately 90 % of insect-eating species followed by population explosion of noctuid moths that caused major crop damage (Makin and Mendelssohn 1985). Massive eradications were also carried out in Cyprus (Hadjisterkotis 2006). Unfortunately, fruit bats are still classified as vermin (Singaravelan et al. 2009) even in countries like Australia where much progress has been made. The New South Wales Farmers Association recently called for a government cull of endangered flying foxes (Cox 2011). Objective documentation and education continues to be of paramount importance in dealing with fruit damage.

18.3.5 Environmental Degradation

Agricultural pesticides and herbicides, industrial and sewage discharges, light pollution, monoculture farming and forestry, and even campfire smoke in parks likely influence bat health and survival, but these impacts are seldom monitored and remain poorly understood.

Despite minimal investigation of insecticide impacts on bats, it is clear that populations have been harmed (Clark et al. 1978; Clark 1981, 2001; Mohr 1972; Reidinger 1972; Sasse 2005). Sublethal effects could seriously compromise immune functions, impair flight, and intolerably increase hibernation metabolism

(O'Shea and Clark 2002), and the spraying of forests for gypsy moths harms nontarget insects that bats consume (Sample and Whitmore 1993). In the UK, bats showed a clear preference for feeding over organic versus conventional farms (Wickramasinghe et al. 2003).

Fortunately, when optimal roosts have been protected, or new ones provided, bat populations typically have grown. The fact that the endangered *M. grisescens* has undergone dramatic recovery based solely on roost conservation, despite mortality from agricultural insecticides (Clark et al. 1978, 1983), clearly indicates that environmental degradation has not yet become a primary cause of decline for this species (Tuttle 1986), and there is no evidence to suggest differently for the endangered Indiana bat (*Myotis sodalis*) (O'Shea and Clark 2002). The industrialized world is on a treadmill that constantly demands stronger pesticides in ever larger quantities to kill increasingly resistant pests (Isenring 2010). When DDT was banned in Missouri, it was replaced with aldrin which also was banned, only to be replaced by heptachlor, then endrin, both of which were banned. All killed bats (O'Shea and Clark 2002; Sasse 2005).

Modern neonicotinoids are so effective at killing insects that insect-eating birds are declining (Gabel 2010). Bat guano may be the most sensitive indicator for environmental monitoring (Clark 1988; Sasse 2005). At every opportunity, conservationists need to help educate a naïve public regarding the benefits of reduced use of pesticides, including Bt toxins (Altieri and Nicholls 2001; Pemsl et al. 2007; Turnbull and Hector 2010). Collaboration with organic growers presents a variety of excellent opportunities for bat conservationists.

18.3.6 Elephant Overpopulation

In Africa, I have found no greater threat to bats than the devastation of their woodland habitats by overpopulated elephants that have nowhere left to go due to human overpopulation. Healthy populations of elephants provide essential ecosystem services, but because they have been pushed into small, restricted parks, overpopulation has led to a crisis. As early as 1983, Barnes noted that overpopulation had caused dramatic reduction in woodlands and that regeneration was no longer possible, raising concerns regarding how the biodiversity of parks and nature reserves could survive (Cumming et al. 1997; Grant et al. 2011) and in some cases damage that cannot recover in a 100 years or more has expanded across vast areas of Southern Africa.

In Botswana, I visited bat roosts in huge, hollow baobab trees in 2000 that had been totally destroyed by 2003. It was sobering to see how quickly giant trees of such vital ecological importance could be entirely eliminated from a large area. There is an urgent need for wildlife managers to establish humane, ethical procedures to control elephant populations. Unfortunately, culling elephants may at times be necessary, but this does not provide a long-term strategy for controlling populations. Contraception may be effective (Delsink et al. 2006) and should be relied upon wherever feasible.

18.3.7 Feral Cats

Feral and unsupervised domestic cats kill a wide variety of wildlife (Dauphine and Cooper 2009), and their numbers have more than doubled since 1970 (Clarke and Pacin 2002). They are especially effective at catching bats as they return to roosts. Their impact can be serious because most bats produce only one young per year. Bat conservationists should support efforts to humanely control feral cats and collaborate in educating the public to keep cats indoors, contributing greatly to the safety of both cats and wildlife. Land managers should be informed regarding the potential threat of cats at bat roosts, especially in front of protective gates that if built in narrow cave entrances force bats to slow and circle, an invitation to unnatural predation. Plans are available for gates that permit safe entry for bats, improving the odds of escaping unnatural predators (Fant et al. 2009).

18.3.8 Habitat Loss

Habitat loss is a well-documented threat, though the specific needs of bats are complex and often poorly understood. Most species require multiple roosts of varied temperature, a mosaic of foraging habitats, and lengthy nightly and seasonal travel may be required to access food or roost resources (Racey and Entwistle 2003). Simply encouraging landowners and managers to protect woodlots, wetlands, hedgerows, multiage forests, and small ponds can be very helpful.

Resource needs vary regionally. Where caves or large, hollow trees have long been available, many bats have become highly dependent upon them. In temperate regions, safe locations for winter hibernation are especially critical, and in tropical and subtropical climates, large populations may require caves year round. Loss of just one can have devastating regional impacts (Tuttle and Stevenson 1978; Stebbings 1988).

Caves and mines that provide the greatest volume and structural diversity have attracted the world's largest, most diverse bat populations because they provide wide temperature ranges and a large volume buffers against extreme weather variations. Caves having multiple entrances also help protect against predators. Unfortunately, such caves are highly used for commercial recreation.

The story of Mammoth Cave, Kentucky (Fig. 18.12), illustrates how huge bat populations have been lost in a manner making recovery exceedingly difficult. This cave once sheltered at least ten million bats and appears to have been dominated by the now endangered Indiana bat (*M. sodalis*), a species once ranked among America's most abundant mammals (Tuttle 1997). Like most caves used by major bat populations in eastern North America, Mammoth Cave was extensively altered during saltpeter extraction for gunpowder as early as the War of 1812 and again during the Civil War. Entrances were blocked to make the cave warmer for humans, and passages were widened for easier access. By 1820 additional alterations were

Fig. 18.12 Tour group entering Mammoth Cave in Mammoth Cave National Park. Millions of bats, dominated by the now endangered Indiana bat (*Myotis sodalis*), relied on this huge and diverse cave for winter hibernation prior to its early commercialization

made for commercial tourism. By the time Mammoth Cave National Park was founded in1941, the bats were gone (Toomey et al. 2002).

Due to the loss of key overwintering caves, Indiana bat populations plummeted from millions in the early 1800s to just 380,000 range-wide by 2001 and have failed to recover despite protection of caves sheltering the largest remaining populations. Unfortunately, early efforts to reverse decline focused almost entirely on protecting hibernation caves from human disturbance, with little or no regard for restoring altered microclimates. Also, substantial efforts were made to protect caves where relatively large numbers of desperate bats were barely surviving marginal conditions. When populations were compared over a 20-year period, those whose roosting temperatures fell within the species' preferred range increased, whereas those that did not declined alarmingly. In fact, some caves protected as "critical habitat" served as periodic death traps during widely spaced, but lethal, weather events.

Illustrative of the dramatic progress made when a bat's roosting needs are met, the endangered gray bat was in such precipitous decline in the 1960s and 1970s that extinction was predicted (Barbour and Davis 1969). Yet, through protection of key roosting caves, mostly with bat gates, millions of gray bats have been restored (Martin 2007). Bellamy Cave's (Tennessee) once large hibernating population had crashed due to intense human disturbance, but began recovering when I convinced the owner to prevent further disturbance. Numbers again plummeted when he partially obstructed airflow through the main entrance resulting in only 65 remaining gray bats in 1974. However, after the obstruction was removed, the hibernation area temperature dropped by an average of 2°C, and the population steadily grew to exceed 150,000 by 2010 (Samoray 2010). Early protective gates often did more harm than good by obstructing essential airflow and/or aiding predators (Tuttle 1977). However, new designs have succeeded in restoring previously declining bat populations (Fant et al. 2009).

Experience illustrates the extreme importance of educating cave owners and managers on how best to protect and manage bat roosts. Increasing numbers of private operators are finding bat conservation to be good business and are setting aside protected areas. Tour cave operators often are willing to cooperate enthusiastically in protecting bats and/or in educating the public to appreciate them. Some species, such as *Perimyotis subflavus*, are adapting to tolerate disturbance, allowing tourists as close as two meters, adding interpretive interest.

When we documented that Saltpetre Cave, a Kentucky state-operated tour cave, had been occupied by a large hibernation population of Indiana bats, managers enthusiastically ended winter tours. As a result, the Indiana bat population grew from 13 in 1999 to 6,088 by 2005. At Wyandotte Cave, Indiana, the once huge population recovered from 2,500 in 1977 to 45,500 in 2008 due to removal of an airflow-blocking gate and cessation of winter tours (Dunlap 2009). Even greater recovery could have been achieved if government administrators had permitted entrance restoration to prevent leakage of cold air (Tuttle 2005).

Although the largest, most complex caves and mines are typically best for bats, when contours provide cold versus warm air traps, even relatively small sites are important. Knowledge of bat needs and how structure impacts airflow and temperature in relation to recognition of stains in formerly occupied roosts are invaluable in selecting the best sites for protection (Tuttle and Stevenson 1978; Tuttle 1979). Rapid population growth in the protected Magazine Mine in Illinois, illustrates how quickly bats find and occupy even artificial sites with ideal conditions.

Hard rock mines, no longer used for mineral extraction, sometimes provide the only suitable refuges for bats displaced from caves. In North America the Bats and Mines Project, a partnership between Bat Conservation International, the mining industry, and government and private entities (Tuttle and Taylor 1998) has protected many important hibernating bat populations (Ducummon 2000; Dutko 1994; Tuttle 1993, 1996), including the most rapidly growing populations of endangered Indiana bats.

Fortunately, appropriate conditions are sometimes easy to restore or to provide artificially (Fig. 18.13). A wide variety of opportunities exist, including modification of existing caves and mines or construction of entirely new roosts ranging from artificial caves (Sander 1997; Locke 2003), to modified highway bridges (Keeley 1997; Keeley and Tuttle 1999), cinderblock towers (replacing ancient tree hollows) (Bayless 2006; Kelm et al. 2007) and bat house crevice roosts (Tuttle et al. 2005). Millions of American bats are now living in artificial roosts that provide the only hope of recovery for some species.

An extra large BCI-designed bat house, built on the University of Florida, Gainesville campus in 1991, now shelters a huge colony, mostly *T. brasiliensis*.

Fig. 18.13 (**a**) Tower roosts, built to provide alternative homes for Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) that have lost their original homes in extra large tree hollows at Mammoth Caves National Park, KY. (**b**) A maternity colony of the same species using an artificial roost in Trinity National River Wildlife Refuge, TX

Due to its popularity with bat watchers, a new roost that can accommodate 400,000 was built in 2010. The combined colony size is already 300,000 and growing rapidly (Reed 2012). More free-tailed bats now live in these two artificial roosts than are known from all the natural roosts in Florida combined, illustrating that loss of roosts is the key bottleneck to restoring bat populations of that area.

A final overriding need involves water. Bats have the greatest surface area per volume ratio of any warm-blooded animal, making them exceptionally susceptible to dehydration. They can lose up to 50 % of their body mass in water in a single day (O'Farrell et al. 1971), likely explaining why most nursery colonies are located near reliable water resources. Also, unlike other animals, most bats must drink in flight, typically requiring an unobstructed swoop zone of at least 3 m, some as much as 30 m (Fig. 18.14) (Taylor and Tuttle 2007). The availability of surface water and associated riparian habitat has declined alarmingly over the past 150 years (Adams 2003), and special watering devices for other animals often fail to meet bat needs.

If forecasts of climate change are correct, without prompt improvement of bat-friendly water resources, widespread extirpation is expected (Adams and Hayes 2008; Adams 2009a, b). The dramatic expansion of bat numbers when suitable roosts and water are provided, whether naturally or artificially, combined with the

Fig. 18.14 California myotis (*Myotis californicus*) drinking at livestock watering trough in arid southwestern US. On hot nights, bats often visited at rates approaching one per second

rapidly growing popularity of bat watching, gives hope for the future. Much remains to be accomplished in educating the public to support such efforts, but growing numbers of nature centers and commercial tour cave owners are now protecting bats because it is simply good business.

18.4 Priority Setting

Early conservationists had little choice but to focus on so-called glamor species, typically the largest or cutest animals. However, too often, multiple organizations competed to protect a few popular animals, while whole groups of ecologically essential species were ignored.

Even now, bats are often neglected despite a rapidly growing body of evidence confirming invaluable eco-services (Chap. 24). There is an urgent need to communicate research findings to conservation decision makers and the public at large (Kunz et al. 2011).

Most private donors, foundations, and government agencies want scientists to clearly define and rank priorities. Nevertheless, gaining objective consensus can be challenging, especially when those attempting to reach agreement stand to gain or lose funding based on how priorities are ranked.

Two approaches have worked for me. In recommending members for the endangered gray bat recovery team, I chose only highly respected bat research colleagues who had nothing to gain or lose from team decisions. We listened to arguments from those who did. Then, following objective discussion among team members, we established clear, quantifiable, and achievable priorities that won broad support.

When it came to cooperation between bat conservationists and the wind industry, a diverse group of stakeholders formed the Bats and Wind Energy Cooperative (BWEC, http://www.batsandwind.org). A governing Oversight Committee is composed of one member each from the five entities who provide primary financial support (American Wind Energy Association, Bat Conservation International, U.S. Department of Energy, U.S. Fish and Wildlife Service, U.S. National Renewable Energy Laboratory). This committee works in close collaboration with a Project Coordinator, under advisement of a six-member Scientific Advisory Committee and a six-member Technical Advisory Committee. The Oversight Committee organizes face-to-face planning meetings, led by a professional facilitator, at approximately 3-year intervals. Progress is reviewed and budgets fine-tuned at least once annually. By assigning final priority setting to a relatively few highly respected individuals, with professionally facilitated input from both scientific and technical advisory committee members, extraordinary consensus has gained broad financial support and commensurate progress.

A final area of conservation planning that deeply concerns me involves finding a balance between priorities for endangered and ecologically essential species. Too often, decision makers focus so heavily on endangered species that the old adage "an ounce of prevention is worth a pound of cure" is ignored. It is now clear that important eco-services can be lost long before a formerly abundant species is endangered (McConkey and Drake 2006).

Just as conservation of biodiversity hotspots has emerged as a strategic planning priority, so should protection of key populations of eco-service providers. A single roost can shelter bats whose loss could threaten regional ecosystem health and thus caves, mines, etc., urgently need to be inventoried and prioritized for conservation. Once protected, these resources can play pivotal roles in conservation with implications extending far beyond bats.

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Chapter 19
Virology and Immunology of Bats

Tony Schountz

Abstract Bats harbor many pathogens of veterinary and human health concern, including several emerging and reemerging viruses such as lyssaviruses, filoviruses, henipaviruses, and SARS-like coronaviruses. Despite immune responses to these viruses, many bats remain infected without disease and likely shed virus to other bats and mammals. Little is known about bat immune systems or how the immune responses of bats control infections. The recent characterization of genome and transcriptome sequences of several bat species suggests they are similar to other mammals. These data indicate that bats possess orthologous genes, antibodies, and cells involved in innate and adaptive immune responses as do other mammals, but bats likely evolved unique mechanisms for controlling viruses that cause disease in other species. It is unclear how these diseases affect bat ecology, and thus, a greater understanding of immunology and infection is needed to understand health impact on bats.

19.1 Introduction

In recent years a number of viruses that cause substantial human and veterinary disease have been detected in or isolated from bats (Calisher et al. 2006). The economic impact of these viruses has been billions of dollars and these diseases have had social and medical impacts (Field 2009). Many viruses persist without pathology in bat populations, whereas some cause diseases. While much is known about the diseases in humans and livestock, virtually nothing is known about bats or how bat immune systems control viral infections.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_19,

Most viruses infect one or a few principal reservoir host species, often with little or no disease (Calisher et al. 2006). Because viruses are dependent upon their hosts for replication, they often limit pathology. The apathogenic virus–reservoir relationship is one of the coadaptations with each becoming biochemically and genetically optimized to allow virus replication without host disease. If hosts become immunocompromised, the balance of this relationship is altered, sometimes compromising both host and virus. Disease usually occurs when viruses inadvertently infect another susceptible species, termed *spillover*, and biochemical processes are no longer optimized between the virus and the new host species.

19.1.1 Reservoirs and Virus Ecology

There are three broad outcomes when a virus is introduced: (1) no infection occurs because the virus cannot utilize the biochemical machinery of the animal; (2) infection that may or may not cause disease, followed by an immune response that may or may not prevent disease; or (3) an apathogenic infection that allows the virus to persist. Oftentimes, viruses have evolved infectious mechanisms that render them innocuous in their reservoir host species that may account for the lack of an immune response, a result of coevolutionary adaptations. However, some viruses may cause disease in some members of the reservoir species depending on the age, genetics, or immune status of the animal. Other viruses replicate slowly and can take months or years to manifest disease, yet healthy-appearing animals can transmit virus to others prior to disease onset.

19.1.2 Immune Evasion, Persistence, and Pathogenesis

Vertebrate cells possess antiviral pathways and proteins that interfere with viral infections. However, many viruses have evolved countermeasures that provide a competitive advantage. Countermeasures have only been studied in a few animal models (e.g., mice, ferrets, or nonhuman primates); however, these countermeasures have evolved in the reservoir hosts, not in spillover or experimental organisms. How they function, qualitatively or quantitatively, in the reservoir may be substantially different than how they function in a spillover or experimental host where disease is often the outcome.

19.1.3 Infectious Diseases of Bats

Microbes play an important role in the health of all vertebrates. Some microbes, termed *normal flora*, provide nutrients in the gastrointestinal tract and help defend against other pathogenic microbes. A bat pathogen, Tacaribe virus, was discovered during a rabies surveillance program near Port of Spain, Trinidad and Tobago (Downs et al. 1963). Many Jamaican (*Artibeus jamaicensis*) and great fruit-eating bats (*Artibeus lituratus*) were found dead or dying from suspected rabies. However, pathological assessment failed to detect rabies virus and subsequent work led to the isolation of Tacaribe virus from 11 bats. Recent experimental infection of *A. jamaicensis* with Tacaribe virus caused a disease in some bats that is similar to human South American hemorrhagic fevers, including neurological manifestations, and the virus was cleared by other bats (Cogswell-Hawkinson et al. 2012). Ebolavirus-like viral RNA was detected in many dead insectivorous Schreiber's bats (*Miniopterus schreibersii*) collected in Spain, Portugal, and France (Negredo et al. 2011). More recently, several pathogenic microbes were detected in organs of European bats collected post-mortem, including pneumonia and myocarditis associated with members of several bacterial families and a protozoan and gastrointestinal disease associated with *Pasteurella multocida* and *Yersinia pseudotuberculosis*, trematode infestation, nematodes, and coccidiosis (Muhldorfer et al. 2010, 2011a, b, c). It is unclear if these microbes caused the diseases or were opportunistic pathogens; the difficulties of conducting experimental infections on bats to test Koch's postulates has hampered disease etiology research.

19.2 The Immune Systems of Bats

19.2.1 Overview of the Immune Response

The vertebrate immune response is responsible for containment of infections using variety of cells (Table 19.1) that operate in a highly orchestrated manner. For many infectious agents, this results in the clearance of the agent. However, others persist for lengthy periods, sometimes the life of the animal. Should the immune response fail to contain the infection, disease often occurs that compromises the host's health leading to its death. In other instances, an aggressive immune response can cause immunopathology that can also lead to death. The balance of the immune response must limit pathogen-induced disease without substantial immunopathology while containing the infection.

The immune response occurs in two principal phases. The *innate response* is initiated by preexisting molecules and cells that recognize products common to infectious agents. The *adaptive response*, which takes several days to begin, provides highly specific long-term immunity and uses two groups of lymphocytes, *B cells* that produce antibodies, some of which can neutralize viruses, and *T cells* that coordinate activities of cells during immune responses and kill cells harboring pathogens.

Currently, the study of bat immune responses suffers from a lack of immunological reagents reactive to bat proteins, the tremendous species diversity of bats (Baker et al. 2013b), and because few captive colonies are available for experimental manipulation. Because bats are often protected by laws, bats cannot be euthanized

Table 19.1 Principal cells of the immune system

Cell	Distribution	Major functions
Neutrophil	Blood	Infiltrates tissues during innate phase and induces inflammation and granulocyte release
Basophil	Blood	During innate phase produces heparin and histamine to mediate inflammation; abundant during ectoparasite infections
Eosinophil	Blood	Granulocytic cells that induce inflammation and secrete inflammatory cytokines and reactive oxygen species; abundant during helminth infections
Mast cell	Blood	Binds to IgE antibodies in tissues and secretes histamine and heparin during inflammatory responses; may be derived from basophils
Monocyte	Blood	Infiltrates tissues and differentiates into tissue macrophages
Macrophage	Most tissues	Resident phagocytic cells that participate in innate and adaptive immune responses; antigen presentation to helper T cells
Natural killer (NK) cell	Blood	Infiltrates tissues during the innate phase and kills cells that are infected with viruses
Myeloid dendritic cell (mDC)	Most tissues	Migrates with antigen to regional lymph nodes to stimulate adaptive immune responses of T and B cells; secretes IL-12
Plasmacytoid dendritic cell (pDC)	Most tissues	Migrates with antigen to regional lymph nodes to stimulate adaptive immune responses of T and B cells; secretes IFN-α
Follicular dendritic cell (FDC)	Secondary lymphoid tissues	Presents protein antigens in their native confirmation to B cells to stimulate antibody production
Helper T (Th) cell (lymphocyte)	Secondary lymphoid tissues	Secretes cytokines during the adaptive phase that modulate local immune responses; provides help to B cells for class switching and affinity maturation and to CTL for sustained antiviral responses
Cytotoxic T cell (CTL) (lymphocyte)	Infected tissues/lymphoid tissues	During the adaptive phase, recognizes and kills cells infected with pathogens (e.g., viruses, some bacteria)
Regulatory T cell (Treg) (lymphocyte)	Lymphoid tissues	Suppresses inflammatory immune responses to mitigate immunopathology
B cell	Secondary lymphoid tissues	Synthesizes and secretes antibodies that bind to antigens and neutralize activity and/or facilitate phagocytosis by other cells, such as macrophages. Requires Th cells for class switching to other immunoglobulin classes and production of high affinity antibodies

Adapted from Abbas et al. 2010. Cell Mol Immunol. 6th Ed. Saunders-Elsevier Press for microbe surveys, for collection of tissues, or for experimental infections. Fortunately, new technologies are emerging that should facilitate understanding of how bats respond to infections.

19.2.2 Innate Immune Mechanisms

Groups of microbes have many molecular structures in common. Gram-negative bacteria have lipopolysaccharide and many viruses synthesize RNA in the cytoplasm of a cell. Vertebrates have evolved *pattern recognition receptors* (PRR) that bind these common microbial motifs for detection of infectious threats. The two broad PRR systems in vertebrate cells are the *Toll-like receptors* (TLR) and *RIG-like helicases* (RLH) and have been identified in *Pteropus alecto*, *Rousettus leschenaultii*, and *A. jamaicensis* using genetic and biochemical analyses (Cowled et al. 2011, 2012; Iha et al. 2010; Papenfuss et al. 2012; Shaw et al. 2012).

Type I interferon (IFN-α, IFN-β) response in mammals is triggered by TLR and RLH signaling and is mediated by more than a 100 proteins, and genes encoding proteins in these pathways have been identified in several bat species (Kepler et al. 2010; Omatsu et al. 2008; Papenfuss et al. 2012; Shaw et al. 2012). When activated by IFN-α or IFN-β, the cell enters an antiviral state and attenuates many of the cellular biochemical pathways needed by viruses for replication, including protein and nucleotide synthesis. It also leads to increased expression of *major histocompatibility complex* (MHC) proteins, which are essential for T cell responses and transition to the adaptive phase of the immune response. *Type III IFNs* (IFN-λ) are encoded by up to three genes in mammals (*Ifnl1*, *Ifnl2*, *Ifnl3*) and also play roles in antiviral immune responses, although their functions are less well characterized. Nonetheless, type III interferons were identified in several bat species (Zhou et al. 2011) and likely are important in viral infection management.

19.2.3 Immune System Cells and Tissues

Development of immune cells occurs in *primary lymphoid tissues*, which include the bone marrow, where most immune cell development begins, and the thymus, the principal site of T cell maturation. *Secondary lymphoid tissues* are where microbial antigens are processed and presented to B cells for antibody production and to T cells for activation of various functions. The spleen is a secondary lymphoid organ responsible for controlling blood-borne infections, whereas the lymph nodes control infections of nearby tissues. All tissues have lymphatic vessels providing a conduit for *antigen-presenting cells* (APCs) and draining lymph fluid to the lymph nodes that act as filters and depots for concentration of antigens and APCs. During infections, the antigen-specific T and B lymphocytes in the lymph nodes have cognate interactions with each other and with APCs, leading to clonal expansion of antigen-specific lymphocytes and activation of the adaptive immune response. Antibodies are produced by the B cells in these lymph nodes and enter the lymphatic vessels or the blood for rapid distribution. *Tertiary lymphoid tissues* are typically small collections of immune cells distributed within solid organs and are involved in organ-specific infections and can traffic to the regional lymph nodes to participate in T cell and B cell activation.

Several subsets of T cells occur in mammals that have specific activities. *Cytotoxic T lymphocytes* (CTL) express the cell surface *cluster of differentiation* (CD) glycoprotein CD8. These cells recognize infected cells and kill them, thereby depriving the virus of the resources necessary for replication. *Helper T (Th) cells* express the surface glycoprotein CD4 and contribute by secreting cytokines that mediate local immune responses. Another subset of T cells is the *regulatory T (Treg) cell* that tempers the immune response and controls inflammation. In many infectious diseases, one of these Th cell types is associated with resistance or susceptibility to disease.

Little work has been conducted on bat lymphocytes, but the presence of high-titer IgG during immune responses demonstrates both B cells, which secrete immunoglobulins, and Th cells, which direct *class switching* and *affinity maturation* as an immune response evolves, are found in bats. The presence of B and T cells in *Pteropus giganteus* (surface immunoglobulins and cells sensitive to T cell mitogens) has been described (Chakraborty and Chakravarty 1983, 1984; Chakravarty and Sarkar 1994; Paul and Chakravarty 1987; Sarkar and Chakravarty 1991). However, virtually no work has been conducted to examine recall T cell functions of bats in response to antigens.

Cells resembling *follicular dendritic cells* (FDC), which present antigens in their native conformations to B cells, occur in *P. giganteus* (Sarkar and Chakravarty 1991) and are distinct from *dendritic cells*, which present peptide antigens to Th cells as part of the transition from innate to adaptive immunity. Reports of these and other cell types in bats are limited; although bats have MHC class II antigens for presenting peptide antigens to helper T cells (Mayer and Brunner 2007; Schad et al. 2011), no work on MHC class I molecules for cytotoxic T cell antigen presentation has been described even though bat transcriptomes reveal their presence (Papenfuss et al. 2012; Shaw et al. 2012). It is likely that bat immune systems are largely similar to other mammals in regard to cell types and functions.

19.2.4 Immunoglobulins

Five major classes of immunoglobulins are found in mammals: IgM, IgG, IgA, IgE, and IgD, and bats examined also have some or all of these (Butler et al. 2011). IgG, IgM, IgA, and IgE are secreted by B cells; IgD is typically a membrane-bound surface immunoglobulin receptor that is not secreted. These bivalent antibodies bind to the antigens by noncovalent interactions and have several activities that impair the microbes' abilities to sustain infection, including neutralization of viruses or toxins, or by marking the antigen for destruction by phagocytic cells or complement proteins that are ever present in blood.

IgM is the first antibody produced during infection and has low affinity because it cannot undergo affinity maturation in the B cells that synthesize it. However, because it is a pentamer (five IgM molecules covalently linked by a *J chain*) and has a valency of ten, it has high avidity and interacts with up to ten antigens, leading to complex aggregates that facilitate immune responses. Within days to weeks, IgG antibodies appear in the blood and the IgM response typically wanes. Importantly, during an immune response many of these antigen-specific IgM-secreting B cells undergo two critical events: class switching to IgG, IgA, or IgE, and affinity maturation that leads to antibodies with such high affinity that they effectively bind to antigen irreversibly under physiologic conditions. While IgG is found in the blood, lymph, and tissues, IgA is secreted into the mucosal tissues and is effective in combating infectious agents in those sites. In other mammals, both class switching and affinity maturation are driven by T cells; thus, the occurrence of high-titer antibody of these classes implies T cell participation in bat antibody responses.

The evolution of antibody genes has been examined in only a few bat species. In agreement with other mammals, the divergence of IgG appears to have occurred after speciation. *Myotis lucifugus* has five IgG subclasses, *Eptesicus fuscus* has two IgG subclasses, *Carollia perspicillata* appears to have a single IgG, and *Cynopterus sphinx* has three IgG subclasses (Butler et al. 2011). The biological functions of these subclasses have yet to be determined. IgM, IgE, and IgA have been detected as well; however, IgD was detected in *M. lucifugus* and *E. fuscus* but not in *C. perspicillata* or *C. sphinx*. It is unclear if IgD is truly absent in these species or if the employed cloning strategies were unsuccessful.

A closer scrutiny of *M. lucifugus* immunoglobulin variable gene segments shows substantial diversity of variable-heavy (V_H) germline gene segments (Bratsch et al. 2011). The somatic mutation rate among these genes appears lower than other mammals; however, the species has many more joining (J_H) and diversity (D_H) segments, suggesting it may rely on combinatorial and junctional diversity and less on somatic hypermutation for antibody diversity.

19.2.5 Complement

The complement (C′) system is composed of proteins having specificity and enzymatic activities for controlling microbial infections. Complement activity occurs in *E. fuscus*, *M. lucifugus*, *Tadarida brasiliensis*, and *Pteropus vampyrus* and many of the functions are similar to other mammals. However, in *E. fuscus* the activity appears less sensitive to cold, perhaps reflecting a need for immunological activity during hibernation or torpor (Allen et al. 2009; Hatten et al. 1973).

19.2.6 Cytokines

Cytokines are a large group of hormone-like proteins essential for immune system development and activities. They are biologically active in picomolar concentrations and provide important noncognate signaling during immune responses. More than 100 cytokines and chemokines have been described in mammals and it is evident that bats have orthologs for most, if not all, of these genes.

Cytokines bind to specific receptors found on certain cells and induce signal transduction that leads to gene expression or repression. The secretion of cytokines is typically brief and without storage because of their potency and potential for inducing immunopathology. They are expressed early in the innate phase through the end of the adaptive phase and activate cells of the immune response for tissue repair after clearance of the microbe.

Many cytokines have been described in bat species (Cogswell-Hawkinson et al. 2011; Iha et al. 2009; Janardhana et al. 2012) and searches of the genomic and transcriptome databases (e.g., GenBank, NCBI Trace Archive, etc.) (Papenfuss et al. 2012; Shaw et al. 2012) reveal many others. Most analysis has been phylogenetic and a significant limitation for evaluating these molecules is the lack of specific reagents for their detection, such as monoclonal antibodies. However, recent advances in gene expression analysis should provide valuable information on the roles of these molecules during viral infections.

19.3 Viruses of Bats

More than 100 viruses from many families have been isolated from or detected in bats (Calisher et al. 2006) (Table 19.2) with few known to be transmitted to humans or other animals. Some cause severe diseases, including rabies virus and other lyssaviruses, ebolaviruses and marburgvirus, severe acute respiratory syndrome (SARS) coronavirus-like viruses, and Nipah and Hendra viruses. Novel herpesviruses were recently detected in bats and other viruses that cause zoonotic diseases have also been detected; however, it is unknown if bats are reservoirs or if infections were incidental.

19.3.1 Rabies Virus and Lyssaviruses

More than 50,000 people die each year from rabies, most in Africa or Asia (Banyard et al. 2011) and most are transmitted by dog bites (see Chap. 18). Persons at risk of rabies exposure, including bat biologists and veterinarians, should be immunized and have their antibody titers checked. An exposure event in a vaccinated person should be followed by booster immunizations to minimize the risk of developing rabies, which is nearly always fatal.

Table 19.2 Virus families detected in or isolated from bats

Virus family	Viruses
Rhabdoviridae	14
Paramyxoviridae	9
Coronaviridae	14
Togaviridae	3
Flaviviridae	19
Bunyaviridae	6
Reoviridae	9
Arenaviridae	1
Herpesviridae	12
Retroviridae	1
Picornaviridae	1
Papillomaviridae	1
Adenoviridae	7
Astroviridae	1
Filoviridae	3
Orthomyxoviridae	1
Unclassified	6

Adapted from Calisher, Childs et al. 2006; C.H. Calisher, Personal communication

Rabies virus and other lyssaviruses belong to the family *Rhabdoviridae* that are widely distributed and infect vertebrates, invertebrates, and plants. The genus *Lyssavirus* has many species that infect bats, including rabies virus, Lagos bat virus, Australian lyssavirus, and two European bat lyssaviruses, and although distinct viruses, all subsequent diseases are termed rabies because of the nearly identical pathology (Johnson et al. 2011).

Rabies likely existed in the Americas prior to European colonization, with reports of Spanish conquistadors dying after vampire bat bites (Blanton et al. 2011). A concerted rabies control program instituted by the Pan American Health Organization has dramatically reduced rabies cases in urban regions (Belotto et al. 2005). Dog bites accounted for 65 % of rabies transmission to humans, whereas bats accounted for 14.7 %. By immunizing dogs, the number of human and canine rabies cases dropped by 90 %. However, because of immunizing companion animals and other wildlife, bats have become an important reservoir for rabies virus. Hematophagous bats, particularly *Desmodus rotundus*, are an important vector for transmitting rabies virus to livestock because of encroachment on bat habitat (Banyard et al. 2011).

The ecology of rabies virus in bats is highly complex and poorly understood. A recent study developed and validated a model of rabies virus transmission in temperate big brown bats (*E. fuscus*) in Colorado (George et al. 2011). Seasonal mechanisms were important in the maintenance and transmission of rabies virus within bat populations. Because of the long incubation period of rabies viruses and the low mortality of bats during hibernation, infected bats survive into the next season as a reservoir for transmission to other bats, particularly naive juveniles, in the spring and summer. As juvenile mortality increased, transmission declined within the population. Thus, the combination of long incubation of rabies virus and lack of biochemical activity of the virus during hibernation likely contributes to some bats remaining persistently infected for years. Because of the diversity of bat ecology, it is likely that lyssavirus transmission and maintenance will vary substantially between bat species.

19.3.2 Coronaviruses

The outbreak of SARS in Southeast Asia in 2002 was caused by a newly discovered coronavirus, SARS-CoV. More than 8,000 cases were reported in 32 countries with a nearly 10 % fatality rate (Field 2009). Chinese investigators initially believed the host was either the masked palm civet (*Paguma larvata*) or the raccoon dog (*Nyctereutes procyonoides*) because SARS-CoV-like viruses were isolated from these animals in local live animal "wet markets" (Ksiazek et al. 2003). However, subsequent fieldwork suggested the Chinese horseshoe bats (*Rhinolophus* spp.) were more likely the original source (Lau et al. 2005; Li et al. 2005, Chap. 18). The prevalence of antibodies to SARS-CoV was as high as 84 % in bat populations. While the genotypes of the bat coronaviruses were distinct from the human SARS-CoV, and thus named *SARS-like coronaviruses*, phylogenetic evaluation indicated the human SARS-CoV was likely descendent from the bat virus. Since, new coronaviruses have been identified in other bat species. A recent outbreak of another coronavirus disease in humans Middle East respiratory syndrome (MERS), was associated with a virus with substantial phylogenetic similarity to coronaviruses isolated from bats in Southeast Asia (van Boheemen et al. 2012), suggesting transmission to humans is a continuing threat. The risk of contracting coronavirus infection from handling bats appears to be low; however, additional studies are needed to fully understand the risks to bat biologists (Stockman et al. 2008).

A survey of Colorado bats identified coronavirus RNA sequences distantly related to SARS-CoV in *E. fuscus* and *M. occultus*, but not *T. brasiliensis*, *M. ciliolabrum*, *M. evotis*, *Lasionycteris noctivagans*, or *M. volans* (Dominguez et al. 2007). Two other coronavirus sequences were also detected in *C. perspicillata* and *Glossophaga soricina* in Trinidad and Tobago (Carrington et al. 2008), while a similar virus occurred in *M. nattereri* and *M. daubentonii* in the United Kingdom (August et al. 2012). Coronavirus sequences were also detected in four species of western European bats (*M. daubentonii*, *M. dasycneme*, *Nyctalus noctula*, *Pipistrellus pipistrellus*) commonly found in urban areas (Reusken et al. 2010), raising the prospect of human or veterinary spillover. Coronaviruses in the same group as the SARS-CoV have been detected in several horseshoe bats (*Rhinolophus hipposideros*) in Slovenia, but not in six other species examined (Rihtaric et al. 2010), and in *D. rotundus* in Brazil (Brandao et al. 2008). Additional coronavirus sequences have been detected in Asia (Shirato et al. 2012; Woo et al. 2012) and there is genetic and serological evidence of coronaviruses in Africa (Muller et al. 2007; Pfefferle et al. 2009). Thus far, no coronaviruses have been isolated from bats; only sequences and antibodies have been detected.

The use of deep sequencing was employed to identify novel coronaviruses in *E. fuscus*, *Perimyotis subflavus*, and *M. lucifugus* (Donaldson et al. 2010). In one night, 41 bats were captured and oral and fecal samples collected for RNA and DNA sequencing resulting in 76 matched coronavirus sequences.

The ecology of bat coronaviruses is unclear. However, one study (Drexler et al. 2011) followed coronavirus, astrovirus, and adenovirus transmission in a maternal colony of *Myotis myotis* in Germany for three years. Coronaviruses and astroviruses amplified within the colony during their best reproductive year, suggesting the viruses had no negative impact on reproduction. This supports the reservoir model of virus maintenance within a vertebrate host.

19.3.3 Henipaviruses

In 1994 an outbreak of an acute respiratory disease with a high fatality rate from encephalitis occurred in 14 horses and a trainer near Hendra, Australia (Field 2009). Several other small outbreaks have since occurred and a paramyxovirus, Hendra virus (HeV), was identified as the causative agent. A similar disease occurred near Nipah, Malaysia, in 1998 at a hog farm that killed hogs and ethnic Chinese abattoir workers. Of 256 cases, 105 people died and over one million hogs were euthanized, at a cost of US$500 million, to prevent further spread of the new paramyxovirus, Nipah virus. Another outbreak occurred in Bangladesh with a high number of cases and fatality rate. Together, these viruses were classified into a new genus, *Henipavirus*, and members have also been detected in Africa. Each virus has been associated with pteropid bats, including the black flying fox (*Pteropus alecto*), gray-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spectacled flying fox (*P. conspicillatus*). Subsequently, other paramyxoviruses have been discovered in bat species from other continents (Kurth et al. 2012; Sasaki et al. 2012; Wilkinson et al. 2012).

In experimental infections of black flying foxes, HeV was detected in the kidneys but none of the bats exhibited signs of disease (Halpin et al. 2011). Virus was isolated from urine and it is thought this is a principal transmission mode. Virus was also detected in the throat swabs, rectal swabs, and blood, and intranasal infection may be a means of acquiring virus.

Transmission to humans was likely a result of anthropogenic environmental changes and encroachment upon bat habitat (Field 2009). Expansion of hog farms by deforestation increased contact of fruit bats with hogs. In addition, planting of fruit orchards near hog farms caused localized habitat sinks that contributed to increased contact between bats and hogs, leading to virus spillover. Once virus entered the hog population, transmission to humans occurred, which lead to human to human transmission. Thus, agricultural intensification likely contributed to the transmission of virus to humans in the Malaysian outbreak (Pulliam et al. 2012).

Henipaviruses have also been detected in Madagascan fruit bats (*Eidolon dupreanum*) in Ghana (Hayman et al. 2008). Serology studies have detected antibodies that recognized Nipah and Hendra viruses and in vitro virus neutralization tests demonstrated cross-reactivity to these viruses. Henipavirus RNA from three putative species was also detected in the African straw-colored fruit bat (*E. helvum*) (Baker et al. 2013a). No human disease has been associated with African henipaviruses; however, considering the pathogenicity of Hendra and Nipah viruses, it is possible these viruses can cause human disease.

The ecology of Nipah virus is unclear, but it is evident that virus can persist in captive *Pteropus vampyrus* bats for over a year (Sohayati et al. 2011). In this study, antibody levels varied substantially among infected bats, with some maintaining high titers and others with low titers for 10 months. Maternal antibody was present in juveniles and persisted up to 14 months. Of particular concern for captive populations, some bats were seropositive, then seronegative, only to become seropositive again after many months, and virus was isolated from one of these bats. This exposes the unreliability of serology for determining the infection status of a bat that could lead to transmission of Nipah virus to unsuspecting handlers. It is prudent to assume the bats are infected even if they are seronegative.

Nipah virus V protein disrupts human type I and type II interferon responses (Rodriguez et al. 2002, 2004) by interacting with STAT1 and STAT2, two critical proteins involved in interferon signaling and transcription of many other antiviral proteins. The V protein appears to bind to one STAT1 and one STAT2 to form a trimer that presumably interferes with STAT1/STAT2 translocation into the nucleus where they normally act as transcription factors that drive the expression of many antiviral genes. However, it is unclear how the V protein behaves in *Pteropus* bat reservoirs where the protein has been shaped by evolutionary pressures.

19.3.4 Filoviruses

Six viruses in the family *Filoviridae* may be hosted by bats: Zaire ebolavirus, Taï forest ebolavirus (formerly Cote d'Ivoire ebolavirus), Sudan ebolavirus, Reston ebolavirus, Bundibugyo ebolavirus, and Lake Victoria marburgvirus. Another unclassified filovirus was also identified during an outbreak in Yambio county, southern Sudan (Onyango et al. 2007). Ebolavirus RNA has been detected in several fruit bat species, including the little collared fruit bat (*Myonycteris torquata*), hammer-headed fruit bat (*Hypsignathus monstrosus*), and Franquet's epauletted bat (*Epomops franqueti*) (Leroy et al. 2005). Antibodies to ebolaviruses were also detected in other bats of these species but viral RNA was not detected in those bats, suggesting the immune responses cleared the virus or reduced viral loads to levels undetectable by PCR. A large serological survey for antibodies specific to filoviruses conducted in Gabon detected antibodies in Egyptian fruit bats, although five other bat species had a lower prevalence. RNA from an eighth ebolavirus-like virus, Lloviu virus, was detected in tissues from dead Schreiber's bats collected in Spain, Portugal, and France (Negredo et al. 2011). Whereas filovirus infection of fruit bats appears to be nonpathogenic, infection of the European insectivorous bats may have caused death.

Only Lake Victoria marburgvirus has been isolated in cell culture from bats (Towner et al. 2009). In 2007 an outbreak of Marburg hemorrhagic fever occurred in workers at Kitaka cave in western Uganda. A survey of the cave revealed large numbers of resident Egyptian fruit bats (*Rousettus aegyptiacus*) and *Hipposideros* species. About 5 % (31/611) of the Egyptian fruit bats had marburgvirus RNA, whereas only one of 609 *Hipposideros* had detectable RNA. Juveniles had a higher prevalence (10.3 %) compared to adults (4.2 %), while pregnant bats had the lowest prevalence (2.1 %). Placentas from pregnant females did not have viral RNA, making vertical transmission unlikely. All bats appeared healthy, suggesting infection had no adverse effect and supporting the hypothesis the species is a reservoir of Lake Victoria marburgvirus.

While the spillover mechanism of filoviruses to humans is unknown, an Ebola fever outbreak in 2007 was traced to consumption of bats (Leroy et al. 2009). More than 260 people were infected with 186 deaths during the outbreak. Each year in April, thousands of migrating bats settle in trees near Ndongo and Koumelele islands in the Democratic Republic of Congo. A palm oil plantation had been established in 1925 near the Lulua River which produced fruit in April and provided a source of food for the bats. Villagers hunted the bats with shotguns as a food source. By mid-May the bats left the area to continue their migration and fewer bats were hunted thereafter, and cases of Ebola disease diminished.

19.3.5 Other Viruses

Many other viruses have been detected in bats, including herpesviruses, adenoviruses, flaviviruses, astroviruses, influenza viruses, bunyaviruses, arenaviruses, alphaviruses, reoviruses, retroviruses, picornaviruses, and papillomaviruses (Calisher et al. 2006; Donaldson et al. 2010; Janoska et al. 2011; Watanabe et al. 2009; Wibbelt et al. 2007). These viruses have been detected in only 104 bat species; thus, more than 1,100 species have yet to be examined (C.H. Calisher, Personal communication). Until surveillance is conducted on bat populations, it is difficult to ascertain if infectious diseases have ecological consequences on bat populations.

19.4 Studying Bat Viruses and Host Response

19.4.1 Virus Detection and Isolation

The great majority of viruses detected in bats thus far have been with serology (testing for antibodies to specific viruses) or PCR amplification. Few bat viruses have been isolated in cell culture or in experimentally inoculated animals. While obtaining serological data or sequences from new viruses is valuable, one cannot perform experimental infection research; the virus must be isolated in an infectious state.

The use of suckling mice has led to the isolation of many viruses from other vertebrate species. This process requires the gentle homogenization of an infected tissue in a buffer, such as cell culture medium, followed by filtration or centrifugation to remove potential bacterial contamination, and inoculation into newborn mice with incompetent immune systems. Oftentimes, intracranial inoculations are required to establish infection. Inoculation of juvenile or adult rodents can be attempted; however, their immune response may clear the virus.

Cell culture is frequently useful and can yield virus free of contaminating substances that come from suckling mouse tissues. Many cell lines from invertebrates and vertebrates have been established that are susceptible to many viruses. However, two lines from the African green monkey, VERO (CCL-81), and a subline, VERO E6 (CRL-1586), are commonly used because of their susceptibility to divergent viruses. These lines are deficient in their type I interferon pathways that likely contribute to their susceptibility to a wide array of viruses. Additionally, cell lines can be established from tissues collected from bats, which may be susceptible to infection and can provide a better understanding of virus-host cell interactions. Because cell lines allow high-throughput screening and isolation, it is typically the first method of choice.

19.4.2 Antibody Detection

During infection vertebrates respond by producing antibodies specific for the agent. Initially, IgM is produced within days after infection and is followed by other isotypes, particularly IgG and IgA antibodies that undergo affinity maturation. Detection of certain isotypes from blood samples can provide clues as to how recent an infection occurred. Detection of only IgM suggests a very recent, ongoing infection, while detection of IgM and other isotypes suggests infection occurred days or weeks ago. Detection of IgG but not IgM usually signifies infection in the distant past, months to years.

The detection of IgM is often performed using a capture ELISA. This assay requires a species-specific anti-IgM antibody, usually produced in goats or rabbits. No commercial anti-bat IgM antibodies are available; thus, it is necessary for investigators to produce their own. This process typically involves immunization of other mammals (e.g., goats, rabbits, mice) with purified IgM fragments followed by purification of the anti-IgM from the serum.

Development of anti-bat IgG antibodies is simpler in most instances. Several companies manufacture protein-A/protein-G columns that have high affinity for IgG, and Bethyl Laboratories produces a goat anti-bat IgG that is reactive to antibodies from ten bat species (and likely more). Once purified, whole bat IgG can be used as an immunogen for producing antiserum for use in ELISA or other immunoassays.

Enzyme conjugates of protein-A/protein-G, such as horseradish peroxidase, are also commercially available and are often suitable for detecting virus-specific IgG in mammals (Schountz et al. 2007). These molecules have very high affinity for IgG of many mammalian species and have an incubation time of about 30 min. This permits the development of rapid field tests for serology and identification of bats that may have been infected with specific agents. This reagent must be carefully examined for reactivity to IgG for each species of interest as it varies in its affinity for antibodies.

19.4.3 Cellular Methods

The detection of antibodies specific to an agent is of limited value for assessment of immune response, but isolation of cells involved in infection and immune responses provides substantially more information. Activities such as cellular responses to infection, innate responses by immune cells, and adaptive responses by T and B cells provide qualitative and quantitative information. In particular, cell cultures can identify subsets of T cells and their cytokines that are active during immune responses, but their cultivation from outbred animals can be daunting and the lack of colonies of bats for experimental infections makes such work difficult. Nonetheless, it may be possible to cultivate virus-specific T cells provided two important cytokines are available: interleukin-2 (IL-2) for propagating T cells and granulocyte-macrophage colony-stimulating factor (GM-CSF) for propagating autologous antigen-presenting cells from the bone marrow. Each of these cytokines is cross-reactive in many species. For example, human IL-2 can be used to propagate *Mus musculus* T cells.

19.4.4 NextGen Sequence Analysis

Advances in genome and transcriptome sequencing have accelerated in recent years (Glenn 2011). The introduction of 454 pyrosequencing has been followed by additional high-throughput sequencing. The Illumina and SOLiD platforms have increased reads and reduction of costs has brought these technologies within reach of many projects. Other technologies, including PacBio, Ion Torrent, Ion Proton, HeliScope, and Starlight, will provide additional options for investigators involved in bat research. The principal difficulty of these technologies is the volume of data generated, approaching 600 gigabases with the Illumina HiSeq2000. Management and reduction of this amount of data requires substantial computational resources and personnel. However, these technologies will allow rapid development of expression assays, such as real-time PCR or cDNA arrays. RNA-seq will also allow a bioinformatics approach to quantifying expression of protein-coding RNAs and microRNAs that may have relevance to host responses.

19.5 Conclusion

Bats are important reservoirs of viruses; however, like other mammals, they are also susceptible to many viruses and infectious agents from other organisms, including other bats, that may have important impact on their health and ecology. The biology of bats and the historical neglect of bats and their viruses make investigation of these viruses difficult. However, new technologies and adapted existing technologies should facilitate the rapid study of host response and disease susceptibility in bat species and should lead to increased understanding of the importance of bats and their viruses, which is mutually beneficial to both bat biologists and public health scientists.

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Chapter 20
White-Nose Syndrome: A Deadly Emerging Infectious Disease of Hibernating Bats

DeeAnn M. Reeder and Marianne S. Moore

Abstract In the winter of 2006–2007, thousands of dead bats were found at a single cave in New York. When mortality spread to nearby hibernacula in the winter of 2007–2008, this emerging infectious disease was dubbed "white-nose syndrome" (WNS), for the white fungi noted around the bat's muzzle. Current estimates suggest that between 5.7 and 6.7 million bats have died thus far (U.S. Fish & Wildlife Service (2012) News release: North American bat death toll exceeds 5.5 million from white-nose syndrome. http://www.fws.gov/whitenosesyndrome/pdf/WNS_Mortality_2012_NR_FINAL.pdf. Accessed 21 Feb 2012), making this one of the most significant conservation threats to bats in history. The goal of this chapter is to review the current state of knowledge regarding WNS and to highlight areas where further research is needed.

20.1 Introduction

Populations of insectivorous temperate bats have historically thrived despite having to adapt to unpredictable environmental conditions, including pathogens. Individuals of these species can live 20 or more years and form colonies that contain thousands of individuals. In the winter of 2006–2007, thousands of dead bats were found at a single cave in New York, and when mortality spread to nearby hibernacula in winter of 2007–2008, this emerging infectious disease was dubbed "white-nose syndrome" (WNS), for the white fungi around the bat's muzzle. Despite the fact that bats have evolved a variety of mechanisms to survive in these variable environments, in the

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_20,

last 5 years, up to 6.7 million bats have died due to WNS (U.S. Fish & Wildlife Service 2012), making this one of the most significant conservation threats to bats in history.

20.2 History and Spread

WNS is now known to be caused by the newly described cold-loving fungus *Geomyces destructans* (Gd) (Blehert et al. 2009; Courtin et al. 2010; Gargas et al. 2009; Lorch et al. 2011; Meteyer et al. 2009; Warnecke et al. 2012). Beginning at the NY epicenter, WNS has spread extensively and continues to be documented at new locations (Fig. 20.1, http://www.fws.gov/whitenosesyndrome). In affected bats, fungal hyphae penetrate the epidermis and dermis, replacing hair follicles, sebaceous glands, and sweat glands and causing major structural damage to the wings and other skin membranes (Fig. 20.2; Courtin et al. 2010; Cryan et al. 2010; Meteyer et al. 2009). There is little evidence of inflammation in response to tissue damage (Meteyer et al. 2009), but wing tissue remote from fungal invasion exhibits tissue damage and death, possibly due to oxygen depletion (i.e., infarcted tissue)

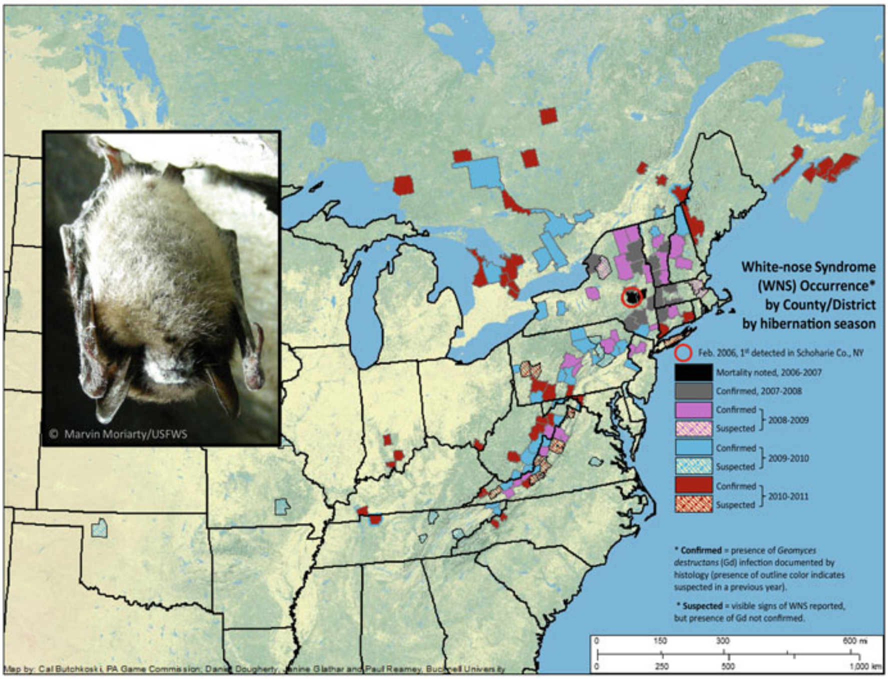

Fig. 20.1 The current and historical distribution of WNS. Inset shows the typical presentation of WNS on a little brown myotis (*M. lucifugus*)

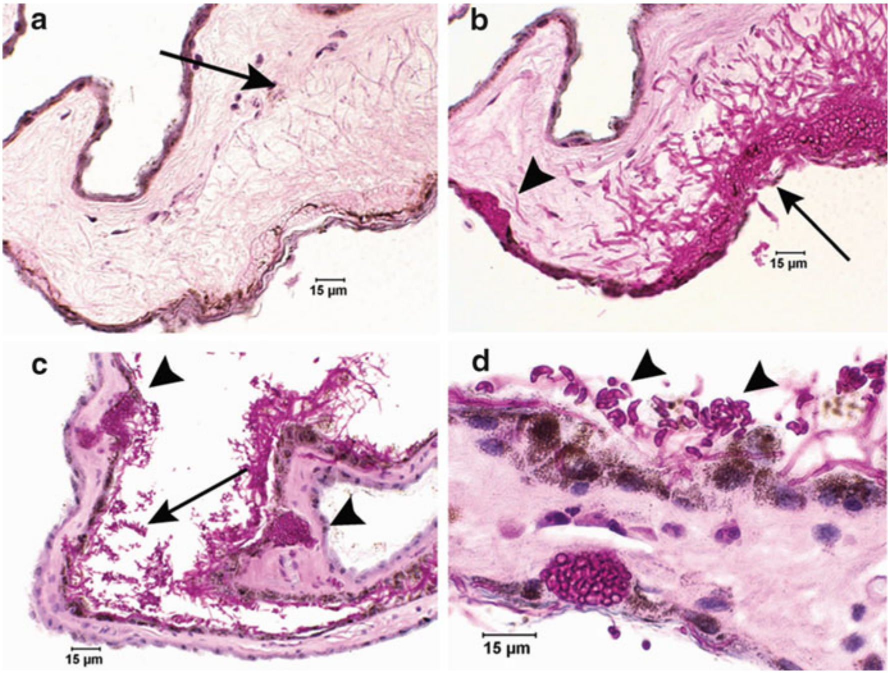

Fig. 20.2 Wing membranes of *M. lucifugus* infected with WNS (from Meteyer et al. 2009, with permission). (**a**) Histologic section of wing membrane from the same bat as in (**b**). Invasive fungus (*arrow*) stains poorly with hematoxylin and eosin stain, and inflammatory infiltrates are not present. (**b**) Periodic acid–Schiff (PAS) stain of serial section from same tissue as in panel (**a**). Fungal hyphae stain *bright magenta*. Hyphae are associated with *cup-shaped* epidermal erosions (*arrowhead*) and ulcers (*arrow*) with invasion of the underlying connective tissue. (**c**) Section of wing membrane, collected while inside the cave, from a little brown bat immediately after euthanasia. Exuberant fungal growth is present on the surface of the skin (*arrow*) and penetrates the wing membrane (*arrowheads*) without associated inflammation. PAS stain. (**d**) Conidia on the surface of the wing membrane of a cave-dwelling little brown bat fixed immediately after euthanasia in the cave. The characteristic curved conidia measure approximately 2.5 mm in diameter and 7.5 mm in curved length, have one or two blunt ends, and have a deeply basophilic central region (*arrowheads*). These conidia are identical to those of *Geomyces* sp. fungus isolated from bats with WNS. A focal cluster of fungal hyphae is present within the epithelium on opposite wing margin. PAS stain

(Cryan et al. 2010). Even outside of hibernation, WNS has lasting effects by extensive wing damage in active season bats. However, some bats have the capacity to heal extensive necrosis and loss of wing membrane (Fuller et al. 2011; Reichard and Kunz 2009; Meteyer et al. 2011). Diagnosed by cutaneous Gd infection, WNS is also characterized by abnormal physiology and behavior during the hibernation season, including altered torpor patterns (Boyles and Willis 2010; Reeder et al. 2012; Warnecke et al. 2012), alterations in immune competence (Moore et al. 2011), decreased body mass in some individuals (Blehert et al. 2009; Courtin et al. 2010), clustering near colder cave entrance, and premature hibernaculum emergence during daylight hours in midwinter (Turner et al. 2011).

Table 20.1 WNS-associated declines of six species of hibernating bats from 42 sites in New York, Pennsylvania, Vermont, Virginia, and West Virginia

	New York (26 sites)	Pennsylvania (6 sites)	Vermont (5 sites)	Virginia (2 sites)	West Virginia (3 sites)	All states combined
Myotis lucifugus						
Pre-WNS count	326,867	14,229	1,943	4,844	394	348,277
Post-WNS count	28,890	198	114	1,032	26	30,260
% change	−91 %	−99 %	−94 %	−79 %	−93 %	−91 %
Myotis sodalis						
Pre-WNS count	54,657	3	2	208	158	55,028
Post-WNS count	15,411	0	3	146	90	15,650
% change	−72 %	−100 %	50 %	−30 %	−43 %	−72 %
Myotis septentrionalis						
Pre-WNS count	619	940	136	7	4	1,706
Post-WNS count	17	2	3	9	0	31
% change	−97 %	−100 %	−98 %	29 %	−100 %	−98 %
Myotis leibii						
Pre-WNS count	1,158	6	131	4	4	1,303
Post-WNS count	1,033	6	91	9	3	1,142
% change	−11 %	0 %	−31 %	125 %	−25 %	−12 %
Perimyotis subflavus						
Pre-WNS count	1,042	284	15	746	1,020	3,107
Post-WNS count	47	28	8	627	73	783
% change	−95 %	−90 %	−47 %	−16 %	−93 %	−75 %
Eptesicus fuscus						
Pre-WNS count	2,573	133	163	19	31	2,919
Post-WNS count	1,478	62	134	25	14	1,713
% change	−43 %	−53 %	−18 %	32 %	−55 %	−41 %
Pre-WNS grand total	386,916	15,595	2,390	5,828	1,611	412,340
Post-WNS grand total	46,876	296	353	1,848	206	49,579
% change	−88 %	−98 %	−85 %	−68 %	−87 %	−88 %

Each site was positive for WNS for at least 2 years, and data were gathered at the end of the 2010–2011 hibernation season. Table modified from Turner et al. (2011)

Thus far, WNS affects six species of hibernating insectivorous bats (Turner et al. 2011), including the big brown bat (*Eptesicus fuscus*), small-footed myotis (*Myotis leibii*), little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*Myotis septentrionalis*), Indiana myotis (*Myotis sodalis*), and tricolored bat (*Perimyotis subflavus*). Also, nucleic acids likely from Gd were found on the southeastern myotis (*Myotis austroriparius*), gray myotis (*Myotis grisescens*), and cave myotis (*Myotis velifer*), but without histological evidence of tissue damage or reports of mortality. Gd and WNS were found in hibernacula of the endangered Virginia big-eared bat (*Corynorhinus townsendii virginianus*), but infection has not been documented. Population declines vary among affected species (Table 20.1). In absolute numbers, the highest declines occurred in the little brown myotis, which was once the most common hibernating bat in the American Northeast. It has declined on average 91 % at sites affected for at least 2 years (Turner et al. 2011, Table 20.1), and mathematical models suggest extirpation in the Northeastern USA by 2026 (Frick et al. 2010).

The presence of Gd, including cutaneous infection, has been documented widely across Europe but without significant mortality (Martínková et al. 2010; Pikula et al. 2012; Puechmaille et al. 2010, 2011a, b; Šimonovičov et al. 2011; Wibbelt et al. 2010a). Martínková et al. (2010), examining archived photographs taken since 1994 of >6,000 bats in the Czech Republic and Slovakia, suggested that Gd may have been present since at least 1995. They also noted that, despite an increase in incidence of visible fungus on greater mouse-eared bats (*M. myotis*, 2 % in 2007, 14 % in 2010), the population of bats increased. However, Pikula et al. (2012) documented tissue invasion by Gd diagnostic of WNS in *M. myotis* biopsies collected in the Czech Republic, and genetic variability occurred in Gd isolates from the Czech Republic and Slovakia (Martínková et al. 2010), which contrasts with clonal population evidence within the affected regions of North America (Chaturvedi et al. 2010; Rajkumar et al. 2011). This genetic disparity between isolates suggests Gd in Europe predates those in North America. Genetic differences among fungal isolates, along with lack of mortality in European bats, have led to speculation that Gd was introduced from Europe to North American bats that are naïve to the pathogen. This is strongly supported by WNS infection and mortality in *M. lucifugus* experimentally infected with the European Gd isolate (Warnecke et al. 2012). Although it has been demonstrated that bat-to-bat transmission of Gd occurs (Lorch et al. 2011), the large geographic leap from Europe to New York is most likely the result of anthropogenic transmission (Turner and Reeder 2009).

20.3 WNS Within the Context of the Disease Triangle

While we have learned much about Gd and WNS, by necessity, most work has focused on aspects of the disease in isolation. However, a classic tenet of studies in disease ecology is that disease reflects the intersection of host dynamics, properties of the pathogen, and environmental conditions (Fig. 20.3; the "disease triangle"; Van der Plank 1963). WNS may be especially deadly due to a "perfect storm" of susceptible hosts (North American temperate insectivorous bats, which apparently downregulate immune responses during hibernation), a highly virulent pathogen (a cold-loving fungus that invades and destroys skin membranes), and an environment conducive to pathogen proliferation (cold, humid caves and mines) (Blehert 2012). Because of this, WNS must be studied in the *eye of this storm*—considering all three factors. For example, what loading dose is needed to establish infection will depend upon the bat species (and potentially sex and age), the time of year infection occurs, and the nature of the bat's hibernaculum.

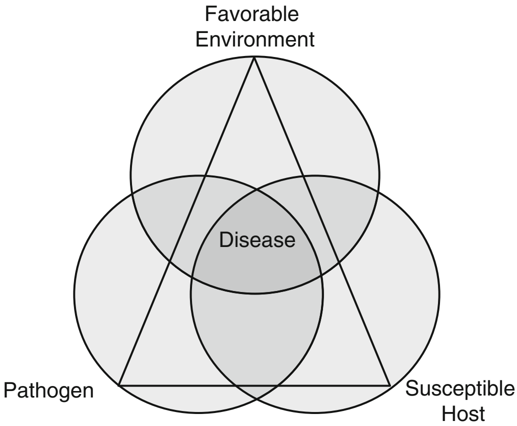

Fig. 20.3 The "disease triangle," indicating that disease occurs at the intersection between a host, a pathogen, and environmental conditions that bring the three components together (Van der Plank 1963)

20.4 Biology of *G. destructans*

Morphological characterization and phylogenetic analysis of fungal isolates from bats with histological evidence of the cutaneous infection revealed an isolate genetically related to the anamorphic genus *Geomyces* (teleomorph *Pseudogymnoascus*) within the inoperculate ascomycetes (order Helotiales) (Rice and Currah 2006). A distinct morphology of curved conidia and genetic dissimilarity warranted recognition of the new species *G. destructans* (Gargas et al. 2009). The phylum Ascomycota, including Gd and relatives, contains about 75 % of all described fungi, is extremely diverse, and includes species that cause plant and animal pathologies. Fungi, including members of Ascomycota, may exist in a range of forms (i.e., pleiomorphic). A considerable number of Ascomycota, including Gd, are known only by their asexual stages (the anamorph). Gd, through mitosis, produces asexual spores (conidia) on conidiophores. Whether Gd's sexual stage (the teleomorph) exists or not is unknown (Gargas et al. 2009).

Representatives of *Geomyces* are terrestrial, psychrophilic (cold loving) saprophytes found in soil samples worldwide, particularly in Arctic and Antarctic regions (Duncan et al. 2008; Kochkina et al. 2007; Mercantini et al. 1989; Sigler et al. 2000). Gd grows optimally between 5 and 10 °C (Blehert et al. 2009), well within the temperature range of bat hibernacula. Most fungi have higher optimal growth temperature ranges and cannot successfully and significantly infect mammals (Robert and Casadevall 2009). A close relative of Gd is the ubiquitous *Geomyces pannorum*, a keratinophilic fungus isolated from numerous environmental samples (DeBellis et al. 2007; Kochkina et al. 2007) that can colonize mammal skin (Chabasse 1988; Gianni et al. 2003).

20.5 Biology of Hibernating Insectivorous Bats

For insectivorous bats, food availability can be extremely variable, and species, populations, and sexes cope with latitudinal insect abundance and temperature variability due to seasonal migration (Humphrey and Cope 1976); changes in metabolic rate (Studier and O'Farrell 1976); seasonal increases in fat deposition (Kunz et al. 1998); thermoregulatory changes, including torpor or prolonged hibernation (Humphrey and Cope 1976; Racey and Speakman 1987; Studier and O'Farrell 1972); and disassociated patterns of reproduction (Gustafson 1979) (Fig. 20.4). For example, *M. lucifugus* mate in autumn near their hibernacula (and also periodically during winter) and females store sperm until spring arousal, after which ovulation and fertilization occur. Females leave their hibernacula and migrate to maternity colonies, containing up to several thousand pregnant females. In males, spermatogenesis is inactive during hibernation and resumes upon spring arousal with a peak in late summer (Gustafson and Shemesh 1976), and most males spend the summer in roosts separated from females.

In order to prepare for the winter, hibernating bats store body fat in late summer and early autumn. In *M. lucifugus*, body fat increases from approximately 7–27 % total mass prior to hibernation (Kunz et al. 1998; Reynolds and Kunz 2000). This fat is the only energy source during hibernation, when body temperature (T_b) and metabolic rate are both greatly reduced. Female *M. lucifugus* enter hibernation with

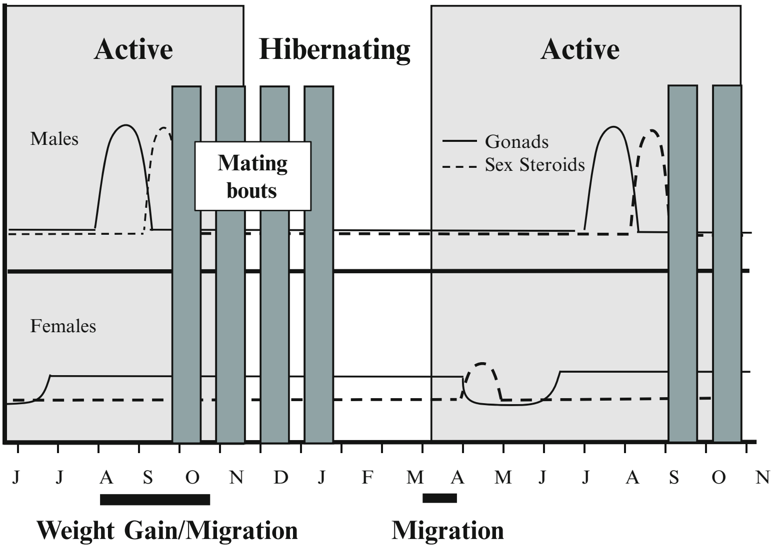

Fig. 20.4 The annual, dissociated reproductive and life-history pattern of little brown myotis, which both hibernate and migrate in the NE United States. Modified from Wimsatt (1969), Gustafson (1979), Oxberry (1979), Rowlands and Weir (1984), and Mendonça et al. (1996)

higher body mass indexes (BMI) and manage energy stores during hibernation more efficiently than males (Jonasson and Willis 2011), presumably because their energetic costs in spring are greater than males. Minimum torpid metabolic rate can be <5 % of basal metabolic rate, with T_b as low as 2 °C (Carey et al. 2003; Geiser 2004; Kayser 1965), and hibernators periodically arouse to euthermic body temperatures (Carey et al. 2003; Geiser 2004). For healthy, free-ranging *M. lucifugus*, torpor bouts last between 12 and 15 days (occasionally 30 or more days), and arousal episodes last 1–2 h (and occasionally longer) (Brack and Twente 1985; Jonasson and Willis 2012; Reeder et al. 2012; Thomas 1995).

Although euthermic periods account for about 1 % of the total time budget during winter, approximately 80–90 % of fat energy is consumed during periodic arousals (Kayser 1965; Thomas et al. 1990). The amount of depot fat expended during each arousal episode (not including flight) for hibernating *M. lucifugus* is about 107.9 mg (Thomas et al. 1990). Jonasson and Willis (2012) recorded an average daily fat expenditure of 7.6 mg. Arousal episodes in hibernators likely serve multiple functions (Humphries et al. 2005; Cryan et al. 2010) and that every mammalian hibernator periodically arouses suggests that they provide some benefit (Carey et al. 2003).

In the hibernaculum, bats select roosts within their preferred microclimate, which may vary over winter and influence fat store usage. For example, the optimum hibernation temperature for *M. lucifugus* is 2 °C; below and above, greater fat stores are required (Boyles et al. 2007; Humphries et al. 2002; McManus 1974). One mechanism by which certain bats fare better than others with WNS may be differences in thermal preferences (Table 20.2). Bats that roost at colder temperatures arouse less often and are able to have lower T_b than those roosting at warmer temperatures. These bats conserve energy, but greater energy is required to arouse to euthermy (Boyles et al. 2007; Humphries et al. 2002). In contrast, bats that roost at warmer temperatures expend more energy during torpor but spend less energy arousing and while euthermic, which may explain why they arouse more frequently (Dunbar and Tomasi 2006). Additionally, bats that cluster during hibernation (*M. lucifugus*) gain significant energetic benefits (Boyles and Brack 2009; Boyles et al. 2008; Clawson et al. 1980), but clustering likely increases disease transmission. Ultimately, optimal hibernation temperature likely depends upon a number of factors, including body condition (Humphries et al. 2002; Kokurewicz 2004) and body size. Microclimates selected during hibernation vary not only by temperature but also by relative humidity. Some species prefer roosts in high relative humidity, presumably to decrease evaporative water loss (Cryan et al. 2010)—this high humidity may support fungal growth.

A number of other variables are likely related to susceptibility, such as torpor bout length, metabolic rate during torpor and euthermy, behavior, prehibernal body fat content, immune response to Gd infection, and lipid composition of the wings. By understanding the role that each of these variables plays, we may be able to predict which bat species are at greater risk for WNS and how quickly they may succumb. For example, Langwig et al. (2012) demonstrated significantly greater mortality in socially gregarious species (likely due to increased transmission) that decreased as population size decreased. From a physiological perspective,

Table 20.2 Comparisons in hibernation characteristics and WNS susceptibility between six infected species

Species	Weight	Hibernation information[a]	WNS susceptibility (declines from Turner et al. 2011; see also Table 20.1)
M. lucifugus (Fenton and Barclay 1980; McManus 1974)	7–10 g	Prefers areas of high humidity (>70 %) and temperatures between 2 and 8 °C. Nearly always clusters in large groups (5–100 s)	Decline ≈ 91 %. Visible during hibernation, thus hibernation counts likely highly accurate. Clustering may spread infection
M. septentrionalis (Caceres and Barclay 2000; Layne 1958)	5–9 g	Hibernates solitarily or in small clusters (sometimes clusters with *M. lucifugus*); may hibernate in deep crevices or move between hibernacula in the winter; variable temperature preference: 2–13 °C	Decline ≈ 98 %. Because this species hibernates in deep crevices, winter counts may be inaccurate
M. leibii (Best and Jennings 1997)	3.8–5.5 g	Have been found hibernating singly and up to groups of 30. Found in caves and mines, in narrow crevices, wall, ceiling, or tucked between rocks on the floor. Often found in "drafty open mines and caves… near the entrance where T drops below freezing." Conditions similar to *E. fuscus*, but not as tolerant of cold and dry; 4–11 °C. Last in, first out. Move between sites during winter	Decline ≈ 12 %. Potentially an underestimate due to rarity in hibernacula. Changes in summer counts should be used to better approximate mortality
M. sodalis (Thomson 1982)	7–10 g	Tend towards site fidelity; cluster size is inverse to temperature, called "the cluster bat." Tend to be easily aroused by disturbances. Prefer cold microclimates; 66–95 % RH	Decline ≈ 72 %. Because of federal requirements for monitoring Indiana bat populations, hibernation counts likely highly accurate. Clustering may spread infection
P. subflavus (Fujita and Kunz 1984; Briggler and Prather 2003)	4–8 g	Hibernates solitarily in deeper parts of caves with stable warmer temperatures (9–12 °C typical, but range from 5 to 16 °C) and high relative humidity (>80 %); highest hibernacula site fidelity. First to enter hibernacula and last to leave	Decline ≈ 75 %. Easily located during hibernation and low levels of movement within hibernacula suggest mortality estimate is accurate
E. fuscus (Kurta and Baker 1990)	11–23 g	Last to enter hibernacula and first to leave, prefers colder, drier, more exposed locations with higher air flow within hibernacula than other species; nearly always solitary	Decline ≈ 41 %. Easy to identify in hibernacula but may move within and between hibernacula, making counts difficult

[a] Species that hibernate in colder, drier locations, and do not cluster may be less susceptible to WNS (Langwig et al. 2012), as Gd does not grow well below 5 °C (Gargas et al. 2009) and as fungi require moist environments for optimal growth. Data from a variety of sources including references at left, Raesly and Gates (1987) and Boyles et al. (2006)

Willis et al. (2011) demonstrated that *M. lucifugus* exhibits higher rates of evaporative water loss than the European Natterer's myotis (*Myotis nattereri*), presumably making it more susceptible to dehydration and thus to adverse effects of Gd. Ultimately, an understanding of variable susceptibility may facilitate development of species-specific mitigation strategies as WNS spreads westward and affects an additional 19 species of North American hibernating bats. Variability in susceptibility likely is both inter- and intraspecific conditional.

Hibernation profoundly affects immune system regulation (Luis and Hudson 2006) and bats are increasingly recognized as important hosts for emerging infectious diseases (Bouma et al. 2010; Calisher et al. 2006; Omatsu et al. 2007; Schountz, Chap. 19). There is little data on how bat immune systems function and vary seasonally and the vast majority focus on viral infection (e.g., rabies and Hendra viruses), with little exploration of the immune system (Allen et al. 2009; Sulkin and Allen 1974; Turmelle et al. 2010; Wibbelt et al. 2010b). Calisher et al. (2006) suggested that the innate and adaptive immune responses in bats may have important differences from primates and rodents, supported by studies of several species of flying foxes (*Pteropus*) (Chakraborty and Chakravarty 1984; Chakravarty and Paul 1987; Paul and Chakravarty 1986).

Seasonal variability in immune competence is nearly ubiquitous among vertebrates (Bouma et al. 2010; Martin et al. 2008; Nelson and Demas 1996) because maintaining robust energetically costly defense systems varies according to life-history state, seasonal resource availability, and disease risk (Lochmiller and Deerenberg 2000). Due to seasonal variation in pathogen exposure, animals are adapted to "predict" (e.g., via photoperiod cues; Bilbo and Nelson 2003) when enhanced immune function outweighs costs. During hibernation, when the conservation of energy is critical, mechanisms associated with immunity are reduced (Bouma et al. 2010; Jaroslow and Serrell 1972; Kurtz and Carey 2007; Larsen 1971; Manasek et al. 1965; Maniero 2000, 2002) and adjustments include decreased primary (Cahill et al. 1967) and secondary (Burton and Reichman 1999; Jaroslow 1968) humoral response, absence of T and B lymphocyte proliferative ability (Maniero 2000), and decreased complement activity (Maniero 2002). Torpor can also result in increased (Emmons 1966; Galletti et al. 1982; Kalabukhov 1958) or decreased levels of infection (Cahill et al. 1967; Kalabukhov 1958), depending on optimal growth conditions of the pathogen (Luis and Hudson 2006).

Immune function in relation to hibernation was studied using experimental infection of *M. lucifugus* and *E. fuscus* with Japanese encephalitis virus, followed by housing at cold temperatures, and resulted in the maintenance of viremias for ~100 days. After transfer to 24 °C, bats rapidly developed antiviral antibody titers (Sulkin and Allen 1974) which supports that arousals from hibernation and immune function are linked (Burton and Reichman 1999; Prendergast et al. 2002). Periodic arousals during hibernation may activate a dormant immune system, which can then combat pathogens. However, bat arousals are much shorter than those observed in other mammals (Carey et al. 2003), confounding comparative models. Despite the possibility that bats are constrained to short arousal bouts, *M. lucifugus* affected by WNS do show signs of elevated immune responses. Specifically, complement proteins produced by bats hibernating in WNS-affected sites were, on average, better able to lyse *Escherichia coli* and *Staphylococcus aureus* compared with those produced by bats at unaffected sites (Moore et al. 2011). However, complement proteins from these same affected bats were less able to kill the fungus *Candida albicans,* and the underlying mechanisms of the observed changes remain unknown.

20.6 The Environment of Hibernacula

Most bat species hibernate under constant conditions provided by caves, mines, or other human-made structures. The attributes of hibernacula most important for bats are only partly understood. The most common caves used by bats are formed when water dissolves dolomite bedrock or limestone, creating sinkholes and solution caves that form a karst system (Palmer 1991). A number of environmental factors determine suitability for bat hibernation, including ambient aboveground temperature, elevation, depth of a cave, entrance shape, water, and air flow (Swezey and Garrity 2011; Tuttle and Stevenson 1978). Most species of bats hibernate deeply in caves where stable conditions occur above freezing (Kokurewicz 2004) and where ambient temperature is often only 1 °C warmer than wall temperature (Tuttle and Stevenson 1978). However, species preferences differ (Table 20.2), and the more complex a cave, the greater the range of microclimates within it and thus the greater number of species present. Complex caves also contain a higher number of crevices, providing greater microclimate stability and energy savings (Solick and Barclay 2006). Because they typically lack structural complexity, mines usually have less microclimate variability and thus less bat species richness.

Both caves and mines can presumably act as reservoirs of Gd, as fungal spores are hardy and found on hibernaculum walls (Puechmaille et al. 2011a) and in sediment (Blehert et al. 2011; Lindner et al. 2011). Whether Gd conidia that persist in the environment can infect or reinfect bats is not yet known. Although WNS is clearly a disease of hibernating bats, summer habitats likely also play an important role in disease ecology. Bats that survive WNS leave their hibernacula in the spring and migrate to summer roosts (Fig. 20.4). Bats from a single hibernaculum may migrate to a number of geographically dispersed summer roosts containing bats from multiple hibernacula (Davis and Hitchcock 1965; Tuttle and Stevenson 1977). Within summer maternity colonies, females may cluster for thermal benefits and possibly spread Gd to uninfected individuals.

20.7 Host–Pathogen–Environment Interactions and Epidemiology of WNS

Exploring the interface of the disease triangle, it is clear that Gd may have found the Achilles' heel of hibernating bats. Although histological, experimental, and physiological evidence clearly demonstrates that Gd is the causative agent of WNS

(Blehert et al. 2009; Chaturvedi et al. 2010; Cryan et al. 2010; Lorch et al. 2011; Meteyer et al. 2009; Warnecke et al. 2012), mechanistic links between this fungus and death have not been experimentally determined. Despite its name, the detrimental effects of WNS are not thought to be due to the visible fungus observed around the muzzle but rather to the effects of Gd on the wings (Cryan et al. 2010; Meteyer et al. 2009).

Bats are unique among mammals in their amounts of exposed skin, having between four and eight times the amount of exposed skin on their wings than other body parts (Reeder and Cowles 1951). Bat wings are not simply flaps of skin (Swartz et al. 2003), but are comprised of blood and lymphatic vessels, nerves, muscles, and connective tissue within two layers of epithelial cells (Makanya and Mortola 2007; Quay 1970). Healthy bat wings are important for maintaining homeostasis, most notably water balance, and hibernating bats are especially susceptible to dehydration (Cryan et al. 2010; Davis 1970; Kallen 1964; Thomas and Cloutier 1992).

Cryan et al. (2010) hypothesized that the damage to the wing caused by Gd (erosions of the epidermis filled with fungal hyphae, destruction of apocrine and sebaceous glands and hair follicles, and destruction of blood vessels and other connective tissues) leads to dysregulation of fluid balance. Blood concentrations of sodium and chloride decreased as wing damage increased, supporting the notion of hypotonic dehydration (Cryan et al. 2013). Loss of homeostasis, particularly water balance, is likely why hibernating mammals periodically become euthermic (Davis 1970; Speakman and Racey 1989; Speakman and Thomas 2003; Thomas and Cloutier 1992; Thomas and Geiser 1997; Thomas et al. 1990). Thus, if bats arouse because of dehydration, and dehydration increases due to Gd infection, WNS-affected bats would arouse more frequently than normal (Boyles and Willis 2010; Britzke et al. 2010; Cryan et al. 2010; Reeder and Turner 2008; Turner and Reeder 2009; Willis et al. 2011). Reeder et al. (2012) found a clear link between torpor bout length and both the severity of infection and date of death due of *M. lucifugus* in that bats with WNS aroused about twice as frequently as those unaffected. Warnecke et al. (2012) found similarly shortened torpor bouts in experimentally infected bats and rapidly depleted stored body fat, and emaciation within 3–4 months. In addition, arousals from torpor may initiate other physiological responses that contribute to death, but unfortunately little is known in this regard (Carey et al. 2003). In any event, while progress to better understand the epidemiology of WNS is made, knowledge of the exact mechanism(s) by which cutaneous infection with Gd causes death remains elusive. Beyond Gd, other factors may potentially contribute. For example, pesticide exposure was ruled out as a cause of WNS but, along with other environmental contaminants, may still contribute to the demise of infected bats (Kannan et al. 2010).

Given what we know about how destructive Gd is, it is surprising that species vary from highly susceptible to apparently not susceptible. These differences can only be understood by considering each species within the context of Gd and the environment (Table 20.2). For example, we know that large-bodied species, such as *E. fuscus* and Virginia big-eared bats, can store and carry more depot fat, hibernate for shorter periods, and have lower surface area-to-volume ratios, slowing heat loss. These species also select colder roost sites within the hibernacula and these characteristics taken together should confer an advantage against Gd. Other species, such as the *M. lucifugus*, have behaviors that may increase susceptibility to WNS, including hibernating for a longer period than the larger species, hibernating at temperatures more conducive to Gd growth, and clustering, which likely increases disease transmission.

Species differences in other physiological systems may also explain differential susceptibility. For example, despite that hibernation is a period of immune suppression in mammalian hibernators (Bouma et al. 2010), Moore et al. (2011) found that some hibernating WNS-affected bats increased activity of part of their immune system. Species differences in immune competence and other physiological attributes (including potential differences between North American and European bats) likely underlie some of the species differences in the ability to survive Gd infection.

Given the broad distribution of bats throughout North America, it is clear that the hibernation (and non-hibernation) environment is not heterogeneous and this heterogeneity likely influences the progression of WNS. One hope is that environmental conditions will restrict the distribution of Gd and its infection patterns. Flory et al. (2012) found that mortality occurs most likely in topographically heterogeneous and higher elevation landscapes that are colder and drier during winter and seasonally variable. Hallam and Federico (2012) indicated that cave temperature and the ability to lay down more body fat (increased feeding opportunities in some environments) both increase survivorship at southern latitudes. Similarly, Langwig et al. (2012) found a survival advantage in colder, drier hibernacula. Understanding these sorts of interactions will greatly improve our ability to make informed management decisions (Flory et al. 2012; Hallam and Federico 2012).

20.8 Critical Research Gaps

Many general aspects of bat population dynamics, ecology, behavior, and life history are still not well described, all of which may affect the outcome of disease when Gd is introduced into a population. Little is known about the ecology of Gd, transmission dynamics, and epidemiology of outbreaks. Additionally, environmental constraints affecting the dynamics of host and pathogen interactions are just being uncovered.

Controlling the spread and mitigating the effects of WNS is of highest priority. To date, no effective mitigation strategy has been developed, nor has any antifungal treatment appropriate for widespread application been identified (Chaturvedi et al. 2011; Reeder et al. unpublished data). The greatest challenges include the fact that bats are widespread and dispersed across the landscape, many treatments may have significant side effects, and applying treatments to hibernating bats may cause considerable disturbance during severe energy constraint. Boyles and Willis (2010) suggested that localized “thermal refugia,” or artificially warm areas, inside hibernacula could reduce heat loss and energy expenditure during periodic arousals. This strategy, however, would require that bats detect and travel to refugia and that the conditions within other parts of a hibernaculum are not inadvertently altered. Culling has also been suggested for reducing transmission (Arnold Air Force Base 2009), but this approach generally has little effect on controlling wildlife diseases (Bolzoni et al. 2007; Carter et al. 2009; Lachish et al. 2010) and modeling efforts predict that this strategy would have little effect on disease spread (Hallam and McCracken 2010). Finally, the distribution of Gd in the environment remains unknown (Lindner et al. 2011), only some aspects of growth requirements are understood (Blehert et al. 2009; Gargas et al. 2009), and little is understood about genetic relationships between Europe and North America isolates.

WNS has decimated many bat hibernation colonies, but it does not appear to affect all species or all populations equally (Turner et al. 2011; Table 20.1). When WNS first affects an area, large colonies tend to experience high mortality earlier than smaller colonies. Species composition also appears to affect rates of mortality, with hibernation colonies composed of greater proportions of species preferring humid microclimates tending to show early mortality (Wilder et al. 2011). Although measurable and dramatic declines have been observed in active season bat populations (Brooks 2011; Dzal et al. 2011; Ford et al. 2011), some persistence of maternity colonies occurs, and there is evidence of reproductively active females within affected areas for several years (Dobony et al. 2011). Additionally, bats confirmed with natural infections of Gd can survive, and clear infection from their tissues in captivity (Meteyer et al. 2011) and some free-ranging bats have also demonstrated rapid regeneration of damaged wing membranes (Fuller et al. 2011). The fundamental question of why and how some bats survive infection is one of the most pressing issues. Traits that facilitate survivorship may allow certain bats (at the individual or species level) to resist tissue invasion by Gd and provide increased resilience against WNS, either in the context of avoiding Gd infection or being capable of more rapid and effective recovery. These traits may be related to behavior during hibernation [i.e., microclimate preferences (Davis 1970), dispersal throughout a hibernaculum (Davis and Hitchcock 1965)], differential production and use of energy stores (Jonasson and Willis 2011), ability to mobilize components of the innate and/or adaptive immune function, and/or wound healing and tissue regeneration capacities (Fuller et al. 2011). It is also possible that some WNS survivors have developed immunological resistance to Gd infection (Blanco and Garcia 2008; Romani 2008; Shoham and Levitz 2005) via genetic resistance indicative of allelic diversity and makeup at major histocompatibility complex (MHC) genes. Determining whether survivors are resilient, resistant, or both is critically important to predicting which species and populations are more or less at risk of experiencing WNS-associated morbidity and mortality.

Understanding differences in susceptibility and resistance between North American and European bats is paramount to understanding whether or not WNS is a threat to European bats (Puechmaille et al. 2011a, b), and differences in virulence within and between fungal isolates from Europe and North America may also help predict region-specific effects. For example, it is possible that the identification of gain-of-function mutations in certain isolates could identify populations at greater risk for succumbing to WNS, information paramount to developing mitigation strategies for preventing further spread and for protecting vulnerable populations and/or species.

Identifying the direct, or proximate, cause of mortality will tell us how the disease develops in and affects individuals and would increase our ability to respond by developing specifically directed mitigation strategies and identifying the most at risk species and populations. A potential direct cause of mortality includes death by starvation, supported by observations of emaciation in affected bats (Blehert et al. 2009; Courtin et al. 2010).

The U.S. Fish and Wildlife Service released a national response plan in May of 2011 to guide the reactions of federal, state, and tribal agencies and their partners (http://www.fws.gov/WhiteNoseSyndrome/). This plan established an organizational structure with defined roles for agencies, stakeholders, and the research community. It institutes seven working groups focused on specific needs, which include communication and outreach, conservation and recovery, data and technical information management, diagnostics, disease management, disease surveillance, epidemiological, and ecological research. Implementation of national strategies will help standardize management practices and ensure consistency and will facilitate interpretation of results at the continental scale.

20.9 Implications of Large-Scale Extirpation

The impacts of large-scale extirpation of insectivorous bats due to WNS are largely unknown; however, regional predictions (Frick et al. 2010) and the fact that bats play critical roles in ecosystems worldwide (Kunz et al. 2011) suggest far reaching effects. With the exception of a few bird species, no other taxa is adapted to nocturnal flight, and therefore the disappearance of millions of bats could have significant effects on nocturnal insect populations. The loss of bats could also destabilize whole ecosystems as bats are both significant predators and prey of other species and contribute to nutrient cycling through dispersal of guano. North American bats provide important ecosystem services by consuming vast quantities of insects, many of which are significant agricultural pests (Boyles et al. 2011; Cleveland et al. 2006).

Overall, the predictions for WNS are dire. As many as 25 species of hibernating bats in North America, representing millions of individual animals, are at risk of exposure to Gd and development of WNS. Without further knowledge, we may be left observing a severe population bottleneck and surviving groups with little ability to recover. Scientific advances provide the greatest hope for developing management strategies to protect survivors within the affected region as well as bats not yet affected by the disease.

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Chapter 21
Impacts of Wind Energy Development on Bats: Implications for Conservation

Edward B. Arnett and Erin F. Baerwald

Abstract At a time of growing concern over the rising costs and long-term environmental impacts from the use of fossil fuels, wind energy has become an increasingly important sector of the electrical power industry. However, large numbers of bats are being killed at utility-scale wind energy facilities, and these fatalities raise important concerns about cumulative impacts of proposed wind energy development on bat populations. We discuss our current state of knowledge on patterns of bat fatalities at wind facilities, present new information on cumulative fatalities in the USA and Canada, and present findings from mitigation studies. Given the magnitude and extent of fatalities of bats worldwide, the conservation implications of understanding and mitigating bat fatalities at wind energy facilities are critically important.

21.1 Introduction

Given a changing climate (Inkley et al. 2004; Schlesinger and Mitchell 1987) and rising costs and long-term environmental impacts from use of fossil fuels, the world is increasingly exploring alternatives to supply emission-free energy (Bernstein et al. 2006; Kunz et al. 2007; McLeish 2002). Wind power is one of the fastest growing renewable energy sources (Fig. 21.1), in part due to recent cost competitiveness with conventional energy sources, technological advances, and tax incentives (Bernstein et al. 2006). At regional to global scales, the effects of wind energy on

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_21,

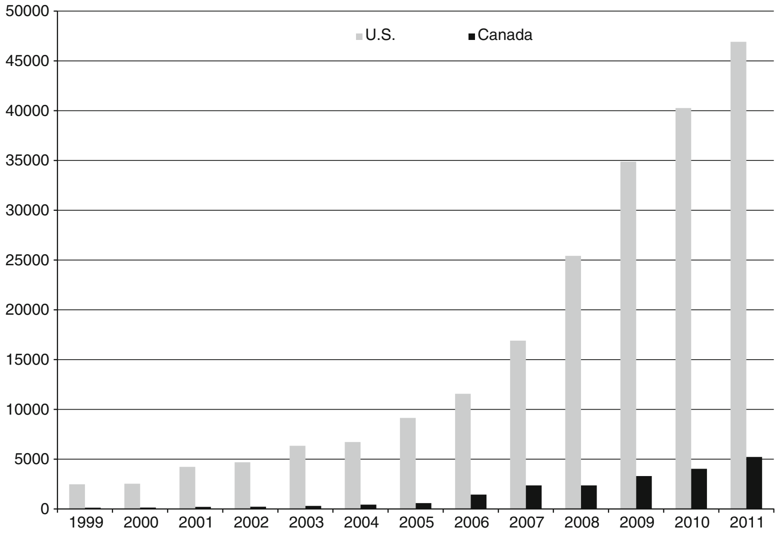

Fig. 21.1 Installed wind energy capacity (MW) from 1999 to 2011 in the USA and Canada (from the Energy Information Administration 2011, http://www.eia.gov/forecasts/aeo; and the Canadian Wind Energy Association, http://www.canwea.ca/farms/wind-farms_e.php)

the environment are positive, owing to reductions in mining activities, air pollution, and greenhouse gas emissions associated with nonrenewable energy sources (NRC 2007). However, wind energy development is not environmentally neutral and impacts on wildlife and their habitats are of increasing concern (Arnett 2012; Arnett et al. 2007; Kunz et al. 2007).

Fatalities of bats (Fig. 21.2) occur at wind energy facilities worldwide but generally received little attention until 2003 when high fatalities were documented at the Mountaineer Wind Energy Center, West Virginia (Arnett et al. 2008), followed by numerous other studies documenting similar losses (e.g., Arnett et al. 2008; Baerwald and Barclay 2009; Dürr and Bach 2004; Rydell et al. 2010). These fatalities raise concerns about population-level impacts (Frick et al. 2010; Racey and Entwistle 2003; Winhold et al. 2008) with wind energy development cited to increase worldwide (Energy Information Administration 2011). Bats are long lived and have exceptionally low reproductive rates, and their population growth is relatively slow, which limits their ability to recover from declines and maintain sustainable populations (Barclay and Harder 2003). The high mortality caused by wind facilities poses a serious threat to bats unless solutions are developed and implemented.

21.1.1 Background

Arnett et al. (2008) reviewed 21 post-construction fatality studies conducted at 19 facilities in the USA and Canada. In this chapter we further synthesized information

Fig. 21.2 A hoary bat found dead at a wind energy facility in the Northeastern Deciduous Forest region (Photo by E.B. Arnett, Bat Conservation International)

from 122 post-construction fatality studies (2000–2011) from 73 regional facilities using only data from studies published in scientific journals or unpublished reports made publicly available through an organization, agency, or online sources. We do not focus extensively on characteristics of surrounding habitat, turbines used, duration of studies, methods employed, and how field sampling biases were accounted for, as did Arnett et al. (2008), but rather focus on patterns of fatality, unifying themes, estimates of fatality and potential cumulative impacts, mitigation, and future research needs.

We categorized regions based on broad habitat characterizations (e.g., forest, shrub-steppe habitats) that potentially influence how bats may generally use an area and considered features that would serve as migration corridors (i.e., topography, geographic landscape, riparian corridors) or affect bat behavior relative to the amount of installed wind capacity. This resulted in 12 regions across the USA (including Alaska and Hawaii) and Canada (Table 21.1). We only present information for five regions because publically available studies were not available from remaining regions or no wind energy facilities have yet been constructed.

We did not have access to data required for quantitative meta-analysis to develop estimates using the published techniques and estimator (Huso 2011). Therefore, we compiled empirical results but only included reported estimates of fatalities where bias corrections (e.g., searcher efficiency and carcass removal) were quantified and used to adjust estimates (Strickland et al. 2011). We report estimated fatalities per turbine and per megawatt (MW, number of fatalities per turbine/megawatt capacity of each turbine type). We caution that studies had varying levels of effort, used different estimators (Huso 2011), and different methods to quantify bias (Arnett et al. 2008).

Table 21.1 Regions of the USA and Canada defined for establishing fatality rates of bats at wind facilities and their installed capacity (MW) as of 31 December 2011

Region	Installed MW (as of 9/30/2011)	States/provinces
Great Basin/Southwest Open Range-Desert	10,037	Southern California and Central Valley; west Texas Pecos region; non-forested Arizona and New Mexico; Nevada; eastern Oregon, Washington, and Idaho; western Utah and Colorado
Great Plains	19,033	Southern Alberta, Saskatchewan, and Manitoba; eastern Montana; North and South Dakota; Nebraska; Kansas; Oklahoma; North and Central Texas; eastern Colorado; unforested portions of Wyoming
Gulf Coast	1,217	Coastal Texas and Louisiana (inland 200 km)
Midwestern Deciduous Forest-Agricultural	13,361	Southern Ontario, Minnesota, Wisconsin, Iowa, Michigan, Illinois, Missouri, Indiana, Ohio
Northern Boreal-Taiga	<1	Central Alaska; most of Northwest and Yukon Territories; northern portions of British Columbia, Alberta, Ontario, Saskatchewan, Manitoba, and Quebec; most of Newfoundland; southern portion of Nunavut
Northeastern Deciduous Forest	4,872	Delaware; Maine; Maryland; Massachusetts; New Hampshire; New Jersey; New York; Pennsylvania; Rhode Island; Vermont; West Virginia; New Brunswick; southern portions of Newfoundland, Ontario, and Quebec; Prince Edward Island; and Nova Scotia
Northern Tundra	10	Northern portions of Alaska, Northwest Territories, Yukon Territory, Nunavut, Quebec, and Newfoundland
Southeastern Mixed Forest	29	Tennessee, Kentucky, Virginia, East Texas, North and South Carolina, Georgia, central and north Louisiana, Arkansas, Mississippi, Alabama, and Florida
Tropical Forest	93	Hawaii
Tropical Open Range-Grassland	0	Hawaii
Western Temperate Forest	247.5	Forested portions of eastern Oregon, Washington, and British Columbia; Idaho; Montana; Wyoming; Colorado; Utah; New Mexico; Arizona
Coastal Temperate Forest	0	West Cascades Oregon and Washington, coastal British Columbia and Alaska, northern California redwood forests

21.2 Reasons for Bat Mortalities

Kunz et al. (2007) proposed several hypotheses relating to potential attraction factors (e.g., insects, heat, visual, sound, roosting opportunities) of turbine arrays. Cryan and Barclay (2009) proposed proximate (how bats were being killed) and ultimate (why bats were being killed) causes of bat fatalities that included random chance events, coincidental fatality during migration, and attraction factors.

Thermal imaging studies confirm that bats are attracted to turbines (Horn et al. 2008). Kunz et al. (2007) proposed a roost attraction hypothesis that seemed potentially plausible. However, Cryan (2008) noted that *Lasiurus* species may use the tallest trees in a landscape as rendezvous points or possibly lekking sites during the migration/mating period and therefore may mistake turbines for trees, resulting in a bias toward fatalities of migratory species (Cryan and Barclay 2009). Baerwald and Barclay (2011) suggested that if the mating behavior hypothesis is correct, then we would expect correlation between timing of adult male/female migration; however, they reported asynchronous migration between sexes of hoary bats (*Lasiurus cinereus*) in southwestern Alberta, suggesting that mating behavior was not likely causative of wind turbine mortality in that region. Baerwald and Barclay (2011) did report concurrent timing of adult male/female silver-haired bats during (*Lasionycteris noctivagans*) migration and that mating behaviors could be associated with fatalities. As is, the roost tree and mating hypotheses may account for mortality in some species, but clearly does not explain fatalities of other species such as the Brazilian free-tailed bats (*Tadarida brasiliensis*) (Miller 2008; Piorkowski and O'Connell 2010) whose mortality may be a coincidental outcome of migration (Cryan and Barclay 2009) or be due to periodic insect aggregations. Although testing hypotheses regarding causation of large-scale bat kills by wind turbines is challenging, it is paramount for preventing future fatalities, especially during early planning and risk assessment phases of development (Cryan and Barclay 2009).

Baerwald et al. (2008) found that the cause of death in many bats at turbines was due to barotrauma, resulting from sudden decompression due to low-pressure air pockets produced by rotating turbine blades (Kunz et al. 2007). Approximately 90 % of dead bats found had no visible signs of injury (Fig. 21.3), but had internal hemorrhaging consistent with barotrauma, whereas direct contact with turbine blades accounted for about half of the fatalities (Baerwald et al. 2008). Grodsky et al. (2011) found that 74 % of 23 bat carcasses examined using radiology had bone fractures, primarily in wing bones, but that using visual inspection only resulted in 33 % fewer detected bone fractures. They also reported that more than half of the specimens had mild to severe hemorrhaging in the middle and/or inner ears, and thus, it is difficult to attribute individual fatalities exclusively to either direct collision or barotrauma. Rollins et al. (2012) found that 73 % of bats they examined had lesions consistent with traumatic injury by physical contact with turbine blades but that 20 % had ruptured tympana, likely from barotrauma (Rollins et al. 2012).

In order to fully account for bat mortality at turbines, we must consider possible crippling effects resulting from inner ear damage and thus echolocation ability due to barotrauma or bone fractures that may cause death far from the offending site. Although Grodsky et al. (2011) suggested that delayed lethal effects could result in underestimating fatalities, they found that 71 % of bat carcasses found were within 30 m of turbines, and the majority of those having the fewest broken bones were found within 40 m of turbines. This finding is consistent with a unifying pattern in all regions we reviewed. Approximately 85 % of bats grounded but still alive found by Klug and Baerwald (2010) were discovered within 35 m of the turbine base, and 69 % of these had no obvious external injuries but fell rapidly to the ground after nonlethal encounters with only a minor number falling outside search plots. While individuals experiencing delayed lethal effects might bias fatality estimates, the

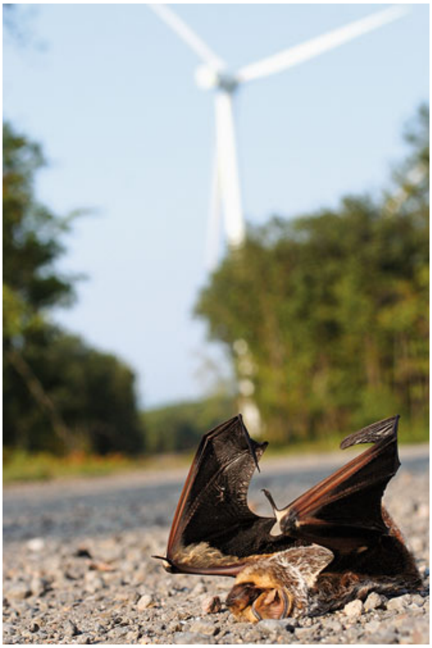

Fig. 21.3 A dead hoary bat found beneath a turbine showing no visible sign of injury (Photo by M.R. Schirmacher, Bat Conservation International)

only feasible method to test this is by using trained dogs (Arnett 2006; Fig. 21.4) to search excessively large plots around turbines (Strickland et al. 2011). However, even dogs cannot search entire landscapes and certain habitats (e.g., forests), so while we do not feel that delayed lethal effects contribute greatly to underestimating fatalities, the true influence may never be known.

21.3 Estimates of Bat Fatalities

We again caution that patterns and estimates of fatalities reported here are affected by many factors (Arnett et al. 2008), but for regions having multiple studies, estimates of bat fatalities were highest at facilities located in the Northeastern Deciduous Forest (Fig. 21.5) and Midwestern Deciduous Forest-Agricultural regions (Table 21.2). The Midwestern region generally had been considered to have low–moderate fatality rates relative to the eastern USA and Canada (Arnett et al. 2008; Johnson 2005); however, this is no longer the case. The Great Plains region (Fig. 21.6) has highly variable (0.16–21.6 individuals/MW) but moderately high fatality rates on average (Table 21.2). While several studies included in this region had low fatality rates, our stratification of this region also included the southern part of the Canadian prairie provinces and facilities in northern Texas, which have reported high fatality rates. The Great Basin/Southwest Open Range-Desert region consistently has reported the least variable and lowest fatality rates for bats (Arnett et al. 2008; Johnson 2005). Wind energy facilities in this region occur in habitats generally offering few if any

Fig. 21.4 The senior author with his chocolate Labrador retriever, Sage, after discovering a tricolored bat at a wind energy facility. Trained dogs can find more than 80 % of bats during searcher efficiency trials and are especially effective in dense, low-visibility vegetation (Photo by M.D. Tuttle, Bat Conservation International)

Fig. 21.5 Wind energy facilities on forested ridges in the eastern USA have consistently documented high fatality rates of bats (Photo by M.D. Tuttle, Bat Conservation International)

Table 21.2 Number of sites and studies and estimates of bat fatality (mean and 95 % confidence limits) for each region in the USA and Canada, 2000–2011

Region	No. sites w/data	No. studies	Mean fatalities	Lower 95 % CL	Upper 95 % CL
Coastal Temperate Forest	–	–	–	–	–
Great Basin/Southwest Open Range-Desert	17	24	1.39	1.02	1.76
Great Plains	20	32	6.04	3.98	8.10
Gulf Coast	–	–	–	–	–
Midwestern Deciduous Forest-Agricultural	14	23	7.94	4.92	10.96
Northern Boreal-Taiga	–	–	–	–	–
Northeastern Deciduous Forest	21	44	8.30	6.08	10.52
Northern Tundra	–	–	–	–	–
Southeastern Mixed Forest	1	2	41.17	28.61	53.73
Tropical Forest	–	–	–	–	–
Tropical Open Range-Grassland	–	–	–	–	–
Western Temperate Forest	–	–	–	–	–

Fig. 21.6 Wind energy facilities in shrub-steppe habitats in the western USA consistently document fatalities of bats, but rates are usually considerably lower than other regions (Photo by E.B. Arnett, Bat Conservation International)

roosting resources, possibly (but untested) poor foraging opportunities, and may not be in migratory pathways, thus rendering these sites less risky to bats. However, we caution that this pattern could change as development increases and if facilities are poorly sited in areas with higher use and densities of bats.

21.4 Bat Fatalities and Cumulative Impacts

The context of fatalities remains a mystery, in part because little population data exist for most species of bats (O'Shea et al. 2003) and has hindered understanding of population-level impacts, as well as effectiveness of mitigation measures. Estimating exposure risk of bats to collisions with turbine blades is problematic but necessary to understand the context of fatalities. The role of abundance, relative to exposure of bats to collisions, may be modified by behavior within and among species and likely varies across locations, although avoidance behavior is complicated by possible attraction to turbines (Cryan and Barclay 2009; Horn et al. 2008). Studies using radar, thermal imaging, acoustic monitoring, and other technologies simultaneously and concurrent with fatality studies would help determine exposure risk. Model-based analysis is also helpful, but empirical data are generally lacking for developing such models (Arnett et al. 2007), but high bat fatalities at some wind energy facilities raise concern about biologically significant additive mortality (Kunz et al. 2007). At the end of 2011, there was 5,265 MW of wind energy installed in Canada and 46,919 MW in the USA, for a total of 52,184 MW (Table 21.1, Fig. 21.1). We calculated mean bat fatalities and 95 % confidence intervals for each region (Table 21.1) and then calculated a weighted mean and confidence interval (based on regional means and weighted by installed capacity) for each year from 1999 to 2010 in the USA and Canada that was multiplied by the total installed MW for each year. The total estimated fatalities for each year were then summed to determine cumulative fatalities between 2000 and 2011 (data from the prior year were used to develop the weighted means and estimates for the following year; no estimate of kills for 1999, but studies from that year were used to derive estimates for the following year in 2000, based on 1999 data and installed capacity). Assuming fatality rates are (1) representative of all regional sites, and (2) consistent from year to year without behavioral modification or mitigation, cumulative bat fatalities in the USA and Canada ranged from 650,104 to 1,308,378 over the past 12 years (Table 21.3). This estimate is projected to increase by 196,190–395,886 bats in 2012. Three species of migratory tree bats (hoary, eastern red (*Lasiurus borealis*), and silver-haired bats) accounted for 78 % of cumulative fatalities, ranging from 538,902 to 1,229,547 individuals killed since 2000. Eastern red bat populations are already thought to be in decline (Winhold et al. 2008), and almost nothing is known about population status and mortality factors affecting this species or populations of hoary and silver-haired bats.

However, some evidence suggests significant population declines of migratory tree bats. Historical accounts of large flocks (>100 individuals) of migrating eastern red bats and hoary bats no longer occur (Winhold et al. 2008). Furthermore, capture rates of lasiurine bats (although perhaps somewhat biased by capture probability) have declined across North America (Carter et al. 2003). Finally, although also biased, the number of lasiurine bats submitted for rabies testing across the USA has decreased; in Arkansas from 1938 to 1998, the number of eastern red bats submitted for rabies testing has fallen by approximately 3 bats per year (Carter et al. 2003); in Michigan a tenfold decrease occurred over 38 years (Winhold et al. 2008); in Indiana, the proportion of eastern red bats submitted has declined 7 % between

Table 21.3 Estimates of cumulative fatalities of each species of bat from 2000 to 2011 for all regions combined in the USA and Canada

Scientific name	Common name	% of total fatalities	Lower range	Upper range
Eptesicus fuscus	Big brown bat	4	26,004	52,255
Lasiurus blossevillii	Western red bat	<0.01	69	143
Lasiurus borealis	Eastern red bat	22	143,023	287,403
Lasiurus cinereus	Hoary bat	38	247,040	633,822
Lasiurus cinereus semotus	Hawaiian hoary bat	<0.001	4	8
Lasiurus ega	Southern yellow bat	<0.01	69	143
Lasiurus intermedius	Northern yellow bat	<0.01	553	1,145
Lasionycteris noctivagans	Silver-haired bat	18	148,839	308,322
Lasiurus seminolus	Seminole bat	<0.01	1,106	2,290
Lasiurus xanthinus	Western yellow bat	<0.01	622	1,288
Myotis evotis	Long-eared myotis	<0.01	3,731	7,730
Myotis lucifugus	Little brown myotis	6	51,617	106,925
Myotis septentrionalis	Northern myotis	<0.01	1,175	2,433
Myotis sodalis	Indiana bat	<0.01	69	143
Myotis velifer	Cave myotis	<0.01	69	143
Myotis volans	Long-legged myotis	<0.01	69	143
Nyctinomops femorosacca	Pocketed free-tailed bat	<0.01	69	143
Nycticeius humeralis	Evening bat	<0.01	1,589	3,292
Perimyotis subflavus	Tricolored bat	6	45,260	93,756
Parastrellus hesperus	Canyon bat	<0.01	69	143
Tadarida brasiliensis	Brazilian free-tailed bat	3	21,282	44,087
Unknown spp.	Unknown spp.	2	20,036	41,505
Total	Total		650,104	1,306,378

1990 and 2000, and hoary bat submissions declined from 3.8 to 1.8 % during the same period (Whitaker et al. 2002).

Turbine fatalities for little brown bats (*Myotis lucifugus*) were estimated to be between 51,617 and 106,925 since 2000 (Table 21.3), and while these estimates are low relative to migratory tree bats, the cumulative impact for cave-hibernating bats is significant and important in light of massive fatalities from white-nose syndrome (WNS; Frick et al. 2010; Turner et al. 2011). Although estimated wind turbine-related fatalities occurred over a longer period than calculated for WNS bat fatalities (Turner et al. 2011), continued wind turbine effects may further compound population declines. Furthermore, wind-related fatalities are skewed toward migratory tree bats, whereas WNS affects hibernating species.

21.5 Composition of Fatalities

Of the 47 species of bats known to occur north of Mexico, individuals of 21 have been reportedly killed at wind energy facilities (Table 21.3) and, as mentioned, fatalities are skewed toward migratory, foliage-roosting species including hoary bats (38 %), eastern red bats (22 %), and silver-haired bats (18.4 %) that comprised a total of 78.4 % of all bat turbine fatalities (Table 21.4). Hoary bats constituted the highest proportion at most facilities (range = 12–51 %; Table 21.44), whereas tricolored bats constituted nearly 25 % of fatalities at some facilities in the eastern USA but only 5.6 % across all fatalities (Table 21.4). Similarly, at some facilities in the Northeastern Deciduous Forest and Midwestern Deciduous-Agricultural regions, the little brown bat comprised up to 60 % of fatalities but only about 6 % of total fatalities. Brazilian free-tailed bats encompass up to 90 % of fatalities at some facilities in Texas (Miller 2008) and Oklahoma (Piorkowski and O'Connell 2010), and at five sites in California, they constituted 31–57 %. Since 2007 when Arnett et al. (2008) reported that 10 species of bats had been found killed at wind facilities, 11 new species have been documented: long-eared myotis (*Myotis evotis*), long-legged myotis (*Myotis volans*), southern yellow bat (*Lasiurus ega*), northern yellow bat (*Lasiurus intermedius*), western yellow bat (*Lasiurus xanthinus*), evening bat (*Nycticeius humeralis*), cave myotis (*Myotis velifer*), canyon bat (*Parastrellus hesperus*), pocketed free-tailed bat (*Nyctinomops femorosacca*), and the US federally endangered Indiana bat (*Myotis sodalis*; Good et al. 2011) and Hawaiian hoary bat (*Lasiurus cinereus semotus*, two fatalities; R. Roy, First Wind, personal communication). Recent reports of Indiana bat fatalities precipitated the development of site-specific habitat conservation plans (HCPs) at the US Fish and Wildlife Service's Midwest region under Section 10 of the US Endangered Species Act (T.J. Miller, US Fish and Wildlife Service, personal communication). Site-specific HCPs have been developed for the Hawaiian hoary bat. Although no other federally threatened or endangered species has been found killed at a wind facility in the USA, anticipated facility expansion within the range of listed species such as the lesser long-nosed bat (*Leptonycteris yerbabuenae*) may yield fatalities. Several of the species found killed at wind facilities have either state of federal agency management status or both, although such status rarely provides a nexus for mandatory survey or mitigation requirements. In Canada, three species (hoary, silver-haired, and eastern red bats) found killed at wind facilities are considered to be of special management concern provincially. Additionally, the Committee on the Status of Endangered Wildlife in Canada recently announced that WNS poses a serious and imminent threat to the survival of bats and recommended that the Federal Environment Minister issue an "emergency order," placing the tricolored bat, little brown myotis, and northern myotis on Canada's list of endangered species.

21.6 Temporal and Spatial Patterns

21.6.1 Seasonal Timing of Fatality

Consistently, the highest bat fatalities occur during late summer and early fall (Arnett et al. 2008; Rydell et al. 2010) which coincide with autumn migration (Cryan 2003; Fleming and Eby 2003), although some fatalities during spring migration have also been reported (Arnett et al. 2008). One hypothesis explaining different seasonal fatality rates is that migratory tree bats may exhibit different behaviors and follow alternate migration routes in each season (Cryan 2003). Baerwald and Barclay (2011) found that fatalities of hoary bats began in late July and early August and were followed by silver-haired bats in mid- to late August, implying that variation in timing of migration is due to different geographic distributions of populations during summer. Piorkowski and O'Connell (2010) reported spring-summer fatalities of Mexican free-tailed bats that included pregnant females during searches conducted in May and June, whereas Miller (2008) reported highest kills of Mexican free-tailed bats in fall with peaks in September and October, indicating that the number of fatalities reported by Piorkowski and O'Connell (2010) was low.

21.6.2 Spatial Patterns

The spatial context of bat kills, both among turbines within a facility and among different facilities, could be useful for developing mitigation strategies. If, for example, kills were concentrated at specific turbines, then curtailment, removal, or relocating that turbine may reduce bat deaths. However, if fatalities are broadly distributed, then facility-wide mitigation strategies would be necessary (Arnett et al. 2008). Although most studies indicate that fatalities generally are distributed across a facility, Piorkowski and O'Connell (2010) found a cluster of turbines to be a hot spot of collision mortality. Baerwald and Barclay (2011) found no differences in fatalities on the east vs. west side of a facility in southern Alberta, but the fatality rate was higher at the north end. Fielder et al. (2007) also observed a general north–south trend of bat fatalities, and Gruver et al. (2009) found slightly higher mortality in the northern portion of their study area for both migratory and nonmigratory species. Baerwald and Barclay (2011) hypothesized that because fall migrations are from north to south, higher fatality rates could be expected at the more northerly turbines first encountered by migrating bats. This pattern likely varies from site to site depending on the facility's location relative to the direction of fall bat migrations.

In addition, Baerwald and Barclay (2009) documented higher activity and fatality rates of bats at wind facilities near the foothills of the Rocky Mountains as compared to eastward prairie grasslands. They hypothesized that turbine proximity to stopover and roost sites in foothills habitat significantly increased fatality rates assuming that geographical landmarks are used for navigating migration routes and that bats judge nightly travel distances between suitable diurnal roosting sites (Baerwald and Barclay 2009; Cryan and Veilleux 2007; Fleming and Eby 2003). There also appears to be a pattern of latitudinal decline in bats fatalities in the Northeastern Deciduous Forest region from the Mid-Atlantic area in Pennsylvania and West Virginia (highest fatalities) northward to Maine and the eastern Canadian provinces (lowest fatalities). It seems plausible that seasonal abundance, distributions, and migratory patterns, any of which may be influenced by climatic conditions and food availability, could impact mortality rates resulting in a pattern of declining kills with increasing latitude in this region.

If fatalities are related to habitat or topographic characteristics, then understanding these relationships may help in developing mitigation strategies (e.g., avoiding placement of turbines near open water sources or known roosts; Arnett et al. 2008). Many wind energy facilities occur in settings with too little habitat or topographic variation among turbines to allow an evaluation of landscape relationships with bat fatalities. However, Johnson et al. (2004) did not find a significant relationship between the number of bat fatalities and any of the 10 cover types within 100 m of turbines at facilities in Minnesota or any relationship between fatalities and distance to nearest wetland or woodlot. Distance to wooded area, regardless of woodlot size, did not predict number of fatalities at wind turbines in Wisconsin either (Gruver et al. 2009). In Oklahoma, Piorkowski and O'Connell (2010) found no consistent pattern in bat fatalities relative to ground cover or topographic position, but did find that fatalities were higher at several individual turbines, all of which were located near the heads of eroded ravines. In New York, Jain et al. (2007) found no significant relationship between bat fatalities and distance to wetlands using daily and 3-day carcass searches, but did find moderate evidence of higher fatalities in proximity to wetlands when using 7-day searches. Conversely, they found no relationship with proximity to woodlands at a different facility in New York. Interestingly, Grodsky (2010) found a significant relationship between fatalities and distance to the Horicon Marsh, but fatalities were actually lower near the marsh.

Piorkowski and O'Connell (2010) documented the first evidence of collision mortality of Mexican free-tailed bats at a North American wind farm that could be attributed to the site's proximity (~15 km) to a large maternity colony. In Wisconsin, Grodsky (2010) found no relationship between distance of turbines from a large hibernaculum (Neda Mine). Given that the majority of bat fatalities appear to be active during migrations, it may not be enough to consider the proximity of a facility to a maternity or hibernation site, but rather where it is located relative to movement corridors between these important sites.

21.7 Effects of Turbine Size

Barclay et al. (2007) reported that taller turbines had significantly higher fatalities on bats than did smaller ones. However, the sites used in this analysis were not sampled simultaneously during the same years, and the reported difference could have resulted from annual variation, increased survey effort at sites with taller turbines, or some other factor. Notwithstanding, Arnett et al. (2008) reported that height and dimensions of the rotor-swept area of turbines appeared to influence bat fatalities. During the second phase of study at Buffalo Mountain, Tennessee, 0.66-MW turbines with 65-m-tall towers and 1,735-m^2 rotor-swept area killed fewer bats per turbine but more bats per MW than adjacent 1.8-MW turbines with 78-m towers and nearly three times the total rotor-swept area (Arnett et al. 2008; Fielder et al. 2007). At the Buffalo Ridge site in Minnesota, taller turbines with greater rotor-swept areas killed more bats per turbine and per MW compared to smaller ones

(Arnett et al. 2008:63). Baerwald and Barclay (2009) found that bat fatality rates varied in part due to differences in turbine height, with taller turbines yielding higher fatalities.

21.8 Fatalities in Relation to Weather Variables

Arnett (2005) employed daily carcass searches and related them to weather variables and found that most bats were killed on low-wind nights when power production appeared insubstantial, but turbine blades were still moving (often times at or close to full operational speed at 17 revolutions/min [rpm]). In addition, 82–85 % of bat fatalities at two facilities in the eastern USA were estimated to have occurred on nights with median nightly wind speeds of <6 m/s (Arnett et al. 2008).

In Iowa, Jain et al. (2011) found that maximum wind speeds when bat collisions likely occurred ranged from 2.4 to 5.3 m/s, and Good et al. (2011) demonstrated for every 1 m/s increase in wind speed, bat fatalities decreased by 14 %. Grodsky (2010) used weekly power output as a surrogate for wind speed and found a significant negative relationship between fatalities and power, indicating lower wind speeds yield higher bat fatalities. Indeed, fatalities are typically highest during lower wind speeds, usually <6.0 m/s (Arnett 2005; Rydell et al. 2010; Young et al. 2010, 2011). In addition, fatalities increased as ambient temperature rose to some threshold (Grodsky 2010; Young et al. 2011), and Baerwald and Barclay (2011) reported that species-specific fatalities were affected by greater moon illumination. They also observed that falling barometric pressure and the number of deaths were correlated and that whereas fatalities of silver-haired bats increased with increased activity, moon illumination, and southeasterly winds, hoary bat mortality increased most significantly with falling barometric pressure. Interestingly, neither hoary bat activity nor fatality was influenced by any measured variables other than falling barometric pressure, possibly because migrating bats are less selective of environmental conditions at the northern end of their migration (Baerwald and Barclay 2011).

21.9 Offshore Wind Facilities

Offshore wind facilities occur throughout Europe, but few studies have determined impacts on animals, and although virtually these relate only to birds (Arnett et al. 2007), observations in Europe and anecdotal accounts of bats occurring offshore suggest probable impacts. Ahlen et al. (2009) recorded 11 species of bats flying over the ocean up to 14 km from shore. They observed both migrant and resident bats foraging over water on abundant insects and observed these bats rapidly changing altitude to forage around turbine blades at an offshore facility. Cryan and Brown (2007) observed hoary bats readily migrating over open ocean between islands that are stopping points along the migratory route. Johnson et al. (2011) recorded five species of bats, including eastern red bats, big brown bats (*Eptesicus fuscus*), hoary bats, tricolored bats, and silver-haired bats, on a barrier island off the coast of Maryland along their migratory route. Use of such islands would have implications for wind energy development near- and offshore.

We suggest that impacts of the first several offshore wind energy facilities proposed and built in North America, including those on inland waters such as the Great Lakes, be evaluated extensively for both fatalities and displacement effects, although finding and retrieving dead birds and bats from water bodies will be a considerable challenge (Arnett 2012; Arnett et al. 2007).

21.10 Mitigating Bat Mortality

21.10.1 Operational Mitigation

As mentioned, most bat fatalities occur during relatively low-wind conditions over a relatively short period of time during bat migration periods (Arnett et al. 2008). Operational adjustments under these conditions and during this time period have long been proposed as a possible means of reducing impacts to bats (Arnett 2005; Arnett et al. 2008; Kunz et al. 2007). In southern Alberta, Baerwald et al. (2009) reported that raising turbine cut-in speeds (i.e., wind speed at which wind-generated electricity enters the power grid) above the manufactured speed (usually 3.5–4.0 m/s for modern turbines) and altering blade angles to either stop or slow rotor movement in low wind speeds significantly reduced mortality by up to 60.0 %. Arnett et al. (2011a) found that nightly reductions in bat fatality ranged from 44 to 93 % when turbine cut-in speed was raised from 3.5 m/s to either 5.0 or 6.5 m/s. The resulting economic loss was less than 1 % of the total annual energy output for the facility. In Indiana, Good et al. (2011) slightly modified the Arnett et al. (2011a) study design and reported an approximate 50 % reduction in overall bat fatalities when turbine cut-in speed was raised from 3.5 to 5.0 m/s and approximately 78 % fewer fatalities when cut-in speed was raised from 3.5 to 6.5 m/s.

Interestingly, some turbine models actually spin, sometimes at full rotational speed, below the turbine's cut-in speed, which can kill bats even when no electricity is being generated. Young et al. (2010) found that by simply pitching turbine blades parallel to the wind and stopping turbine blades from spinning below the manufacturer's cut-in speed (4.0 m/s; Baerwald et al. 2009), fatalities were reduced significantly, and by excluding nights that treatments were not in effect, the odds of a bat casualty was 3.69 times less likely at curtailed turbines during the first 5 h past sunset and approximately two times less likely at turbines curtailed during 5 h prior to sunrise than under normal operations. This represented 50–72 % fewer bat kills at curtailed turbines with little financial cost beyond operational time to implement treatments. Such operational costs could be minimized if turbine computer systems are reconfigured to account for such adjustments and automatically implemented on turbines. While costs of lost power due to mitigation can be factored into the economics, financing, and power purchase agreements of new projects, altering turbine operations even on a limited-term basis potentially poses difficulties on existing projects, so there is considerable interest in developing other solutions that do not involve turbine shutdowns.

21.10.2 Turbine Color

Insect attraction to and activity near wind turbines remains a valid, but untested, hypothesis (Cryan and Barclay 2009; Kunz et al. 2007). Long et al. (2010) found that, at ground level, common turbine colors, white and light grey, attracted significantly more insects than other colors tested; however, tests at hub height and/or at operating wind facilities have not been conducted to date.

21.10.3 Electromagnetic Signals

Studies in Scotland suggest that bat activity may be deterred by electromagnetic signals from small, portable radar units. Nicholls and Racey (2009) reported that bat activity and foraging effort per unit time were significantly reduced during experimental trials when their radar antenna was fixed to produce a unidirectional signal that maximized exposure to foraging bats. The effectiveness of radar as a potential deterrent has not been tested at an operating wind facility; thus, it remains unclear if electromagnetic signals would deter bats that are migrating as effectively as those that are foraging. Moreover, the effective range of electromagnetic signals as well as the number of radar units needed to affect the most airspace has not been determined, but must be to fully evaluate effectiveness and the cost-benefit analysis relative to other potential deterrents (Arnett et al. 2011a; Baerwald et al. 2009).

21.10.4 Ultrasonic Broadcasts

Griffin et al. (1963) showed that broadband random ultrasonic noise could partially mask bat echolocation, and Mackey and Barclay (1989) concluded that ultrasound broadcasts reduced bat activity by increasing the difficulty for bats to hear and interpret the echoes from insects. Arnett et al. (2011b) tested a newly designed ultrasonic broadcasting device and found that, after accounting for inherent variation among sample turbines, bat fatalities were reduced up to 64 % at turbines with deterrent devices relative to control turbines (see http://www.batsandwind.org) and that broadband, ultrasound broadcasts may discourage bats from approaching a sound source that interferes with their echolocation. However, effectiveness is limited by the distance and area that ultrasound can be broadcast. Ultrasound attenuates quickly and is heavily influenced by humidity, and thus ultrasonic deterrent devices are still in the experimental and modifications phase. Future studies should attempt to optimize both placement and number of devices on each turbine that would affect the greatest amount of airspace in the rotor-swept area and evaluate the cost-effectiveness of deterrents in relation to different operational strategies.

21.11 Implications for Conservation

The negative effects of turbines on bats are troubling because in a larger context assaults on bat welfare are many, and most importantly WNS is rapidly spreading and decimating populations of several cave-hibernating species of bats (Frick et al. 2010; Turner et al. 2011). If taken cumulatively, the overall impact on bat communities is predictably devastating and will have profound ecological and economic impacts (Boyles et al. 2011). Animals that migrate tend to be more vulnerable to extinction than those that do not (Fleming and Eby 2003; Pimm et al. 1988) and also require appropriate habitat in several, spatially disjunct locations including breeding/summering sites, hibernation/overwintering sites, stopover sites, and linked migratory corridors. Although the distributions, genetic structure, and migratory routes of bats are mostly unknown, if certain routes have an increased risk of mortality at wind facilities, then this may lead to genetic isolation and endangerment of specific subpopulations. Migratory tree-roosting species killed most frequently by turbines in North America are not protected under federal, state, or provincial laws (Arnett 2012; Cryan 2011). Further, while bats may be protected under state laws pertaining to “nongame” animals, most states do not enforce the killing of bats by wind turbines (Arnett 2012).

21.12 Conclusions

Whereas predicting patterns of fatality based on habitat types and other covariates is confounding, it may be possible to predict high-risk facility locations based on possible migratory pathways (Baerwald and Barclay 2009). As such, we strongly encourage future research efforts that identify migratory pathways and stopover sites and the establishment of buffers where turbines may not be constructed near maternity roosts, hibernacula, and other important areas.

Population data are lacking for most species of bats (O’Shea et al. 2003) and particularly migratory tree bats (Carter et al. 2003). Not only does this impede our understanding of the true impacts of wind turbines but also makes it difficult to determine if, for example, a 50 % reduction in bat fatalities from changing turbine cut-in speed is an adequate mitigation strategy or is simply delaying inevitable population-level impacts. The lack of population data also make it difficult to set triggers for mitigation (i.e., number of bats killed per turbine or MW that requires mitigation). However, such data are not likely to be available for most bats species in the near future, and thus, wind operators should practice the precautionary principle and implement operational mitigation at sites where bat fatalities are high, even in the absence of population data.

Future mitigation experiments should be designed to determine which factors (e.g., temperature, wind, humidity, moon illumination) or combination of factors will best improve predictability of bat fatalities while minimizing economic costs (Weller and Baldwin 2012). Developers must avoid building wind energy facilities in high-risk areas based upon pre-construction activity data, even though analyses linking pre-construction activity with post-construction fatality data are still lacking. More detailed meta-analyses of existing data may yield important relationships, but lack of data disclosure from many sites by some companies hinders such analyses.

We encourage developers to follow guidelines (Strickland et al. 2011) consistently when implementing pre- and post-construction monitoring and allow for these data to be published in refereed journals or placed into the public domain to avoid unnecessary skepticism regarding the quality of such efforts. There have been relatively few studies of wind energy effects on wildlife in peer-reviewed scientific journals (Arnett et al. 2007), although this trend is changing (Arnett 2012), allowing for decision-making based on solid science (Kunz et al. 2007; NRC 2007). Research partnerships among diverse players help generate common goals and to provide adequate funding for research [Arnett and Haufler 2003; Bats and Wind Energy Cooperative (http://www.batsandwind.org), American Wind and Wildlife Institute (http://www.awwi.org)] as well as garner support from government agencies, industry, NGOs, and academia that can leverage dollars and logistical support needed and provide peer-review, dissemination processes, and transparency that yield credibility (Arnett 2012).

The Canadian Wind Energy Association is calling for wind energy to provide 20 % of Canada's electricity, an additional 50,000 MW by 2025 (http://www.canwea.ca/images/uploads/File/Windvision_summary_e.pdf). The US Department of Energy (2008) estimated that 241 GW of land-based wind energy development on approximately five million hectares will be needed to reach 20 % electricity production for the USA by 2030. Kiesecker et al. (2011), however, estimated that there is 3,500 GW of potential wind energy that could be developed across the USA on already disturbed lands which would avert development of 2.3 million hectares on undisturbed lands while generating the same amount of energy. While we agree with this strategy, we caution that development in disturbed landscapes may not alleviate bats fatalities because high bat fatalities are already reported in agricultural settings in the Midwestern Deciduous Forest-Agricultural region (Grodsky 2010) and Great Plains (Baerwald and Barclay 2009). Thus, operational mitigation may be required at facilities constructed in disturbed landscapes.

There are a number of policies, regulatory, and communication challenges we face in protecting bats while developing wind energy responsibly (Arnett 2012). Unless there is a federal, state, or provincial nexus, most research, siting, and mitigation efforts by wind energy developers and operating companies will be voluntary, likely without regard for cumulative effects. Sites that do trigger a regulatory nexus will be driven by endangered species issues, and associated operational mitigation may also benefit other bat species. We encourage continuing cooperation with stakeholders, gathering needed information, avoiding construction in high-risk sites, considering cumulative effects, and implementing mitigation where needed even when no regulatory process is triggered. We do, however, recognize that companies must be treated fairly and consistently to ensure proactive measures are implemented. That some companies may choose to cooperate while others may not (Arnett 2012) creates unnecessary angst and deters resolving wildlife impacts and other issues. Decision-making based on the best available science, consistent policy, accountability, effective siting and mitigation strategies, and a "level-playing field" for the industry (i.e., consistent requirements and incentives for all companies) is fundamental for successfully developing wind energy that protects bats and other wildlife.

Acknowledgments We appreciate reports shared with us by Western Ecosystems Technology, Stantec, Golder, TransAlta Wind, and several other companies. We are grateful to C. Martin and N. Quintana, Texas Tech University, for assisting with the development of our stratification criteria for regions. This chapter was greatly enhanced by reviews from R. Adams, S. Pedersen, and an anonymous reviewer. We dedicate this chapter to our friend and colleague David Redell, whom we lost way too soon. We also dedicate our work to Dr. Thomas Kunz for all of his wisdom, knowledge, and efforts in regard to wind energy impacts on bats.

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Chapter 22
Responses of Bats to Climate Change: Learning from the Past and Predicting the Future

Gareth Jones and Hugo Rebelo

Abstract Climate change is widely regarded as being of major and growing importance for influencing the future distribution and abundance of organisms. However, the potential effect on bats has received little attention. Herein we provide some general background for climate change and its broader context to biodiversity. We use predicted climate-induced 'universal responses' by organisms and data from historical (Holocene) events to better predict how bats may respond. We also outline how the effects of climate change on bats will create challenges for populations in the future, how best to mitigate impacts, and identify research needs.

22.1 The Evidence for Climate Change

The overwhelming consensus among climatologists is that the Earth is experiencing a rapid increase in global annual mean surface temperature (Hansen et al. 2006) (Fig. 22.1), and overwhelming evidence indicates that warming over the last 50 years is attributable to human use of fossil fuels that has increased concentrations of greenhouse gases (Houghton et al. 2001). In addition, the rate of warming appears to be increasing and is most pronounced at high latitudes (Hansen et al. 2006). The IPCC predicts temperature rises of 1.1–6.4 °C by the end of this century (Bernstein et al. 2007), and regional climatic shifts could match or exceed the magnitude experienced over the past 21,000 years, since the Last Glacial Maximum (LGM) (Jackson and Overpeck 2000). Further, temperatures may increase beyond the maximum of

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_22,

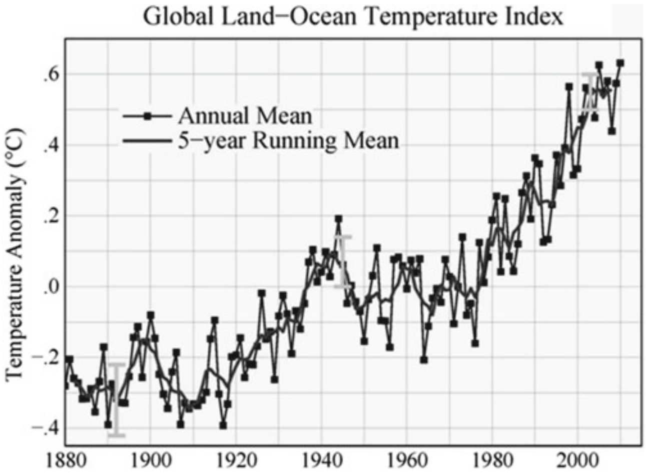

Fig. 22.1 Global mean land-ocean temperature index from 1880 to present (base period 1951–1980). The *line* joining *black squares* is the annual mean, the *solid lighter line* is the 5-year mean, and the *lightly shaded vertical bars* show uncertainty estimates (update from Hansen et al. 2006)

the last 40 My (Houghton et al. 2001), and the IPCC predicts the melting of pack ice will cause rises in sea level, increased frequency of extreme heat, episodes of heavy precipitation and increase in cyclone intensity. In addition, positional shifts of jet streams will alter the global distribution of precipitation. Already, human-induced increases in greenhouse gases have apparently contributed to intensified precipitation events in the Northern Hemisphere (Min et al. 2011), increased flooding in the UK (Pall et al. 2011) and an increase in the frequency of exceptionally heavy precipitation in North America (Peterson et al. 2008). Although precipitation may increase at high latitudes, it may decrease in subtropical land regions (Bernstein et al. 2007) and novel climates may arise that are warmer and precipitation may shift unpredictably. Future climates may lack modern analogues and lead to the appearance of animal and plant communities that are compositionally different from today (Williams and Jackson 2007). The likely impacts of climate change will be profound.

22.2 Climate Change as an Environmental Stressor

Indicators of global change show that global biodiversity is declining, whereas pressure indicators, including climate change, are increasing (Butchart et al. 2010). Rockström et al. (2009) identified a set of planetary boundaries, defined as the safe operating space for humanity, and showed that climate change and biodiversity loss exceed these maximums by a considerable margin (Fig. 22.2).

Current extinction rates are estimated to be 100–1,000 times expected natural levels, and climate change is an important driving factor in previous mass extinction events (Barnosky et al. 2011). On average 52 species of mammals, birds and

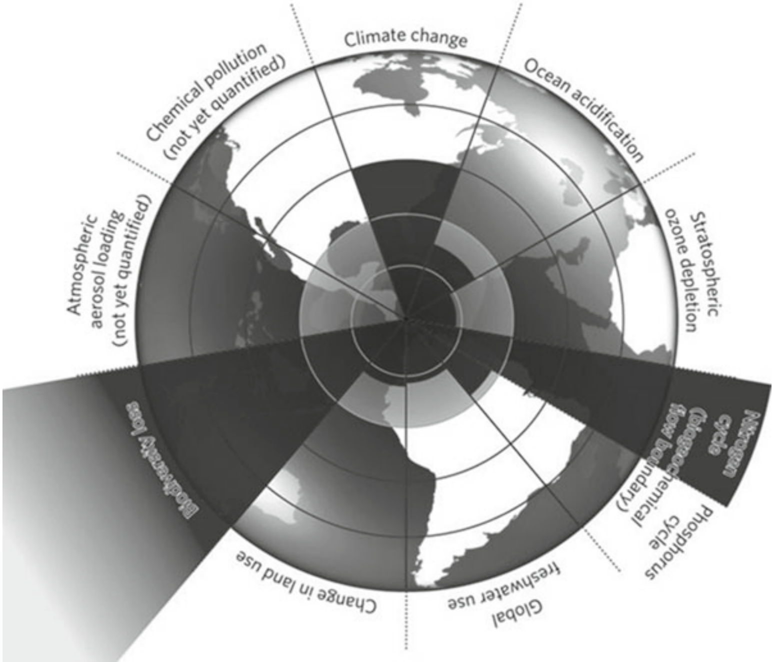

Fig. 22.2 Planetary boundaries (Modified from Rockström et al. 2009, reproduced with permission, Macmillan Publishers). The second *inner circle* delimits the proposed safe operating zones with *dark wedges* estimating current position of each system. Safe operating zones for rates of biodiversity loss, human interference of nitrogen cycle and climate change are seriously exceeded

amphibians move one category closer to extinction each year (International Union for the Conservation of Nature) (Hoffmann et al. 2010). Mammals show a declining trend (Red List Index), and more species move into categories of higher extinction risk annually; these negative trends are set to continue into the twenty-first century (Pereira et al. 2010). The long-recognised threats to biodiversity of expanding human populations and consumption leading to habitat loss, pollution, overhunting, invasive species and pathogens have been greatly multiplied by the onset of climate change. Thomas et al. (2004), using mid-range climate warming scenarios, predict that 15–37 % of species in a sample of regions covering 20 % of the planet's terrestrial surface will be 'committed to extinction' by 2050.

22.3 'General Responses' to Climate Change and Bats

Climate has been a major force on the evolution of life (Erwin 2009). Since the beginning of the Quaternary period (ca. 2.6 million years ago), the Earth's climate has been oscillating between long glacial periods (stabilised duration of 100,000 years) with intermittent shorter, warmer interglacial periods usually lasting between

10,000 and 20,000 years (Tzedakis et al. 2009). Moreover, transitions between periods can occur over mere decades (Alley et al. 2003). Abrupt climate changes have a strong impact on biodiversity (Araújo et al. 2008), and species respond in a variety of ways, although at least four 'general responses' have been proposed (Dawson et al. 2011; Gardner et al. 2011).

22.3.1 Toleration

The ecological niche of some species seems stable or changes little over time, known as niche conservatism (Peterson 2011). Molecular analysis and species distribution modelling have provided evidence that many species have tended to occupy areas that experience similar climatic conditions over millennia (Martinez-Meyer et al. 2004; Peterson 2011; Waltari et al. 2007; Wiens and Graham 2005). Tolerating a changing climate requires physiology and/or ecology flexibility. Adaptive mechanisms for coping with climate change may evolve via natural selection, and there are recent examples of rapid evolution due to climate change (Angert et al. 2011; Parmesan 2006). For example, Franks et al. (2007) found that California field mustard populations have advancing flowering time in response to warming, and in the UK two European butterflies (*Hesperia comma* and *Aricia agestis*) have broadened their dietary requirements, enabling them to expand northwards (Thomas et al. 2001).

Toleration responses include altering phenology, for example, breeding earlier (Parmesan 2006), shifting habitat preferences or changing migration times (Miller-Rushing et al. 2008). There is growing global evidence for earlier flowering seasons and spring green-up (Cleland et al. 2007), earlier arrival times in bird migration (Miller-Rushing et al. 2008) and an overall advance of spring and summer events for 726 taxa in the UK (Thackeray et al. 2010). However, changes in phenology do not necessarily mean better adaptation to climate change in populations. In temperate climates, winters are becoming shorter and warmer (Walther et al. 2002). As with many other organisms, the life cycles of bats may be altered by regional and local climate changes with unpredictable consequences.

Although little information is available on how bats are responding to climate change (Sherwin et al. 2012), some studies have shown phenological changes in birth timing in affected populations. Greater horseshoe bats, *Rhinolophus ferrumequinum*, advanced average birth date by 18 days with a 2 °C increase in spring temperature (Ransome and McOwat 1994). In southern Spain, greater mouse-eared bats, *M. myotis*, gave birth in midwinter, up to 4 months earlier than expected, and hibernation was apparently absent (Ibáñez 1997). In Australia winter activity of tree-roosting bats increased whenever warmer temperatures occurred (Turbill 2008). It is likely that a number of bat species will use shorter torpor periods or that hibernation may disappear in some regions as climate warms. Additionally, phenological changes in the life cycle can potentially create temporal mismatches with food availability as occurred in great tits, *Parus major*, that feed on caterpillars (Visser et al. 2006). For example, lactation periods in *Myotis blythii* and *M. myotis* coincided with high insect prey abundance (Arlettaz et al. 2001), and if insects shifted phenology rapidly, reaching peak abundance before predators with longer generation times can adapt, the consequences would be devastating. Temporal mismatches occur across trophic levels and thus have profound consequences (Thackeray et al. 2010), and thus, communities may shift compositionally with unpredicted consequences for ecosystems (Williams and Jackson 2007).

Bat species life cycles are temporally synchronised with highest food availability, and temporal mismatches affect not only bats but also their interacting species. For example, over 500 species of flowers are pollinated by bats, with many species totally dependent on bats for pollination (Fleming et al. 2009), and fruit-eating bats play an important role in forest regeneration and seed dispersal in the Neotropics (Muscarella and Fleming 2007). Moreover, seasonality of food resources drives over 30 bat species to migrate between breeding and non-breeding sites (Bisson et al. 2009; Rodrigues and Palmeirim 2008), the timing of which is critical. As is, bat species will not likely react equally to changes in climate and ecosystems. Generalist species might have a greater probability of finding suitable resources in a new area (Thomas et al. 2004), whereas specialised species would predictably face significant difficulties (Thuiller et al. 2005).

22.3.2 Changes in Body Size

Because larger organisms have a smaller surface area to volume ratio, they may experience problems dissipating heat in hot conditions, and thus, selection may favour decreasing body sizes over time. Many species show clines in body size, with largest individuals at coldest latitudes (Bergmann's rule). Though the causes of this relationship are still debated, it is interesting to note that the fossil record of horses shows that body size decreased during warming periods (Secord et al. 2012). However, some data suggest increases in temperature extremes may actually select for increased size (Gardner et al. 2011). Although evidence for Bergmann's rule in bats is marginal (e.g. Bogdanowicz 1990), Tupinier (1977), Palmeirim (1990) and Storz et al. (2001) detected a decrease in body size in warmer regions in some taxa, but could not determine if differences were the result of microevolutionary processes or simply phenotypic plasticity (Husby et al. 2010).

22.3.3 Changes in Distribution and Abundance

Organisms may move to new locations in response to changing climates by latitudinal shifts, and many taxa in the Northern Hemisphere are expanding northwards (Hickling et al. 2006). It is expected that current bioclimatic zones will shift towards currently cooler areas in higher latitudes or altitudes (Franco et al. 2006). Ranges may also contract, as seen in some butterflies and amphibians (Thomas et al. 2006), and areas once suitable for some species may disappear (Araújo et al. 2006; Thomas et al. 2006). Those species currently occupying cooler biogeographic regions where the magnitude of climate change is greater will probably go extinct (Virkkala et al. 2008). Of course, some species may benefit from climate change by increasing density or by expanding distributional ranges as seen in some European birds (Gregory et al. 2009). Moreover, the rapid rate of current climate change may preclude adaptive change in many species (Loarie et al. 2009). Traits such as dispersal ability or life history parameters (e.g. rate of reproduction) could limit the ability to move away from current ranges and become established in newly suitable habitats. For example, passerine birds with greater dietary breadth and alpine plants with longer seed dispersal periods alter distributions quickly, whereas mammals with greater longevity change less rapidly (Angert et al. 2011).

Evidence for changes in the distribution and abundance of bats due to climate disruption is already emerging. Upward altitudinal shifts in Costa Rican cloud forest seem to be partly a response to climate change, although forest clearing by humans also contributes to this pattern (LaVal 2004). Rebelo et al. (2010) hypothesised that 28 European bat species will undergo a major range shift towards northern latitudes by the end of the century. For example, *Pipistrellus kuhlii* has expanded its range northwards since the 1990s, presumably in response to increasing temperatures (Sachanowicz et al. 2006). Nevertheless, the expansion of bat populations may be hampered because suitable foraging habitats may take considerable time to develop (McLachlan et al. 2005), and roosts with adequate microclimates will take time to establish. Indeed, roost availability played an important role on the colonisation patterns of greater mouse-eared bats in central Europe where human settlements created novel roosting opportunities (Rodrigues et al. 2003). Species with narrow ecological niches and whose roost conditions are highly restricted will probably experience greater challenges than their more generalist counterparts (Safi and Kerth 2004). In fact, European bat species having greater predicted decreases throughout the twenty-first century are those that are currently threatened or occupy narrow ranges in northern latitudes (Rebelo et al. 2010). Some bat species have a relatively large capacity to migrate (Riede 2001) and explore new areas, but this does not necessarily imply that bat populations will simply follow climatically suitable areas as climate changes. In fact, major movements could create isolated and fragmented populations.

22.3.4 Extinction

If organisms are unable to cope, adapt or move in response to climate change, they may become extinct (Franco et al. 2006; Thomas et al. 2004). In the past, climate change has contributed to several extinctions and even mass extinctions (Nogués-Bravo et al. 2010; Kaiser et al. 2011) and is comparable in importance to habitat loss in driving some butterfly extinctions in recent decades (Franco et al. 2006). It is expected that some bat populations will experience severe declines due to slow reproductive rates.

22.4 Proximate Effects of Climate Change on Bats

The ways in which bats may respond to climate disruption may be better understood by highlighting some key aspects of physiology, population dynamics and ecology that are especially sensitive to climate (Jones et al. 2009). Because bats are a diverse group, specific data on natural history traits will be important in predicting how species will react to change.

22.4.1 Effects on Bat Physiology

Climate change has the potential to affect several aspects of bat physiology, some having a strong influence on survival. Mortality rates are affected by extreme temperatures, drought and rainfall. For example, Welbergen et al. (2008) reported over 30,000 deaths of flying foxes in Australia since 1994 due to at least 19 periods of high temperatures. Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) has a low tolerance to high ambient temperatures (Downs et al. 2012), and most bat species have a 'thermal optimum' where metabolic rate and water loss are minimised (Webb et al. 1995). In conditions outside that 'thermal optimum', metabolic rate and water loss increase, imposing greater stress on survival. The high surface area to volume ratio of naked wing membranes causes evaporative water loss rates in bats to be higher than in similarly sized mammals (Webb et al. 1995). Reduced water availability is another potential effect of climate change in that IPCC scenarios predict a decrease in rainfall and an increase in frequency of droughts for several regions (Nakicenovic et al. 2000). Water availability is important for successful lactation in bats (Adams and Hayes 2008), and in arid environments, most bat activity and diversity occurs near aquatic sources (Razgour et al. 2010; Rebelo and Brito 2007). Moreover, survival probability increases in little brown bats (*Myotis lucifugus*) in wet years with high cumulative summer precipitation (Frick et al. 2010).

22.4.2 Effects on Phenology

Phenological changes in parturition can be driven by changes in torpor patterns and can be manipulated by up to 3 months in the laboratory (Racey 1972). This ability to adjust the rate of foetal growth is unique among mammals (Racey 1982) and is a function of the time spent in torpor during pregnancy that extends gestation under inclement conditions (Racey 1973; Racey and Swift 1981). Predictably, the use of torpor during pregnancy is likely to reduce under climate warming, and in some regions bats may begin parturition earlier in the season.

Climate change is also likely to affect the energetics of hibernation and consequently alter the distributions of hibernating bat species (Humphries et al. 2002).

In temperate (e.g. Hope and Jones 2012; Park et al. 2000) and even subtropical (e.g. Liu and Karasov 2011) regions, hibernation behaviour is sensitive to temperature. Typically torpor bouts are shorter at higher ambient temperatures, so an increase in arousal frequency may have implications for the risk of white-nose syndrome, as the causative fungus *Geomyces destructans* appears to increase arousal frequency causing mortality (Boyles et al. 2006; Cryan et al. 2010; Chap. 19).

Insect abundance may increase due to warmer winters, but whether they will become sufficiently abundant to offset the increased energetic costs associated with more frequent arousal by bats is questionable. Insects have a short life cycle strongly dependent on temperature (Wilson and Maclean 2011), and many species have narrow distributional ranges and limited ability for expansion into future climatically favourable regions. With predicted increases in twenty-first-century temperatures, changes in insect community composition are expected (Cannon 1998). In fact, insect pests are expected to respond quickly to climate change and colonise new habitats (Cannon 1998), although several lepidopteran species are already showing both range contractions and population declines (Wilson and Maclean 2011). These changes in insect communities will have unknown consequences for bat populations because most species hunt certain types of prey (Goerlitz et al. 2010) that may be especially at risk.

22.4.3 Effects on Bat Habitats and Roosts

Long-term changes in habitats may affect roost availability, and in the short term, events such as hurricanes destroy roosting structures or foraging areas, paramount for bat survival. Bats with higher levels of habitat specialisation are at greater risk of extinction than generalist species (Safi and Kerth 2004). For forests that require a long time to reach maturity, the pace of climate-induced disruption may be too fast to accommodate required shifts in bat distributions. For example, it is not expected that large areas of mature broadleaf forest could develop in northern Europe until the end of the century (McLachlan et al. 2005). Besides the disappearance of suitable roosts, climate change could modify the microclimatic condition within roosts, which could affect crucial phases of a bat's life cycle such as breeding and hibernation.

The frequency of hurricanes is predicted to increase under most scenarios, and such storms majorly impact island populations of pteropodids (nearly the entire population of *Pteropus rodricensis* on Mascarene Islands was eradicated by one hurricane) (Powell and Wehneh 2003) as well as phyllostomids (Gannon and Willig 2009; Pedersen et al. 2009). Responses to hurricanes are species-specific, depend on the severity of the storm and can affect bat populations through direct mortality or by habitat alteration that affects food resources and roosts (Gannon and Willig 2009).

22.4.4 Studying the Effects of Climate Change on Bats

Short-term changes in population sizes and distributions can be monitored under rapid periods of changing climate if methods are sufficiently comprehensive. Changes in distributions over geologic time can be inferred from fossils in relation to contemporary genetic data that together help model how future climate conditions may affect bats.

22.4.5 Monitoring Distributions and Populations Under Rapid Change

As discussed, there is evidence for recent range expansions of *Pipistrellus kuhlii* in Europe as has recent increases in population size. Since 1997, a large-scale network of volunteers in the UK has contributed to the National Bat Monitoring Programme for 11 species co-ordinated by the Bat Conservation Trust. Monitoring includes standardised counts at maternity sites and hibernacula and also measuring bat activity acoustically at field sites using bat detectors. The 2011 report (http://www.bats.org.uk/pages/results_and_reports.html) shows trends of longer-term increases of 56–90 % since 1997 for two species of horseshoe bats (*Rhinolophus ferrumequinum* and the lesser horseshoe bat *R. hipposideros*) estimated from maternity colony counts. It is highly likely that warmer conditions have contributed to these considerable increases in abundance.

22.4.6 Population Trends and Reproductive Success Under Rapid Change

Three recent long-term studies have analysed the impact of climate on the population composition and demography of bat populations. Adams (2010) analysed trends in the capture frequency of reproductive and non-reproductive females for *Myotis* species and big brown bats (*Eptesicus fuscus*) between 1996 and 2008 in Colorado, USA. Populations showed slight to profound reductions in reproductive output depending on the severity of drought conditions, and significant consequences were predicted if current climate scenarios play out in western North America. The availability of drinking water is of major importance to lactating female bats that visited to drink at a water source 13 times more frequently than did non-reproductive females. Such a dramatic surge in the need for water during lactation demonstrates how increased intensity and frequency of regional droughts will negatively and quickly affect reproductive success of several bat species (Adams and Hayes 2008). The immediate response of female reproductive frequencies to yearly weather conditions makes bats a potential early warning indicator of widespread, long-term climate change effects.

Frick et al. (2010) analysed mark-recapture data to infer survivorship in little brown bats, *Myotis lucifugus*, between 1993 and 2008. Although females survived best in wet years, young born earlier in the summer showed higher survival than late-born pups and were more likely to return to their natal colony to breed the following summer. Although no effects of average summer temperature on survival were detected, Frick et al. (2010) argued that aerial insects may be more abundant in wetter summers and this may increase survival rates (Frick et al. 2010). Barclay (2012) studied three maternity roosts of *Eptesicus fuscus* in Canada over 14 years and found that early parturitions led to skewed sex ratios favouring females. This effect is expected to intensify under climate warming, though the consequences for population viability are still unknown.

22.5 Learning from the Past

Understanding how bat communities have responded to climate change can be gained by radiocarbon dating skeletal material found in cave deposits and relate these findings to climatic reconstructions and palaeobotanical data since the Last Glacial Maximum (LGM, 19,000–20,000 years ago) as deglaciation increased northern summer insolation (Clark et al. 2009). Hence, changes in climate that altered land cover can be used to determine temporal changes in ranges of bat species in the Swiss Alps (Blant et al. 2010) (Fig. 22.3). Species adapted to warmer conditions (e.g. Bechstein's bat *Myotis bechsteinii* and *Rhinolophus hipposideros*) were found most abundant during the Holocene climatic optimum 5,000–9,000 years ago when mixed forest cover was extensive. Cold-tolerant species such as Brandt's bat, *Myotis brandtii*, also occurred, but most samples were found from the Late Holocene when summer temperatures were declining; humans used fire to convert forests into open areas for cultivation, and forest bat species like *M. bechsteinii* became infrequent (Blant et al. 2010).

Major changes in the distribution of organisms resulted from the LGM as animals and plants move away from the poles into glacial refugia, and recent advances in molecular ecology have allowed for reconstruction of recolonisation routes as glaciers receded (Hewitt 2000). Refugial populations harbour the highest genetic diversity because populations across a wide geographical range become concentrated there. As populations disperse from refugia, founding populations lose alleles through genetic drift, thereby decreasing genetic diversity with increasing distance from refugia (Fig. 22.4). By analyses of evolutionary relationships among neighbouring populations, recolonisation routes can be identified (Flanders et al. 2009; Kerth et al. 2008; Rossiter et al. 2007).

Because microsatellites evolve faster than mitochondrial genes, they can reveal more recent patterns of population expansion. In Europe, major refugia important to bats during the LGM have been identified in the Iberian Peninsula (composed of Spain and Portugal), Italy and the Balkans (Hewitt 2000). Recolonisation routes are species-specific, and bats have transcended potential dispersal barriers such as the

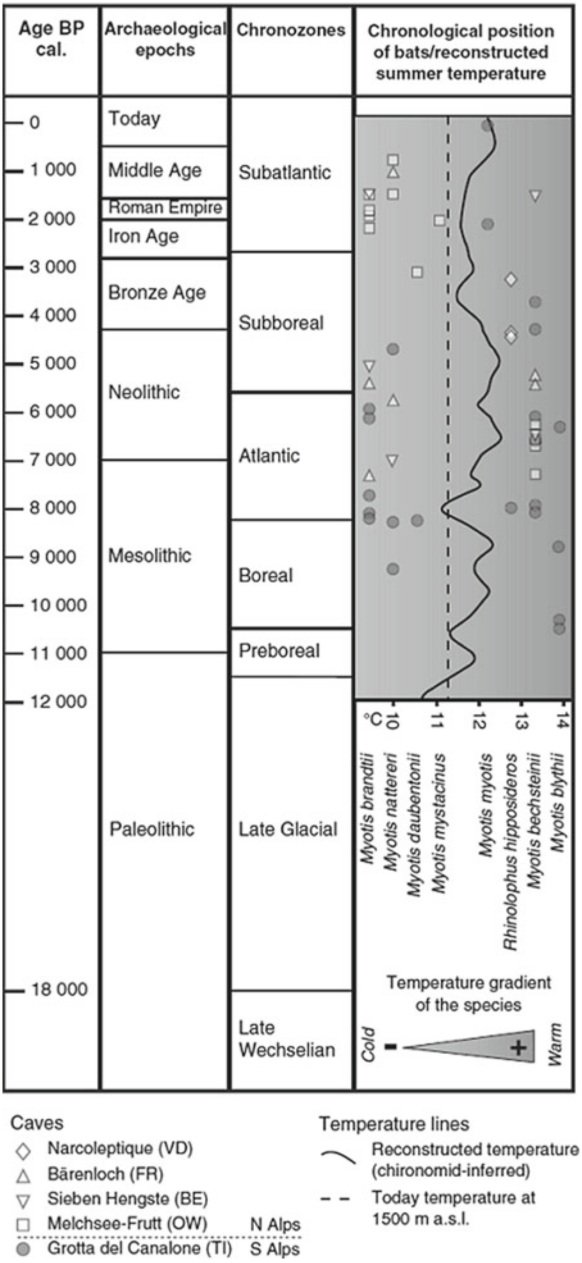

Fig. 22.3 Chronology of radiocarbon-dated bats found in alpine cave deposits with summer temperatures reconstructed based on past chironomid assemblages corroborated by tree-line oscillations. *Myotis bechsteinii*, *Rhinolophus hipposideros* and *Myotis blythii* were considered thermophilous (warm demanding) based on current distributions, with *M. myotis* as 'meso-thermophilous', and *Myotis brandtii*, *M. nattereri*, *M. daubentonii* and *M. mystacinus* as psychrophilous (cold tolerant). Thermophilous species occurred most frequently when climate was warmest, psychrophilous species when climate was warm, but persisted under colder conditions in Late Holocene (from Blant et al. 2010, reproduced with permission, Sage publications)

Alps and the Pyrenees mountains in different ways (Ruedi and Castella 2003). For example, *Barbastella barbastellus* spread to the UK from refugia located in today's Italy (Rebelo et al. 2012), although the Alps acted as a barrier to gene flow out of Italy for refugial populations of *M. myotis* (Ruedi et al. 2008; Ruedi and Castella 2003).

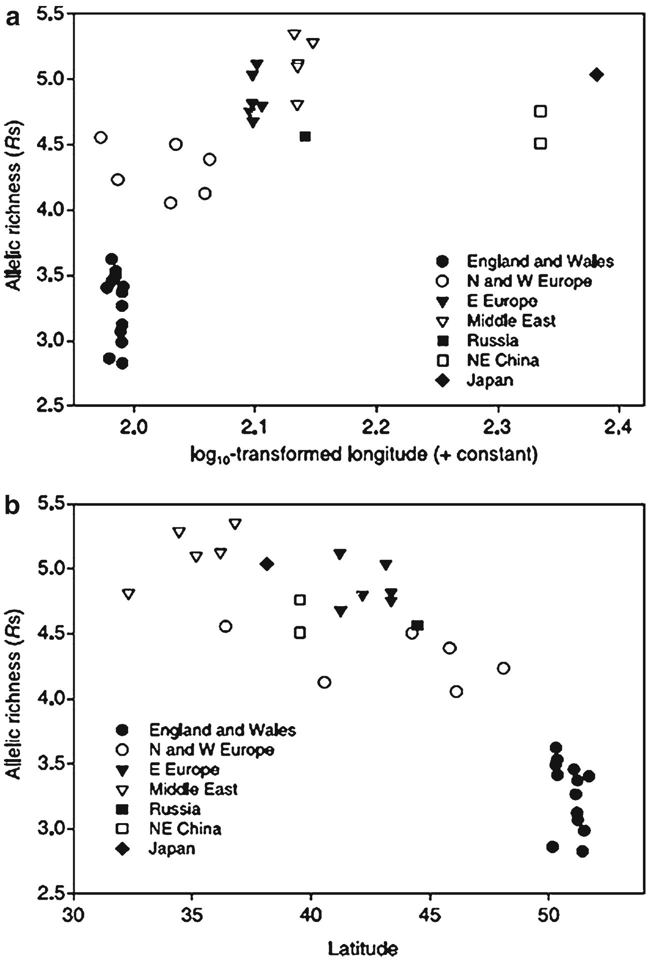

Fig. 22.4 Relationships between allelic richness for 17 microsatellite loci of *Rhinolophus ferrumequinum* populations across its geographic range against (**a**) longitude (**b**) latitude. Highest allelic richness occurred at eastern longitudes where founding populations were from southern latitudes wherein past glacial refugia harbour substantial genetic diversity. The trend is consistent with rapid northern expansion (Rossiter et al. 2007, reproduced with permission from Blackwell Publishing Ltd.)

By combining analyses of mitochondrial sequence data and microsatellites, Flanders et al. (2009) showed two patterns of expansion in European populations of *Rhinolophus ferrumequinum*. There is little diversity in the mitochondrial *ND2* gene in European bats and one haplotype dominates, but diversity at this locus is considerable in western Asia. Demographic analyses suggest an expansion event out of Asia Minor 40,000–60,000 years ago during a period of global warming in the Middle Pleniglacial. More recently, during the LGM, bats occupied Iberia and/or

Italy and the Balkans/Greece, expanding to colonise western and central Europe from these refugia, respectively (Rossiter et al. 2007).

Although extensive work has been performed on expansions of bat populations out of past glacial refugia in Europe, recent studies have also identified expansion patterns in North America from a large southern refugium in today's southeastern USA and several isolated refugia in western North America for *Myotis lucifugus* (Dixon 2011). In eastern continental Asia, refugial areas have been documented for David's myotis, *Myotis davidii* (You et al. 2010) and *Rhinolophus ferrumequinum* (Flanders et al. 2011).

Flanders et al. (2011) and Rebelo et al. (2012) used ecological niche modelling to predict the locations of historical refugial areas by looking at the distribution of climatic conditions during the LGM that were similar to those currently influencing the species' range. This method proved very powerful in predicting refugial areas and can be a powerful way of forecasting how bat distributions may respond to future climate change. We may expect to find the emergence of 'warming refugia' that harbour extensive genetic diversity as bats migrate to them from wide geographical ranges. Identifying geographic barriers to gene flow in the past is important, for example, the Alps could again hinder dispersal of *M. myotis* out of Italy making this species high risk from the effects of climate change.

22.6 Modelling the Future

Although we are increasingly confident of climate reconstructions from the past, we are less sure of the future. The IPCC has produced climate change scenarios ranging from conservative to severe (Bernstein et al. 2007), and one can use those scenarios in ecological niche models to predict effects on bat communities. Rebelo et al. (2010) used spatial principal components analysis to group 28 European bat species according to their current climatic niches. Three groups were identified comprising Mediterranean, temperate/humid and boreal species. Presence-only modelling (Maxent—Phillips et al. 2006) was used to predict changes in species distribution up to the year 2100 under four IPCC scenarios. The most severe scenario was A1FI (a globalised world with intensive economic growth sustained by intensive use of fossil fuel), whereas the most environmentally friendly B1 involved high levels of environmental and social consciousness for globally stable development. Species of boreal climates were predicted to be most affected, with some extinctions possible in the future as suitable niche space disappears. Mediterranean and some temperate species were more tolerant of temperature increases. Hotspots of species richness were predicted to shift northwards, and the area occupied by all species groups was predicted to decline under the A1FI scenario, with Mediterranean and temperate taxa increasing their ranges under the B1 scenario (Fig. 22.5). Because Rebelo et al. (2010) modelled only climate change and did not consider projected changes in land use and vegetation, their predictions were probably optimistic.

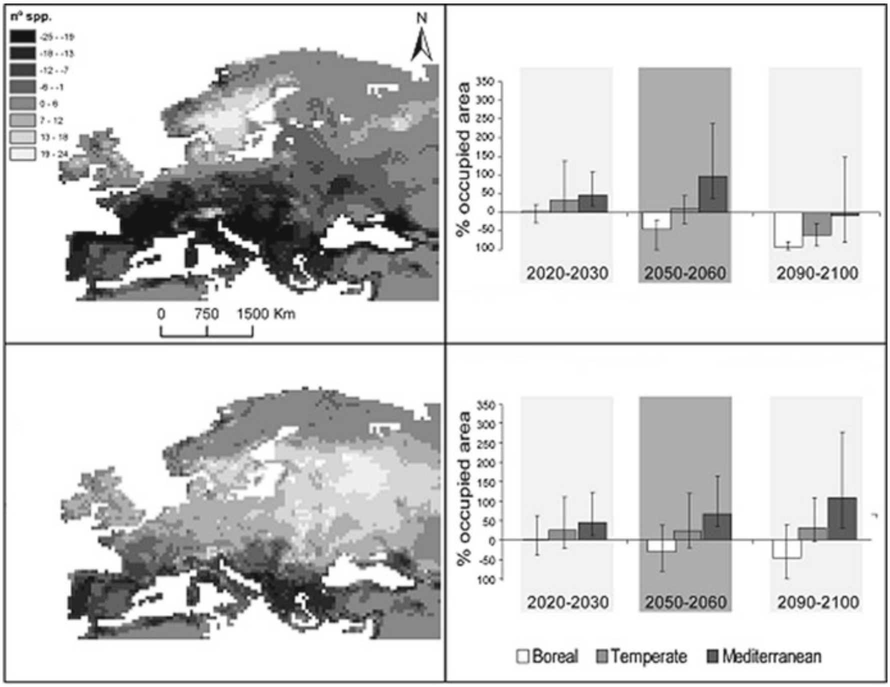

Fig. 22.5 Responses of European bats to two predicted IPCC climate change scenarios. The two row panels show anticipated changes in species richness under the A1FI (*top*) and the greener B1 scenarios (*bottom*). Predicted species loss is greatest in Mediterranean regions under both scenarios (*darker colours* in maps), with the effects more severe under the fossil fuel intensive scenario. The *right panels* show average variation in occupied area throughout the twenty-first century in relation to area currently occupied by three biogeographic groups of bats (boreal, temperate and Mediterranean). *Vertical bars* are maximum and minimum values for a bat species within each group. All biogeographic groups are predicted to show range contractions under the A1FI scenario, whereas range expansions may occur for temperate and Mediterranean taxa under B1 (Reproduced from Rebelo et al. 2010, with permission, John Wiley & Sons Inc.)

Interactions between land use changes and climate are important in predicting future scenarios for tropical biodiversity (Brodie et al. 2012). Projected land use change with climate disruption was used to predict alterations in bat species richness in Southeast Asia using recent distribution records of 171 species (Hughes et al. 2012). When potential vegetation and climate changes were combined, only 1 % of bat species showed no alterations in predicted ranges by 2050, and only 1–13 % of species were projected to show no reduction in current range under the bioclimatic scenarios. Many bat species were predicted to move north in response to climate change. Hughes et al. (2012) suggested that improving forest connectivity may help mitigate the effects of climate change in a region where at least 30 % of mammal species are bats (Kingston 2010), with over 320 species already described (Simmons 2005) and with many cryptic species being discovered (Francis et al. 2010).

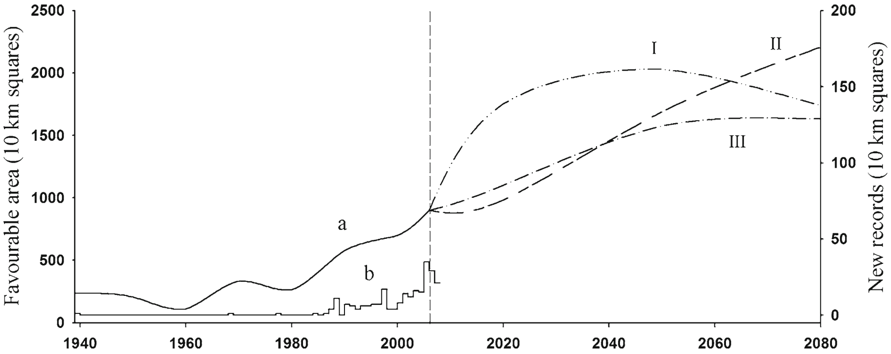

Fig. 22.6 Modelled habitat suitability for Nathusius's pipistrelle *Pipistrellus nathusii* in the UK (*a*) from 1940 to 2000 and three future climate projections (2020–2080). The frequency distribution (*b*) shows an upward trend in annual frequency of new records within 10-km squares (From Lundy et al. 2010, reproduced with permission, John Wiley & Sons Inc.)

Pipistrellus nathusii was formerly considered to be a vagrant in the UK, but records now show that breeding colonies exist and this range expansion may be due to climate change. Lundy et al. (2010) used presence-absence modelling encompassing all UK records between 1940 and 2006 and found that presence was positively related to warmer winter temperatures and predicted a further twofold increase in suitable range in the UK by 2050 under IPCC scenario A2a (a scenario that involves a heterogeneous world with slow but continued population growth and economic expansion) (Fig. 22.6). Furthermore, *P. nathusii* was recently found overwintering in Poland, where it was previously considered too cold.

Range expansions of migratory species due to climate change may have implications for the spread of emerging infectious diseases such as rabies in bats via extreme weather episodes predicted to become more frequent throughout the twenty-first century. For example, in 1998 the emergence of Nipah virus in Malaysia was thought to be associated with bats fleeing from forest fires fuelled by intense drought during the largest El Niño event of the century (Epstein et al. 2003).

22.7 Mitigation Against the Effects of Climate Change

Throughout this chapter, we have reviewed and evaluated potential impacts of climate change on bat species, and overall, we expect that some species will face severe challenges. Most mitigation measures for bat populations are directed towards roost and habitat management as these elements are paramount for conservation. In addition, corridors between suitable areas are important to maintain population connectivity (Hughes et al. 2012). Translocation is unlikely to be effective for establishing bat populations in new areas (Ruffell et al. 2009). The protection of populations located in historical glacial refugia and in areas where species are predicted to persist is also pertinent for the maintenance of genetic diversity. Additionally, finding adequate roosts in newly suitable climatic areas could pose challenges, and therefore, pre-emptive construction of artificial roosts should be considered as well as construction and/or maintenance of water sources proximate to maternity colonies may also become increasingly important.

Newson et al. (2009) proposed four indicators for tracking bat populations: monitoring hibernation sites in Europe (see Battersby 2010); monitoring straw-coloured fruit bats, *Eidolon helvum*, for changes in species' phenology and temporal mismatches; monitoring movements of common vampire bats, *Desmodus rotundus* (a species sensitive to temperature), from Mexico and Central America into Texas as an indicator species for climate-induced range shifts and continued monitoring populations of Mexican free-tailed bats, *Tadarida brasiliensis*, for impacts of climate change on population dynamics, including population size.

In parallel, the increasing concern about climate change phenomena and the depletion of traditional energy sources has promoted high investments in the renewable energy (Global Wind Energy Council 2012). Despite its clear benefits, the impacts of wind farms causing large-scale mortality in bats is currently of major concern (Arnett et al. 2008; Kunz et al. 2007; Chap. 20). The consequences of hydropower development on bat populations are largely unknown, but some evidence suggests that conversion of extensive areas into homogeneous water surfaces may cause significant impacts. In immediate years after reservoir construction in Europe, bats moved from flooded landscapes to the margins of the reservoir, initially increasing species richness and abundance that declined after several more years (Rebelo and Rainho 2009).

22.8 Research Needs

Research on climate change has only commenced recently and consequently, a vast array of related topics are in need of investigation. In the previous section we suggested active management of habitat and roosts in current and predicted future bat distributions. Hence, the study of the ecological requirements (e.g. foraging grounds, water requirements and roosts) as well as the capacity of species to colonise new territories (e.g. rates of reproduction and dispersal ability) for the most vulnerable species should be prioritised.

Additionally, in order to better evaluate the impacts of climate change on bat populations, a framework based on biological meaningful indicators should be developed (Jones et al. 2009; Newson et al. 2009; Thomas et al. 2011) and species-specific susceptibility quantified (Dawson et al. 2011). McMahon et al. (2011) suggested developing better monitoring programmes, quantifying the sensitivity of species to climate change, incorporating community dynamics into responses, accounting for evolutionary processes and improving understandings of how functional groups of species are defined.

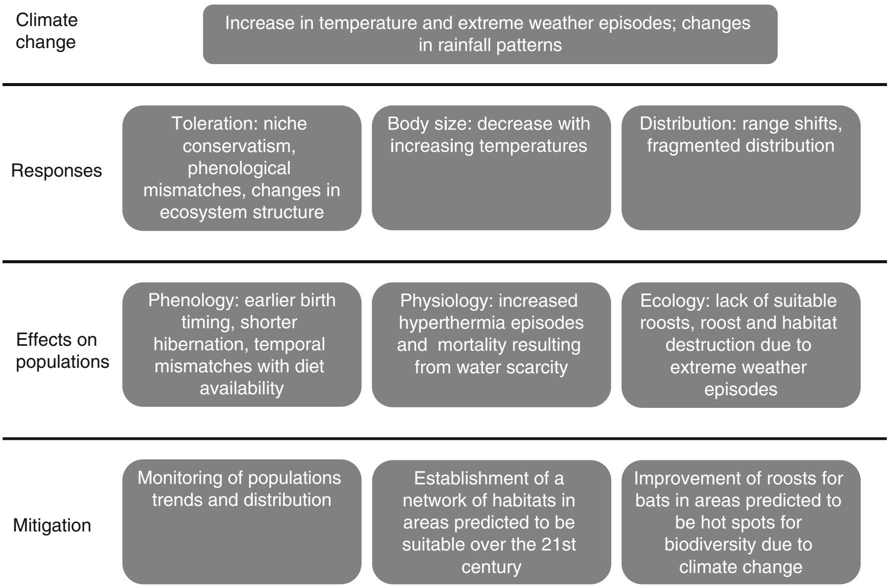

Fig. 22.7 Main threats to bat populations due to climate change and respective mitigations

22.9 Conclusions

Historically, changing climates altered the evolutionary paths of life on Earth (Erwin 2009). Overall, bats are good indicators of ecosystem changes in response to climate warming (Jones et al. 2009). We have underscored in this chapter examples of how climate disruption is affecting birth timing, hibernation duration and the distribution of bat populations. Some species may face immense challenges to their survival, especially those more dependent on colder climates or those with narrow ecological niches. Figure 22.7 provides a summary of the potential threats to bats, and it is expected that future research will provide significant progress towards mitigation. Some bat species are already reacting to climate change even though average global temperatures have increased merely 0.74 °C over the last century with predicted increase of 1.1–6.4 °C by the end of this century (Bernstein et al. 2007). Thus, mitigating the effects of climate change will likely become a top priority for bat conservation.

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Chapter 23
Challenges of Using Bioacoustics to Globally Monitor Bats

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Abstract As bats are important biodiversity indicators, monitoring their populations is becoming increasingly important to understand the impacts of global change. Bats leak information about themselves into the environment in the form of ultrasonic calls. Using these calls to globally survey bat populations may offer a more efficient alternative or addition to traditional methods for bat monitoring. We identify three of the most important challenges to the development of a global acoustic bat monitoring programme: the robust identification of acoustic signals, the ability to develop meaningful population trends from acoustic activity, and engaging a global audience to take part. We discuss the rapid progress in all three of these areas, for example, development of comprehensive call libraries, quantitative regional tools for call identification, new statistical methods to monitor trends and a resurgence of interest in the public participation in science and monitoring of nature. We also discuss the important gaps in our knowledge and where resources could be best focused to build a global programme. Specifically, tropical areas present a particular challenge: they have high species-richness; species acoustic diversity is poorly documented; call similarity of species is very high, making robust call identification more challenging; and traditionally these areas have had a lower citizen engagement in biodiversity monitoring.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_23,

23.1 Introduction

Survey and monitoring bat populations is becoming increasingly important to monitor threats such as disease, habitat conversion, and climate change (Kunz et al. 2007; Puechmaille et al. 2011; Rebelo et al. 2009). Changes in bat population abundances and distributions also act as indicators of global change on biodiversity more generally because of their global distribution, provisioning of ecosystem services, and slow rates of reproduction making populations sensitive to disturbances (Jones et al. 2009a). Historically, bat monitoring has focused mostly on visual counts at roosts, and some bat populations in Europe have been monitored for decades (Battersby 2010; Haysom 2008). Roosts can range from very obvious ones containing millions of individuals (e.g., in trees or caves) to cryptic roosts of single individuals (e.g., in tree cavities, under leaves, underground sites or buildings). Trapping with mist nets or harp traps is also used to monitor bats (Hayes et al. 2009), and this method enables morphometric or genetic measurements to be taken from individuals to assess the impact of disturbance (Struebig et al. 2011).

Acoustic monitoring bat populations has become more widespread in recent years (Ahlen and Baagoe 1999; Brooks 2011; Estrada-Villegas et al. 2010; Fukui et al. 2004; Jaberg and Guisan 2001; Kofoky et al. 2006; Ochoa et al. 2000; Russo and Jones 2003; Vaughan et al. 1997; Wickramasinghe et al. 2003) and exploit the fact that over 80 % of all bat species emit ultrasonic pulses (echolocation) and use the returning echoes to learn about their surroundings, avoid obstacles, and find prey in the dark (Griffin and Galambos 1941; Schnitzler et al. 2003). As well as being noninvasive, acoustic methods offer the unique ability to survey bats remotely so that microphones can be left at a site and set to record echolocation activity at certain times, thus reducing the amount of effort needed for surveying bats compared to the more labor-intensive roost and trapping monitoring methods.

Out of over 1,116 species of bats globally (Simmons 2005), only a tiny proportion are consistently monitored (acoustically or otherwise), and research efforts are biased towards higher latitudes. For example, Europe contains one of the most well-organized, mature bat monitoring programs but only at best covers just over 40 species (Haysom 2008). There must be a rapid increase in the scale of monitoring to understand the status and trends of bat populations globally. Statistical methods can now compile trends from different monitoring methods into a single metric, so that results of different monitoring techniques can be combined. However, there needs to be an increase in the amount of actual monitoring data collected from different species and areas, and acoustic monitoring might be the best way to rapidly scale up. Interest in acoustic monitoring is also growing to monitor other taxa; most noticeably Cornell University's Ornithology Laboratory has been applying acoustic techniques to a broad range of taxa from night migrant birds, the infrasonic calls of elephants, to the ultrasonic sounds of cetaceans (http://www.birds.cornell.edu). Despite the promise of acoustic bat monitoring, substantial challenges remain, and there has been a fair amount of skepticism in the literature about the feasibility of its application (Hayes et al. 2009; Hayes 2000). In this chapter, we discuss the three main challenges to applying a global acoustic monitoring program: the robust identification of acoustic signals, developing meaningful population trends from acoustic activity, and finally engaging global audiences to take part.

23.2 Challenge 1: What Bat Is That?

Perhaps the biggest and most complex issue facing a global bat acoustic monitoring program is the objective and statistical taxonomic identification of bioacoustic signals. Although identification to species or individuals with species is most desirable, monitoring the status of the same recognizable signal over time without specific identification may be sufficient in some situations. Compared to morphological identification, quantifying and identifying acoustic information for any taxa is a relatively new field, particularly beyond using song to recognize bird species. From one perspective, identifying bat acoustic signals is easy because the frequencies bats use (i.e., 9–215 kHz) are not used by many other taxa and are easily recognizable. However, from another perspective, identification is challenging because bat acoustic signals are incredibly diverse and contains a lot of intra- as well as interspecific variation. Added to this complexity is that acoustic signal variation is not well documented, and signal identification tools are in their infancy.

23.2.1 Bat Acoustic Diversity

Bats use a variety of different frequencies and temporal structures in their calls (Griffin 1958; Jones and Teeling 2006), and these have evolved via ecological and perceptual challenges and constraints. Acoustic signals include social calls as well as those used for navigation and localization of prey (echolocation). All but one (Pteropodidae) of the eighteen families of bats use ultrasonic echolocation signals, and some species of Pteropodidae have been found to use audible clicks for navigation.

Echolocation calls are structurally very diverse, despite a strong phylogenetic constraint in some families (Jones and Teeling 2006). Conveying species identity is not the primary purpose of echolocation, and call design has been constrained by the need to effectively detect and identify objects to fulfill the sensory objectives of echolocation (Barclay 1999). Phylogenetic constraints and call convergence have led to overlapping frequencies and temporal structures, making it difficult for humans to distinguish among the calls of some species (Jones and Teeling 2006; Preatoni et al. 2005). Variation in call structure across individuals within species based upon anatomical differences, geographic location, as well as sex and age further confounds species identification, especially on a global scale (Buckley et al. 2011; Heller and Von Helversen 1989; Jones and Siemers 2011; Kalko and Schnitzler 1993; Murray et al. 2001; Obrist 1995; Papadatou et al. 2008; Siemers et al. 2005; Thomas et al. 1987). Individuals of the same species may also vary their call

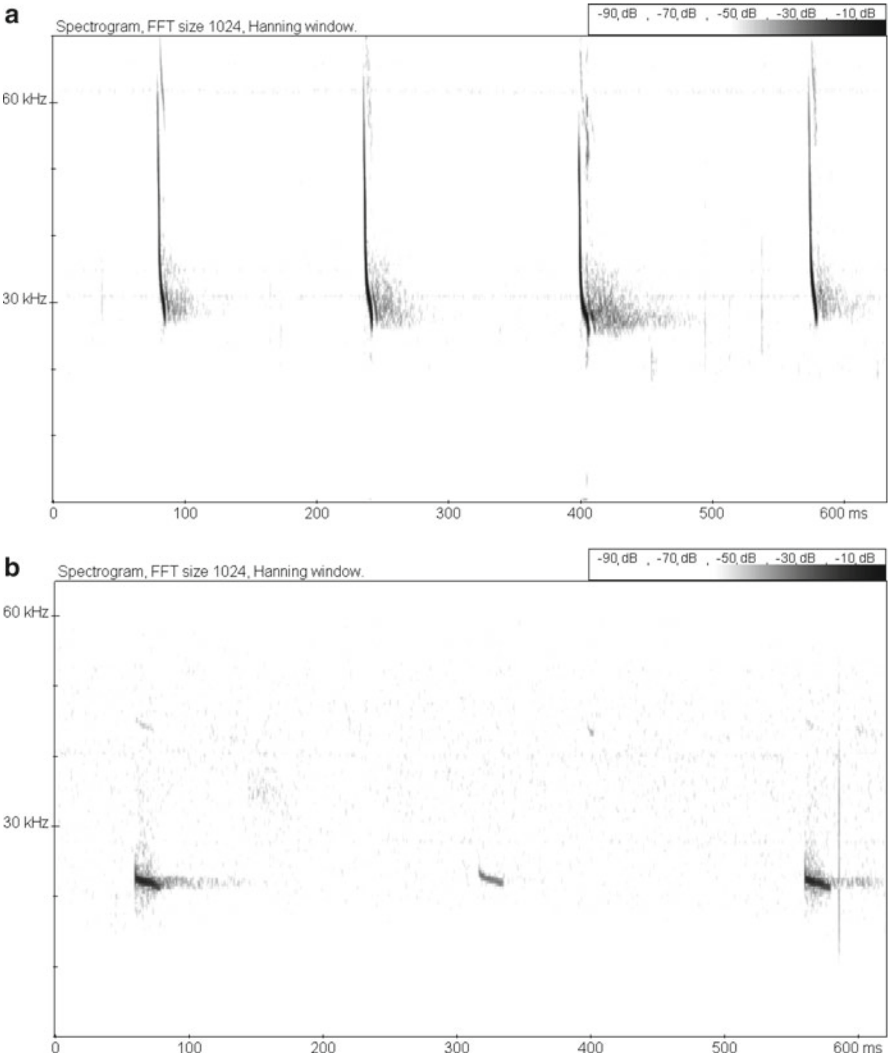

Fig. 23.1 Spectrograms of *Nyctalus leisleri* echolocation calls (Hanning window, FFT size of 1,024) in (**a**) cluttered and (**b**) open environments

structure depending on the situation and the information required. For example, when flying in a cluttered environment, individuals may use shorter duration, higher frequency, and larger bandwidth calls that give more precise information than they would in less cluttered surroundings (Kalko and Schnitzler 1993) (Fig. 23.1).

Variation in echolocation also occurs during foraging. Insectivorous bats that employ an aerial-pursuit strategy normally use relatively longer, slower "search-phase" calls during commuting flight. Calls become shorter and quicker as the bat

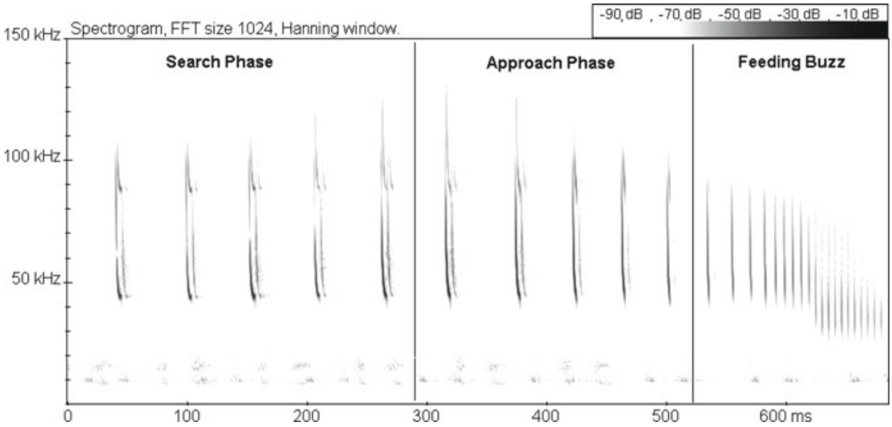

Fig. 23.2 A spectrogram of a sequence of *Pipistrellus pipistrellus* echolocation calls (Hanning window, FFT size of 1,024) showing variation depending on activity

approaches and hones in on a prey item, escalating assessment of the insect's position and movements and culminating in a series of very short, quick calls termed as a "feeding buzz" (Griffin et al. 1960; Kalko and Schnitzler 1993) (Fig. 23.2). Individuals can also alter call frequency in the presence of other bats to avoid overlapping signals (Obrist 1995; Ulanovsky et al. 2004), further adding to the challenge of species identification.

23.2.2 Documenting Echolocation Call Diversity

Call diversity would not be such a challenge to a global monitoring effort if the variation was well documented. However, although a number of call libraries exist (Table 23.1), the data are biased towards echolocation calls of well-studied areas and species, and many of these libraries allow only listening access (British Library Sound Archive) which is useless for the analysis of the spectral content of calls (required for quantitative identification). The need for a global call database has been recognized for some time (Korine and Kalko 2001) and has led to the development of EchoBank, a global, reference call library of time-expanded and real-time call sequences (Collen 2012). EchoBank currently contains 53,488 calls in 3,531 call sequences, from 297 species in 94 genera and 18 families, representing 31 % of echolocating bat species and 95 % of families (Fig. 23.3).

Call files in EchoBank are donated to the library from a consortium of scientists using a variety of different recording equipment and data capture settings, from many different habitats, and using a number of different methods such as recording bats in the hand, in a bat bag, free hanging, after hand release, on a zip line, light tagged, in a flight cage, free flying, in artificial roost sites, in a test room/laboratory, and leaving a roost. This ensures that as much of the natural variation present in the

Table 23.1 Echolocation call reference libraries currently available

Library name	No. of species	No. of records	Recording method	Source
Bat Conservation Trust Sound library	15	27	H	http://www.bats.org.uk
Southeastern Australian Bat Call Library	9	31	FD	http://www.csu.edu.au/batcall/batcall1.html
Pacific Northwest Bat Call Library	10	33	FD	http://www.depts.washington.edu/sdwasm/pnwbat/batcall.html
Wyoming Bat Call Library	14	73	FD	http://www.uwyo.edu/wyndd/data-dissemination/priority-data-comp/wyoming-bat-call-library/index.html
BatCall—Museum of Southwestern Biology	22	3,821	FD	http://www.msb.unm.edu/mammals/batcall
Batcalls.com	26	60	TE	http://www.batcalls.com
Cornell Lab of Ornithology—Macaulay Library	29	258	TE	http://www.macaulaylibrary.org
BatCalls.org	42	91	TE	http://www.batcalls.org
British Library—British Sound Archive	139	~700	TE	http://www.bl.uk/soundarchive
EchoBank	297	3,531	TE and RT	Collen (2012)

Recording methods are *H* heterodyne, *FD* frequency division, *TE* time expansion, and *RT* real time

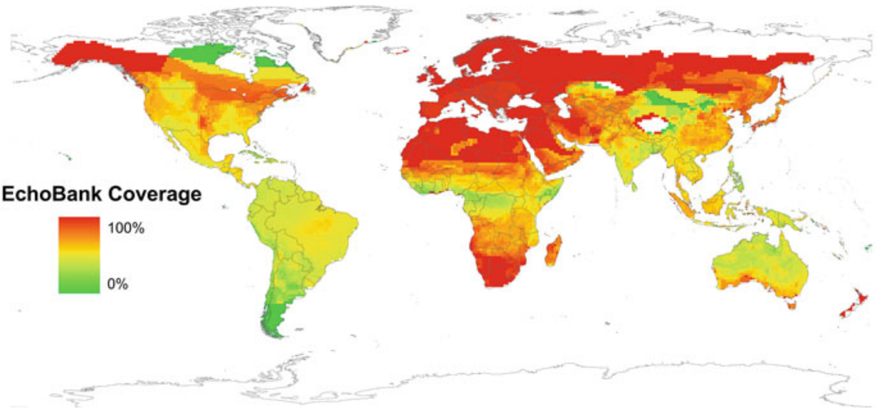

Fig. 23.3 The proportion of species in EchoBank of all echolocating species known present (Simmons 2005), mapped in 1° squares globally. *Red* indicates high percentage coverage of EchoBank, whereas *green* indicates low percentage coverage

calls of each species is represented within the database, and data are not biased by any particular recording situation or method.

EchoBank is a promising resource for a global monitoring program as it represents the most comprehensive library for bat echolocation calls to date and contains full call sequences rather than individual calls, to provide sufficient data for acoustic identification. However, it still only contains calls from 31 % of species and is confined to time-expanded and real-time recordings. Areas with complete coverage or near-complete representation only occur in North America, Europe, Northern Africa, South Africa, the Middle East, and North Asia (Fig. 23.3). Continued data collection should be prioritized for the tropics and in the southern hemisphere as these are species-rich, important regions. Further, the geographic coverage for each species needs to be increased so that different areas of their range are covered, ensuring that geographic variation is represented. EchoBank requires further buy-in from the scientific community to expand its coverage and support to make it accessible online.

23.2.3 Acoustic Identification Tools

Although there has been a recent growth in the development of tools for identifying calls from different taxa [e.g., sea lions, Campbell et al. (2002); insects, Chesmore (2004); whales, Murray et al. (1998); birds, Trifa et al. (2008)], development of bat acoustic identification tools remains a significant challenge. This is not only because of the level of acoustic diversity and lack of documentation but also because of the plethora of different methods developed to convert and record bat ultrasound into frequencies audible to humans. These techniques include narrowband methods (heterodyne) as well as broadband methods (frequency division, time expansion, or real time). The advantages and disadvantages of each technology were discussed in detail elsewhere (Parsons et al. 2000; Parsons and Szewczak 2009), but each represents acoustic signals differently thereby complicating identification. In addition, many different types of equipment and data capture devices are used, all of which can impact interpretation.

23.2.3.1 Narrowband Methods: Heterodyne Detection

Heterodyne detectors work by reducing ultrasonic calls into a series of simple audible clicks of which frequency, tone, and temporal aspects can be used to determine species identity in the field (Limpens 2004). However, this technique requires a high level of skill to master, and therefore error levels depend on the user's experience (Jennings et al. 2008). The inherent subjectivity involved in identifying bats to species using a heterodyne detector means that they do not lend themselves widely useful for large monitoring programs. However, careful training of volunteers has led to the successful implementation of field-based qualitative identification in the UK Bat Conservation Trusts' National Bat Monitoring Program (NBMP), for identifying common pipistrelles (*Pipistrellus pipistrellus*), soprano pipistrelles (*Pipistrellus pygmaeus*), noctules (*Nyctalus noctula*), and serotines (*Eptesicus serotinus*) (Walsh et al. 2001). However, because identification is only based on interpretation of the peak frequency, duration and inter-pulse interval of the calls in the field, these methods are difficult to use in larger and more species-rich areas. In such areas there may be a greater overlap in call structure among species and bat species present may not be well documented. These limitations suggest that heterodyne methods are not well suited for a global monitoring program.

23.2.3.2 Broadband Methods

Broadband methods such as frequency division, time expansion, and real-time recording have a distinct advantage in that they retain much more of the information within each call, allowing detailed post-processing and thus less interpretive error and more standardized identification. This opens up development of automated quantitative methods to extract and classify calls from recordings, removing human error and bias. A number of existing bat monitoring programs use broadband detectors, for example, iBats (Eastern Europe) (Jones et al. 2013), Bat Conservation Ireland (Ireland) (Roche et al. 2011), and BATGRID (USA) (Rodhouse et al. 2012).

Scaling up these broadband monitoring programs to a global scale will require development of automated quantitative call identification methods with capacity to (1) *detect* and *isolate* an acoustic signal from background noise in recordings, (2) *characterize* the acoustic signal (e.g., extract call features), and (3) *classify* it to an individual, species, or other taxonomic group. These tools have an enormous promise to help make the development of a global acoustic monitoring program more realistic.

Detection and Isolation Tools

Traditionally, signal detection has been achieved by manual inspection of sound files, displayed as frequency–time plots (spectrograms) or time–amplitude plots (oscillograms). However, this method is particularly time-consuming, making it impractical for large volumes of data. It is also subjective and highly dependent on the skill and opinion of a particular individual. Therefore, the method has low repeatability and no quantitative measure of certainty (Skowronski and Fenton 2008). The amount of data that needs to be examined manually can be reduced by using inbuilt "silence removers" (e.g., Sound Analysis Pro, SA+) that perform a low-pass filter on the signal in order to remove silent gaps and consequently reduces the volume of data needing examination. However, this filter is relatively ineffective on files having high levels of background noise.

More recently, automated software packages have been developed that use a variety of algorithms (often more than one) to recognize a bat acoustic signal from background noise providing a consistent, objective analysis method (Table 23.2).

Table 23.2 Examples of software using automated species detection packages of broadband bat calls

Software	Platform	Signal detection method	Website
SonoBat	W, M	ATF, ASFC	http://www.sonobat.com
bcAdmin	M	SC	http://www.ecoobs.com
BatScope	M	SC	http://www.wsl.ch
AnaLook	W, M	SC	http://www.titley-scientific.com
SCAN'R	W	CC	http://www.binaryacoustictech.com

W and M represent Macintosh and Windows platforms, respectively; *ATF* amplitude threshold filtering, *ASFC* areas of smooth frequency change, *SC* search criteria, and *CC* cross-correlation

Amplitude threshold filtering (ATF) involves the application of a filter at an amplitude threshold and extracting any signals in the recording that exceed this threshold. However, successful extraction relies on a quiescent signal between calls, so calls will be missed that are at the same amplitude as background noise. As noise may be greater in amplitude at particular frequencies than others, creation of frequency bands in the spectrogram allows individual threshold filters to be added at different energy levels appropriate to the average energy of that frequency band (Brandes 2008). Careful consideration needs to be taken over setting threshold amplitudes, as bats that echolocate at low amplitudes will not be detected at low thresholds. Although calls of amplitude below the threshold may be of too low quality for classification, they may still be useful in signaling the presence of a bat. *Areas of smooth frequency change* (ASFC) is another property of signals that can be used to discriminate animal vocalizations from noise. In general, frequency changes in animal calls are smooth, whereas in background noise they are random. Algorithms can scan through files and detect signals with a smooth trend in frequency. However, these algorithms cannot discriminate between different taxa, so an additional process may be required to remove non-bat signals. The call analysis program SonoBat (http://www.sonobat.com) (Table 23.2) uses a combination of ATF and ASFC to detect and isolate bat calls. *Detection of search criteria* (SC) is an algorithm based on the detection of particular call characteristics (e.g., spectral, amplitudinal, and temporal) to extract calls from sound files, for example, the sound analysis software programs bcAdmin (EcoObs http://www.ecoobs.com), AnaLook (http://www.titley-scientific.com), and BatScope (http://www.wsl.ch) (Table 23.2). *Cross-correlation* (CC) is an algorithm based on cross-correlation of signal spectrograms in a sound file with a reference spectrogram. Signals that have high similarity to the reference signal are detected as calls. This method is used in the sound analysis software program SCAN'R (Binary Acoustic Technology, http://www.binaryacoustictech.com). SCAN'R successfully detected over 99 % of the echolocation calls identified manually by an experienced researcher, within 12,000 sound files, suggesting that this method is highly effective at signal detection (Williams et al. 2007).

Unfortunately, little data has been published regarding the accuracy of detection of most commercially available bioacoustic software programs. As accuracy of detection is a vital consideration when choosing an automated approach, ideally all of these methods should be assessed against a previously hand-inspected dataset

(Williams et al. 2007). Detection success may also vary from species to species as some call types and frequencies may be easier to isolate from background noise than others. The application of these methods to a global acoustic monitoring program is hampered by a lack of transparency of the exact algorithms used and only recognizing single echolocation calls. Ideally, we need an algorithm that is freeware, can recognize the sequence the call has come from, and separate out social calls from echolocation calls and separate out search-phase echolocation calls from approach and terminal phase calls. Development of such tools has begun (Bat Detective http://www.batdetective.org) but is still very much in progress.

Feature Extraction Tools

Once acoustic signals have been detected and isolated, features need to be extracted to enable identification. Traditionally, this has been done by measuring a number of parameters describing the call. A variety of different parameters can be measured: those most commonly considered include highest, lowest, and middle frequency; call duration; inter-pulse interval; and peak frequency (Hughes et al. 2010; Papadatou et al. 2008; Parsons and Jones 2000; Redgwell et al. 2009; Zingg 1990). The parameters most useful for distinguishing between species may depend on the assemblage being studied (Walters et al. 2012) and the method of recording. For example, some frequency division detectors reduce the complexity of calls, thereby losing information about the harmonic structure and amplitude and decreasing the ability to distinguish among species, particularly in species-rich areas (Parsons et al. 2000).

Characterizing signals by reducing complexity to a few descriptive measurements leads to a loss of information which may be informative in distinguishing between species. Developments in the ways that we characterize acoustic signals that move away from series of measurements, to instead characterizing the signal as a whole, show promise in improving quantitative classification of species. For example, Lundy et al. (2011) show that using elliptic Fourier descriptors to characterize call shapes enabled a high level of discrimination of three *Myotis* species, typically difficult to classify. Obrist et al. (2004) developed synergetic pattern recognition techniques to classify 26 Swiss bat species that do not rely on call measurements but instead calculate 159 × 128-point spectrograms of each call producing a 20,352 point feature vector. This method therefore characterizes the entire call within a single vector, and algorithms recognize patterns within vectors which are distinct to a particular species while ignoring patterns common to all species (Obrist et al. 2004). An average classification rate of 86 % was achieved across all 26 species using this method, compared to 75 % using conventional call parameters.

Other methods that investigate whole acoustic signals using automated speech recognition in humans (ASR) (Dudley and Balashek 1958) might also be promising for call identification. Cepstral coefficients (CCs) are features of the spectrogram which are used in ASR. The cepstrum is a smoothed version of the spectrogram, computed by performing another Fourier transform on the logarithm of the spectrogram magnitudes for each time point: the cepstral coefficients are the magnitudes of the cepstrum. To model the human auditory system, scaled versions of these CCs were developed for ASR that reflects the frequency resolution of human hearing. By using Mel-frequency filters before taking the logarithms, the spectrogram is filtered and transformed to produce associated cepstral coefficients (MFCCs) that reduce the resolution for higher frequencies while maintaining high discrimination for lower ones (Davis and Mermelstein 1980).

Due to the similarities between bird and human hearing (Doupe and Kuhl 1999), several studies have used MFCCs for species-specific feature extraction of bird song (Fagerlund 2007; Lee et al. 2006; Tyagi et al. 2006), insects (Ganchev et al. 2007), and bats (Harris and Skowronski 2006). Mirzaei et al. (2012) compared spectrogram features and MFCCs for five bat species and found that they slightly outperformed spectrogram features when identifying species. Further possible directions are CCs tailored to the auditory systems (Greenwood 1961) by emphasizing medium frequencies (Kossel 1993).

Spectrograms also have an infamous drawback because of the trade-off in resolution in either time or frequency, which other representations avoid. For example, Mirzaei et al. (2012) compared spectrogram and MFCC features following a discrete wavelet transform (DWT), also useful for noise reduction. For calls of five bat species, the DWT features outperformed both the MFCCs and spectrogram analyses with near-perfect species recognition, showing promise for automated species identification.

These and other feature extraction techniques being used in ASR show promise for improving classification rates and our understanding of call structure. Feature extraction may be the most crucial aspect of call classification but is currently underdeveloped.

Classification Tools

A number of objective quantitative approaches are available to classify bat calls, including multivariate statistical methods and machine learning techniques (Parsons and Szewczak 2009). Many of these methods have been applied to the classification of time-expanded or real-time recorded echolocation calls (see Armitage and Ober 2010 for a frequency division example). Multivariate statistics like discriminant function analysis (DFA) (Fukui et al. 2004; Hughes et al. 2010; Papadatou et al. 2008; Parsons and Jones 2000) and machine learning methods such as support vector machines (SVM) (Redgwell et al. 2009), synergetic pattern recognition (Obrist et al. 2004), and artificial neural networks (ANN) (Britzke et al. 2011; Jennings et al. 2008; Parsons 2001; Parsons and Jones 2000; Redgwell et al. 2009; Walters et al. 2012) all show good species-level classification, with ANN proving most accurate by comparison (Redgwell et al. 2009). Combining the strengths of many classifiers is more robust and provides better classification than any single algorithm if classification of each individual algorithm is above 50 % (Redgwell et al. 2009).

Although many of these algorithms are able to classify most of the species on which they are trained with a high degree of accuracy, most have been trained on a relatively small number of species or calls collected within a small region (Fukui et al. 2004; Papadatou et al. 2008; Parsons and Jones 2000) and can therefore only classify individuals from a limited number of species recorded within the same region. In order to use these quantitative methods for wide-scale regional monitoring, classification needs to be standardized and algorithms constructed from an entire region. Training regional classification tools has been hampered by the lack of a suitable call reference library. As mentioned, EchoBank contains representative calls from a large number of species covering entire regions and will be crucial to the development of identification tools for use in large-scale survey and monitoring programs (e.g., see Walters et al. 2012 for a Pan-European example).

Although the methods described above show great promise, improvements to the methods used could increase classification accuracy further, particularly for the difficulty to distinguish species and perhaps for individuals within species. Increasing the number and types of recordings available in call libraries will help to provide recordings encompassing all the variation in echolocation calls, making resultant classification tools more likely to be able to identify free-flying bats accurately. Methods of characterizing calls which retain all of the information present in the call should also improve classification. Also, combining our knowledge of species distributions and habitat associations with classification tools may aid in distinguishing between allopatric species (Hughes et al. 2010), although this technique should be used with caution in long-term monitoring schemes given that species distributions are expected to shift under global climate change (Parmesan 2006).

23.3 Challenge 2: Are We Really Monitoring Population Changes?

Although acoustic detection of bat calls may play an important role in monitoring trends and patterns, it is important to understand inherent biases. As with most survey methods, species vary in their likelihood of detection, with acoustic detection depending on the echolocation call type used by the species and the habitat the bat is recorded in (Hayes 2000; Jones and Teeling 2006; Meyer et al. 2011). Bat species that emit high-intensity calls which have a lower directionality and lower frequency have a greater probability of being detected because these calls have slower attenuation through air. High-intensity calls are detected at greater distances, and a less directional call type may be detected at a wider range of angles (Bazley 1976).

Conversely, high-frequency, highly directional, or low-intensity calls may be detected less often as their calls are less likely to be recorded unless they are close to the microphone. Adequate sampling of such species would require a much greater survey effort, and other survey methods may be better suited. These differences in likelihood of detection among species mean that conclusions drawn from comparisons of activity levels may be of limited validity, unless detectability differences are explicitly accounted for (Hayes 2000).

Detectability differences also exist between habitat types, with probability highest in open environments (Meyer et al. 2011; Patriquin et al. 2003). However, detectability of species in particular habitats should remain constant if species abundance remains constant (Meyer et al. 2011); thus, acoustic methods assess within species activity changes and comparisons of relative species-specific activity through time.

Acoustic methods may be more useful for species inventories than are mist netting or harp trapping (MacSwiney et al. 2008; Meyer et al. 2011; Ochoa et al. 2000; O'Farrell and Gannon 1999) because they sample areas that nets and traps cannot reach. Additionally, acoustic methods are noninvasive and do not disrupt, alter the behavior of foraging bats, or suffer from trap-wise bats (Kunz and Brock 1975; Larsen et al. 2007). Acoustic methods may therefore provide less biased activity estimates in situations where the same area is repeatedly surveyed.

With current technology, it is difficult to distinguish whether a single individual has been recorded many times or if many individuals of the same species are present, making it difficult to reliably estimate population abundance (Hayes et al. 2009). Occupancy studies, whereby a set of sites are repeatedly surveyed for presence/absence using acoustics, is one option that avoids the problems of estimating population density from acoustic activity (Kéry et al. 2010).

Another promising development is to use methods to estimate abundance which do not require known individuals, where pseudo-replication is assumed and modeled implicitly (Hutchinson and Waser 2007). For example, methods modeling encounter rates rather than number of individuals have been developed to estimate abundance from camera-trap images (Rowcliffe et al. 2008). These models assume that animals move in straight lines, of random direction, and by considering the camera trap as a cone of detection, the number of expected trapping events is calculated and compared to an empirical number of events, and abundance can be calculated. It is possible to alter these models to take into account the differences between acoustic and image detection, and they can be further modified to account for differences in flight speed and call directionality, intensity, and frequency and used to compare abundances among species. The assumption of random movement is of course a simplification, but the estimate will still be correct if animals move by Brownian motion instead of straight lines, although the variance will increase (Hutchinson and Waser 2007).

23.4 Challenge 3: Who Would Take Part?

To rapidly scale up acoustic monitoring globally, we need a much wider engagement of communities and the public to collect the necessary data. The recent resurgence of "citizen science" broadens who can take part in bat monitoring and how they can participate (Roy et al. 2012). There is a long tradition in some regions where monitoring programs engage interested amateurs to collect bat acoustic data (e.g., Bat Conservation Trust's UK National Bat Monitoring Programme). However, this approach needs to be radically widened and made much more accessible to the general public. Equipment to detect bats ultrasonically is currently expensive, limiting its appeal, and ironically, the cheaper detectors (heterodynes) collect the least useful data for automated analyses.

One alternative is to take an approach where more expensive broadband ultrasonic equipment is purchased for a local environmental organization whose volunteers borrow the equipment to monitor together. Since 2006, thousands of iBats (http://www.ibats.co.uk) (Jones et al. 2013) volunteers have monitored bats using time expansion broadband detectors within national community groups in Romania, Hungary, Bulgaria, Ukraine, Russia, and the UK. These groups include national bat or other taxon-specific organizations (e.g., Romanian Bat Protection Association, the Green Balkans Bulgaria) and environmental groups (e.g., PRESEVET, Russia), whose volunteers monitor transects (Jones et al. 2013). Similar projects occur in other countries (e.g., Ireland, Roche et al. 2011; USA, Rodhouse et al. 2012), and the development of automatic acoustic identification tools for bats in Europe (Walters et al. 2012) allows for quick and objective analysis of iBats data files.

New technologies such as tablets and smartphones could enable this wider engagement to flourish. Smartphone applications for bat monitoring are currently limited to recording sound and geolocation from an attached detector (iBats, http://www.ibats.co.uk) but a prototype to incorporate the European time expansion and real-time recording algorithms (Walters et al. 2012) into an application for automatic identification on the smartphone in development (https://www.batmobile.blogs.ilrt.org). Ideally, future call classification technologies would be able to take signals from any detection systems (broadband or narrowband) and return identifications, with the probability of correct identification adjusted by the level of information within a signal. In this way, identifications from calls recorded using different techniques would be comparable, with higher weight given to calls that retain more useful signal information. Alternatively, classifications from different systems could be combined if the probability of correct identification is known from each classifier and for each region.

National governments are waking up to the importance of citizen science activities, perhaps seeing them as a good deal in this austerity climate (Roy et al. 2012). The public can be mobilized and organized with astonishing speed to provide data on important and topical issues such as the spread of invasive species and diseases. The development of the http://www.ashtag.org application to monitor the spread of the ash dieback disease within a few days of it being reported in the UK is a recent example. More radical citizen science approaches that blur the distinction between "citizen" and "scientist" might also be used to scale up acoustic monitoring. For example, technological advances in the development of customizable computer hardware, such as the Raspberry Pi (http://www.raspberrypi.org), may eventually allow anyone to build a cheap broadband bat detector. Programming on such devices is becoming more accessible, with community hackfests and Epicollect (Aanensen et al. 2009) where anyone can design and launch a smartphone application to collect data.

These approaches to widen public participation bring substantial new challenges to consider: the proliferation of citizen science projects might mean that projects have to compete for attention, efforts may be duplicated, and common data standards may not be agreed upon, which may hamper data sharing and a more scalable and global approach. Recent development of citizen science platforms with common data standards and suites of projects or tools might ameliorate these issues (e.g., http://www.zooniverse.org, http://www.inaturalist.org).

23.5 Future Priorities

Acoustic monitoring efforts for bats are less available in lower latitude, tropical countries which contain high bat species diversity as represented by poor EchoBank coverage (Fig. 23.3). We investigated the difficulty of building accurate acoustic tools to identify bats in these regions by quantifying the degree of overlap echolocation call parameters. Using calls from EchoBank (Collen 2012), along with parameter values from literature searches and parameter estimation techniques based on phylogeny (Bruggeman et al. 2009), we generated an index of similarity in spectral and temporal aspects of echolocation calls within bat communities globally (Box 23.1) (Fig. 23.4).

Box 23.1 Echolocation Call Similarity Index—Methods

Call parameter data. Species median values of four echolocation call parameters: maximum frequency (kHz), minimum frequency (kHz), peak frequency (kHz), and call duration (ms) for 297 species obtained from time-expanded or real-time calls (EchoBank) (Collen 2012) supplemented with parameter data 114 species. Median parameter values for another 481 species estimated using PhyloPars (Bruggeman et al. 2009) and phylogenetic supertree of bats (Collen 2012). Estimates were generated based on 411 species above, body mass data from PanTHERIA (Jones et al. 2009b). Each value was divided by the mean across all species and weighted equally.

Species assemblages. Distribution maps for 892 echolocating species from the IUCN mammal assessments (IUCN 2012) and assemblages within each 1° grid square globally were extracted using enumerate intersecting feature tool in Hawths Tools (Beyer 2004) in ArcMap V9.3 (ESRI 2008).

Similarity index. The Euclidean distance between each species pair, based on the four call parameters, was calculated within each species assemblage (grid square). The mean pair-wise distance was calculated for the species within each grid square and divided by the number of species present to generate a similarity index normalized between 0 and 1. This index accounts for the number of species present and the similarity in the four call parameters across assemblages. Scores of 0 indicate that species' calls within the grid square have exactly the same parameter values, whereas scores of 1 indicate complete difference. This index was mapped per one degree square scale (Fig. 23.4).

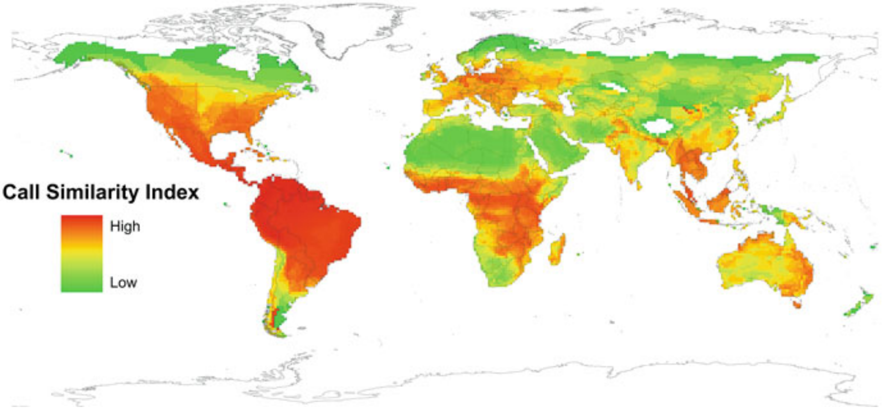

Fig. 23.4 Call similarity in global bat communities for 892 species. *Red* indicates areas (1° grid squares) where calls among species are most overlapping, whereas *green* represents areas of least interspecific call similarity (see Box 23.1 for methodological details)

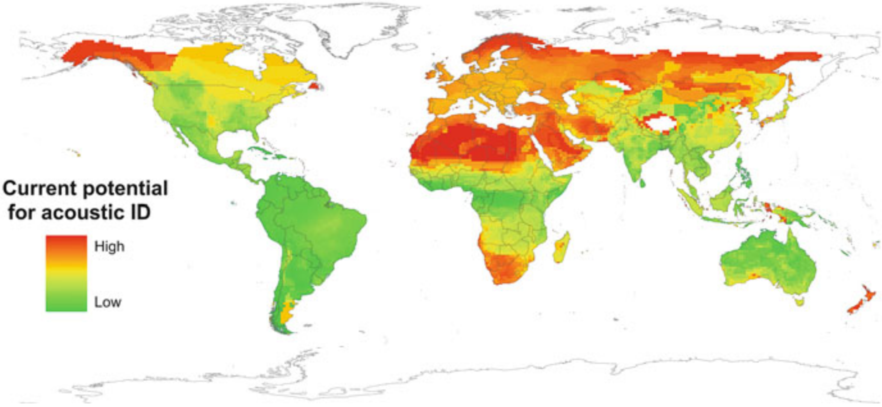

Fig. 23.5 A combined index of call similarity and species representation in EchoBank. *Red* indicates best opportunities for acoustic monitoring, whereas *green* represents areas where monitoring is more difficult

Regions like South America and Southeast Asia have many closely related bat species and a high degree of call similarity which makes acoustic monitoring challenging due to the difficulties in identifying species. In these areas, it may be necessary to classify calls to species groups or functional groups and to monitor changes in presence and distribution, rather than individual species. However, in areas where call similarities are relatively low (North Africa and Eurasia), accurate species identification and effective species-level monitoring are possible (Fig. 23.4), where comprehensive libraries of reference calls for resident species have been compiled.

Combining data on the species coverage of EchoBank (Fig. 23.3) with data on levels of similarity within bat communities (Fig. 23.4) produced an index of best acoustic monitoring opportunities (Fig. 23.5). Areas such as Europe, North Africa, the Middle East, and North Asia have high call library coverage and relatively low call similarity and thus provide an opportunity to generate robust, accurate species identification. Some of these geographic areas are already the focus of monitoring programs, and efforts should be made to establish monitoring in regions not currently covered. For the Neotropics, the priority should be to gather known species call sequences under numerous conditions and establish community monitoring programs.

23.6 Conclusions

We are on the cusp of major advances in the scope and ambition of bat acoustic survey and monitoring. The new and rapidly developing methodological approaches to automated detection, characterization, and classification, coupled with the expansion of comprehensive call libraries mean, will allow for standardized monitoring and the potential for rapid expansion. The increasing interest in citizen science and the application of smartphone technologies attached to cheap ultrasonic microphones may open up bat conservation to vast numbers of ordinary people.

Acknowledgments We thank Michel Barataud, Roger Coles, Christian Dietz, Brock Fenton, Dai Fukui, Gareth Jones, David Jacobs, Richard Jenkins, Nancy Jennings, Elisabeth Kalko, Martin Obrist, Stuart Parsons, Sebastien Puechmaille, and Thomas Sattler for contributing calls to EchoBank, Bernd Brandt for assistance using PhyloPars, and an anonymous reviewer and editor Rick Adams for invaluable comments on a previous version of this manuscript.

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Chapter 24
On Estimating the Economic Value of Insectivorous Bats: Prospects and Priorities for Biologists

Justin G. Boyles, Catherine L. Sole, Paul M. Cryan, and Gary F. McCracken

Abstract Bats are among the most economically important nondomesticated mammals in the world. They are well-known pollinators and seed dispersers, but crop pest suppression is probably the most valuable ecosystem service provided by bats. Scientific literature and popular media often include reports of crop pests in the diet of bats and anecdotal or extrapolated estimates of how many insects are eaten by bats. However, quantitative estimates of the ecosystem services provided by bats in agricultural systems are rare, and the few estimates that are available are limited to a single cotton-dominated system in Texas. Despite the tremendous value for conservation and economic security of such information, surprisingly few scientific efforts have been dedicated to quantifying the economic value of bats. Here, we outline the types of information needed to better quantify the value of bats in agricultural ecosystems. Because of the complexity of the ecosystems involved, creative experimental design and innovative new methods will help advance our knowledge in this area. Experiments involving bats in agricultural systems may be needed sooner than later, before population declines associated with white-nose syndrome and wind turbines potentially render them impossible.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_24,

24.1 Introduction

Bats play many vital roles in ecosystems worldwide as pollinators, major predators of arthropods, and seed dispersers (Kunz et al. 2011). These “ecosystem services” are hugely beneficial to humans, and bats are likely one of the most economically important nondomesticated groups of mammals (Boyles et al. 2011; Kunz et al. 2011). Still, public perceptions of bats are often negative, and this discordance between perception and reality may make bats among the most undervalued of all vertebrate groups. Arguments about the value of bats have historically been limited to anecdotal accounts of how many insects a single bat can consume or the crops that rely on bats for seed dispersal or pollination. Although these arguments are helpful in changing perceptions about bats, they often fail to establish the relevance of bats to the public, a problem which may be solvable by placing the value of bats in a context that is widely appreciated—money. There have been several recent attempts to place monetary values on the pest control services provided by bats, and resulting estimates are impressively large (Boyles et al. 2011; Cleveland et al. 2006; Federico et al. 2008b). Although these estimates have gained traction in the public media and as effective arguments for bat conservation, our scientific understanding of ecosystem services provided by bats is so underdeveloped that all estimates to date have been quite imprecise. Unfortunately, even such imperfect estimates are unavailable for the economic value of bats as seed dispersers and, except for Bumrungsri et al. (2009), as pollinators. These deficiencies in our ability to accurately quantify the value of bats only hinder public appreciation and efforts to justify conservation efforts directed toward this misunderstood group of mammals.

Our goal in this chapter is to provide recommendations on where researchers might focus their efforts in the next decade to more thoroughly describe the economic value of bats. Kunz et al. (2011) have provided a prospectus on the economic methodologies available to estimate the value of those services; therefore, we will expand the discussion with research recommendations regarding the largest functional group of bats, the insectivores (Kunz et al. 2011), and what we need to know to quantify their economic importance.

24.2 The Science of Value Estimation: Insectivorous Bats as Pest Controllers

In general, we see two promising scientific approaches to estimate the value of bats in controlling crop pests. First, we can attempt to study, understand, and parameterize each component of a model describing the value of bats, including the population size of bats in an area, what prey species those bats eat, how many insects of each species they eat, and the effects of bat predation on the insect populations. Second, we can take a more holistic approach and attempt to understand the effects of bats on crop production without attempting to understand the underlying processes. Each approach has advantages. The first approach will greatly increase our understanding of basic bat biology, allow for evaluation of how each factor affects the overall value of bats, and is likely to have greater heuristic value for attempting to evaluate other crop/insect/bat food webs worldwide. However, getting at those underlying processes will be time consuming, logistically difficult, and in some cases (e.g., species of bats that cannot be counted) impractical. The second approach of tracking bat-associated changes in crop production may lead to quicker and more direct answers but, because the underlying processes are ignored, will likely provide less insight into other systems worldwide. We will address each of these approaches in turn, beginning with the individual processes we need to understand to describe the whole system.

24.2.1 What Bats Eat

Given the long history of studies on diets of insectivorous bats (e.g., Agosta 2002; Brack and LaVal 2006; Clare et al. 2011; Kurta and Whitaker 1998; Tuttle et al. 2006; Whitaker 1995, 2004; Whitaker and Clem 1992; Whitaker and Rissler 1993; Whitaker et al. 1997), it may seem odd that we believe one of the most important research areas as we move forward is determining what bats eat. Traditional diet analyses, based on visually identifying insect fragments in bats' feces to the lowest taxonomic level possible and largely pioneered by Dr. John Whitaker, Jr. (Whitaker 1988), provide an incredible amount of information about what insects some bat species consume. However, these traditional analytical techniques have limitations, such as biases caused by differences in digestibility among prey, limited ability to detect very small prey, and difficulty in identifying remains below the ordinal level (Whitaker et al. 2009). Traditional diet analyses are also very time consuming, so mass sampling of bat diets, and thus continent- or global-scale evaluation of bat diets, is unlikely.

Molecular techniques, which are rapidly developing, clearly offer a promising research pathway. A huge number of insect species in the diets of certain bats have been identified using PCR amplification and bar coding sequence analysis of DNA extracted from insect fragments in bat feces (Clare et al. 2009, 2011). Still, these techniques require the separation of fecal material, so digestibility issues might hinder the identification of small or soft prey using this approach. Further, the time involved with mechanical separation of feces means that analyses of bat diets on large geographic scales are limited by sample processing time. In most instances, different genetic markers are needed to identify different insect groups. This not only increases analytical costs exponentially but also limits prey identification to the selected target group and requires a priori knowledge of prey species (King et al. 2008). Whole fecal sample extraction followed by PCR and molecular cloning for DNA bar codes of targeted taxa (Zeale et al. 2011) has recently provided a more efficient approach for identification of phylogenetically diverse insects in the diet of bats, but as in all bar code studies, a reference database of sequences from known available insects is required. Another limitation of this technique is the cost associated with using large numbers of clones per sample, which in turn may result in the failure to detect less abundant prey items (Alberdi et al. 2012). The use of next-generation sequencing techniques allows even more efficient analysis of dietary diversity and resource partitioning in insectivorous bats where reference sequence data are lacking for available insect prey. Two recent studies using Roche FLX 454 high-throughput DNA sequencing techniques allowed PCR of fecal samples from multiple bats using uniquely tagged insect-generic primers in which prey items were identified as molecular operational taxonomic units (MOTU's) (Razgour et al. 2011; Bohmann et al. 2011). In both studies, greater taxonomic precision was obtained by combining the molecular data with traditional identification of insect fragments and additional references to known sequences in DNA bar code databases (e.g., Lepidoptera Barcode of Life; http://www.lepbarcoding.org/). As reference bar code data expand, these techniques will allow increasing specificity in identifying the insect prey of bats. Future advances in sequence technology will only increase our ability to analyze a large number of samples at even lower costs (Pompanon et al. 2012).

As suggested by Whitaker (1988), traditional diet analyses based on identifying insect fragments in bats' feces provide two types of information: "percent frequency" (i.e., the proportion of fecal samples or fecal pellets containing a given prey item) and "percent volume" (i.e., the proportion of the fecal sample comprised of specific prey item or taxon). Although molecular techniques are much refined in identifying dietary diversity in bats, most published molecular assays to date provide information only on "percent frequency" (see Table 2 in Razgour et al. 2011). A major limitation in our current understanding of bat diets, especially for assessing possible impacts on insect populations, is quantifying the amount of each prey type being consumed by bats. To this end, the use of quantitative PCR (qPCR) has been used to estimate numbers of gene copies of sequences from targeted insect species in fecal samples from individual bats (McCracken et al. 2012). Calibrated as numbers of gene copies per mass unit of feces, this approach could provide information analogous to "percent volume" of a prey item. Although captive feeding studies to calibrate this approach indicate that there are many complications in relating gene copy numbers from qPCR to estimates of biomass or numbers of prey consumed (McCracken et al. 2012), with proper controls such an analysis may be feasible. The rapid development of deep-sequencing and high-throughput technologies also suggests that targeted prey soon will be quantifiable using tagged insect-specific primers.

24.2.2 The Energetics of Insectivorous Bats

The study of energetics provides another link connecting what a bat species eats to how many insects are eaten. The modern study of energetics in bats goes back to at least the 1940s (e.g., Hock 1951; Pearson 1947), and interest in the topic goes back considerably further (Hall 1832). Until recently, the vast majority of studies on the energetics of bats have relied on metabolic measurements done in artificial chambers (e.g., Dunbar and Tomasi 2006; Hock 1951; Kelm and von Helversen 2007; Thomas et al. 1990; Willis et al. 2005), which are difficult to extrapolate to free-living bats. More importantly, estimates of the cost of flight in insectivorous bats (e.g., Speakman and Racey 1991) are surprisingly rare and are likely to vary among species because of differences in morphology (Norberg and Rayner 1987), so fully parameterizing models estimating energy expenditure is presently difficult. However, several analytical techniques are becoming more readily available and cheaper and are therefore quickly increasing in use. For example, the doubly labeled water technique is gaining popularity as a way of estimating energy expenditure in free-ranging bats (Kurta et al. 1989; Voigt and Lewanzik 2011; Voigt et al. 2006), and heart rate monitors, commonly used to estimate the cost of flight in free-ranging birds (e.g., Sapir et al. 2010), are being applied to bats as well (Dechmann et al. 2011; Studier and Howell 1969). The data from such empirical studies can then be used to model the energetics of bats under natural, and varying, conditions (Boyles and Brack 2009; Boyles and McKechnie 2010; Boyles and Willis 2010; Federico et al. 2008a). With additional refinement, long-term, holistic estimates of energy expenditure in free-living bats may be possible. These types of studies will be vital in estimating the energy expenditure, and therefore the energy (and biomass) intake, of insectivorous bats.

Describing the energetic expenditure of free-ranging bats will be most useful in this context if those data are supplemented with information about the energy and mineral content available in individual prey insects (Encarnação and Dietz 2006). A few studies estimating the energetic and mineral content of insect prey were done decades ago (Keeler and Studier 1992; O'Farrell et al. 1971; Studier and Sevick 1992), but such studies are rare of late. Combining dietary composition estimates derived using molecular techniques with the energetic intake of free-ranging bats and the energetic content of prey should allow for more thorough and accurate estimates of species-specific biomass intake than have been possible in the past (e.g., Anthony and Kunz 1977).

24.2.3 Estimating Bat Populations

One of the major limiting factors in fully understanding the value of bats to agriculture is quantifying the number of bats in a region. Previous authors have noted both the difficulties in estimating bat populations and the outlook for future techniques that might be useful (O'Shea and Bogan 2003). Recent work has highlighted the problems with some historical population estimates (Betke et al. 2008; Meretsky et al. 2010), and new and creative ways of better estimating population sizes or trends are needed. The need for better monitoring methods is especially pressing in light of the massive population crashes associated with white-nose syndrome (Blehert et al. 2009; Frick et al. 2010; Turner et al. 2011) and the high levels of bat mortality observed at wind power facilities (Cryan and Barclay 2009; Kunz et al. 2007).

The ability to obtain genetic data on bats noninvasively from their fecal samples (Puechmaille and Petit 2007; Vege and McCracken 2001), coupled with statistical analyses based on observed genetic diversity, provides an opportunity to estimate population sizes (Leberg 2005; Luikart et al. 2010). A straightforward approach involves estimating the numbers of individuals in a specified area with data from multiple polymorphic microsatellite loci in which an individual is recognized based on its genetic fingerprint. By relating the number of distinct genotypes to the number of samples and using the asymptote of the curve, minimum population size can be estimated (Creel et al. 2003; Leberg 2005; Luikart et al. 2010). Puechmaille and Petit (2007) successfully applied this technique to estimate the numbers of bats in a colony while also providing perspective on the potential pitfalls and limitations of such analyses. Because bats are highly vagile and some species frequently move between numerous roost sites, estimates of the number of bats in a colony may have little to do with the actual numbers of bats on the landscape that are contributing to pest suppression. Nonetheless, it is easy to envision a situation where estimating the quantity of insects eaten by a given colony of bats could be a compelling message for bat conservation. Other approaches based on likelihood and coalescent methods have been developed and applied (Leberg 2005; Luikart et al. 2010) to provide estimates of effective population sizes (Russell et al. 2011). While these approaches have the potential for estimating total numbers of individuals of a given species on the landscape, there are significant limitations and caveats. First, effective population size differs and is typically some fraction of total population or census size (Leberg 2005; Luikart et al. 2010), and the conversion of census size to effective size depends on a suite of demographic and behavioral characteristics that differ among bat populations and species and are probably poorly known. Second, likelihood and coalescence methods provide historical signatures to population sizes and changes in population size. The molecules employed and their rate(s) of evolution determine the historical “depth” of the estimates obtained, and these estimates may have little to do with the sizes of populations that are presently on the landscape (Russell et al. 2011). Lastly, these estimates typically have confidence limits that span orders of magnitude (Luikart et al. 2010; Russell et al. 2011). In sum, a number of concerns exist around noninvasive genetic sampling techniques and population size estimation (Luikart et al. 2010; Pompanon et al. 2005; Waits and Paetkau 2005); however, as both the molecular techniques and models improve, these limitations will likely become less restrictive.

Bats themselves can add to the complexity of investigating their diet, their population size, and their energetic needs because species of economic importance could possibly be part of a larger species complex. In such cases, it would be easy to imagine a situation where morphologically similar species exhibit behavioral and/or ecological differentiation. This differentiation will be important when studying the suppression of crop pests by specific bat species, so understanding this variation by exploring the genealogical relationships (Avise 2000) between cryptic bat species will be an added critical contribution to our understanding of diets, energetics, and population sizes of cryptic species and the differences between them. Future studies on the intra- and interspecific relationships of insectivorous bats will thus be important as we attempt to distinguish suppression of insects by species of conservation concern from suppression by the bat community as a whole.

24.2.4 The Effects of Bats on Insect Populations

Bats and their insect prey represent a textbook example of a coevolutionary arms race that has evolved over millions of years (Conner and Corcoran 2012). Although work describing predator/prey interactions of bats and insects at the level of individuals is common (e.g., Conner and Corcoran 2012; Corcoran et al. 2009; Pavey et al. 2006), few studies have evaluated the population, community, and ecosystem ramifications of such interactions on the insect prey. Bats face very large energetic demands because of flight and must consume huge amounts of insect biomass each night (Kurta et al. 1989). This fact, coupled with historically large bat populations, leads to the understandable assumption by some that the collective impacts of bat communities on insect populations and community structure may therefore be substantial (Whitaker 1995; Whitaker and Clem 1992). However, there are reasons to believe that bats may not actually suppress insect populations. For example, many bats have broad diets suggesting they forage opportunistically on available prey and may therefore best be classified as “generalist predators” (Clare et al. 2009). As such, it may be that many insectivorous bats, as has been suggested for other generalist predators, do not exert sufficient predation pressure on any one insect species to substantially affect its population dynamics (Knipling 1979). Although this idea has gained traction in the biocontrol literature, an opposing school of thought suggests that generalist predators of insects can suppress prey populations if they can (1) persist on alternative prey when populations of any one insect species decline and (2) rapidly exploit populations of insects as those populations increase (Symondson et al. 2002). The longevity of bats (Wilkinson and South 2002) suggests they must be able to survive as insect populations wax and wane, and many studies have reported that bats often take advantage of transient insect populations (Belwood and Fenton 1976; Fenton and Morris 1976; Storm and Whitaker 2008). In fact, theoretical and empirical work suggests that other generalist predators can suppress insect populations (Riechert and Lockley 1984); therefore, bats deserve study in this area.

It is clear that bats eat substantial numbers of insects (Cleveland et al. 2006; Whitaker 1995), yet surprisingly few if any studies have directly quantified how bat predation affects crop pest populations in natural settings. Simply noting a prey species in the diet of a bat is not sufficient evidence that bats act as biological control agents on that specific pest. Most importantly, the short generation times and high reproductive rates of many insects suggest that adult survival may actually play only a small role in overall population growth. A good example of this disparity in life history characteristics between predator and prey involves predation of Brazilian free-tailed (*Tadarida brasiliensis*) bats on adult cotton bollworm moths *Helicoverpa zea* (Lepidoptera: Noctuidae). Importantly, it is the larval stage of corn earworms that damage crops and wildflowers (Cleveland et al. 2006), and even with predation of adult *H. zea* by bats, far more moth eggs are laid on each plant than can survive. In fact, mortality at the egg stage may account for the major limiting factor in population growth of cotton bollworms in some years (Sansone and Smith 2001). Still, predation by Brazilian free-tailed bats does appear to lessen the number of eggs that actually make it onto plants, which likely slows population growth rate enough to delay much of the damage to harvested crops (Cleveland et al. 2006). This predation on adult *H. zea* is especially important in years when the natural predators on *H. zea* eggs (mostly pirate bugs; *Orius* spp.) are rare.

Estimating the impacts of bat predation on insect populations may be further complicated by both spatial and temporal environmental variation leading to variation in population dynamics of the pest. For example, some pest species may be univoltine (one brood per year) at northern latitudes, but could have multiple broods per year at southern latitudes because of increases in temperature-dependent development rates and longer summers (Nielsen et al. 2008). Likewise, insect egg laying can occur at different times, even in agroecosystems located in close proximity (Sansone and Smith 2001) due to local differences in growing regimes (e.g. planting dates, crop varieties, irrigation, fertilization). Thus, not all predation by bats on crop pests is equal if bats are not eating the pest during the life stage that does damage to crops. For example, predation on adult moths late in the growing season is unlikely to have a strong effect on insect populations if the insects have already laid most or all of their eggs. Thus, estimates of the number of insect eggs that bat predation removes from an agroecosystem (Whitaker 1995) may be oversimplified if the temporal importance of predation events is not carefully considered.

Quantifying the effect of predation by bats (or any biocontrol agent) on a population of insects requires additional experimentation or a thorough understanding of the life history and demography of the pest in question (Stark and Banks 2003). Experimental assessments of the impacts of bats on pest populations may be possible with enclosures, but care must be taken in interpreting such results. For example, northern bats (*Myotis septentrionalis*) suppress mosquito populations in enclosures (Reiskind and Wund 2009), but there is little evidence that northern bats consume mosquitos in natural settings, and even when they do, mosquitos make up only a small percentage of northern bats' diets (Whitaker 2004).

Clear and consistent terminology is an important component of any advancing field of research. Because bats are unlikely to eliminate a crop pest, we suggest the appropriate terminology is that bats may *suppress* insect populations, and not *control* them. Put differently, use of the word "control" intimates that a specific pest should not exist where there is bat predation, while "suppress" more accurately suggests that bats can affect a pest population. Our goal as researchers should be never to mislead the public about the value of bats but instead to set realistic estimates of their economic worth to humans.

24.2.5 Holistic Evaluations of Crop Damage Suppression by Bats

Given the pressing conservation concerns surrounding bats (i.e., white-nose syndrome and high mortality at wind power facilities) and the high likelihood of drastic populations changes in the next few years (Frick et al. 2010), less time consuming and logistically more practical alternative means of assessing pest suppression by bats are needed. Because of the rapidly developing impact of the white-nose syndrome (WNS) epizootic, bat populations have already crashed in the northeastern and mid-Atlantic regions of the USA and Canada, and populations in the Midwest may experience the same fate in the near future. Thus, WNS is acting as a natural, albeit unfortunate, experiment on a scale we could never replicate. Direct comparisons of crop damage before and after population crashes might provide clues to the overall value of bats in pest suppression, but there is a vital need for pre-WNS baseline data. By the time this book reaches publication, it may be too late to collect such data in bat communities of the eastern USA, but an opportunity will likely still exist to do this type of work in the Midwest and western USA.

The most efficient way to address the value of bats to agriculture may be through experimental exclusion of bats in areas with healthy bat populations. Such exclusions have been done in a few forest and agroforest ecosystems (Böhm et al. 2011; Kalka et al. 2008; Morrison and Lindell 2012; Williams-Guillen et al. 2008), but no comparable studies are available from agricultural areas. Exclusion studies in agricultural regions will require researchers to overcome several logistical challenges. First, predation by bats and birds must be separated, likely through the use of exclusions that can be opened and closed at sunrise and sunset (Morrison and Lindell 2012). Second, the exclosures will have to be large enough to eliminate both direct and indirect effects of predation; i.e., echolocation pulses emitted by bats outside of the exclosures must not elicit antipredator responses of insects inside the exclosure. Determining the correct size of enclosures will take some trial and error depending on the echolocation frequencies and intensities of the bats that are being excluded. Third, terrestrial dispersal of crop pests (especially in the larval stages) will have to be controlled so that pest densities within the exclosures are not affected by immigration of larval stage pests from outside. Finally, well-known effects of exclosures, like birds using the frame as a perch and thus increasing predation on insects in the area, will have to be addressed. However, these problems should all be solvable with innovative experimental design. Further, crops which are planted with wide spacing (e.g., fruit trees) may lend themselves to exclusion more readily than field crops like corn, soybeans, or cotton.

Indirect evidence may also provide clues into the overall value of bats in agroecosystems without necessarily leading to an understanding of the process underlying the system. For example, data on pesticides purchased and applied have been collected by the United States Department of Agriculture (USDA). An increase in pesticides purchased after WNS affects an area might provide inferential evidence that bats were important in pest suppression in the area, but such correlative evidence will obviously be confounded by a variety of factors. Regardless, a comparison between pesticide use and bat population declines should be feasible when the pesticide use data for the years since the beginning of the WNS epizootic become available.

24.2.6 Can the Value of Bats in Agriculture Be Increased?

While the dynamics of such complex predator/prey systems might seem beyond our influence, there may be several opportunities to impact the behavior of bats or the dynamics of bat predation on crop pests so as to increase the strength of top–down suppression of insects by bats in agroecosystems. The most obvious prospect is to increase the density of bat populations by providing artificial roosting structures to replace those lost during the conversion of natural habitat into agricultural landscapes (Flaquer et al. 2006). There is evidence that providing such artificial structures may attract bats to agricultural areas (Long et al. 2006), but to our knowledge, there is only anecdotal evidence that such structures increase foraging in the agroecosystems where the structures are placed (Kiser and Kiser 2002). We might also be able to influence the foraging behavior of bats by playing echolocation calls in agroecosystems. Many bat species are attracted by calls of conspecifics, especially calls associated with feeding ("feeding buzzes"; Gillam 2007). Thus, broadcasting recorded or simulated feeding buzzes may increase the density of foraging bats in agroecosystems, which should increase predation pressure on crop pests in the area. Such approaches also might be expected to alter the reproduction and population densities of crop pests, but the only study on the subject failed to find such effects (Gillam et al. 2011). Finally, alterations of the environment may change the dynamics of the predator/prey system. For example, it is well known that lights attract insects, and bats take advantage of the artificially increased insect densities (Rydell 1992). Further, lights also are known to hamper the ability of many moths to evade bat predation (Svensson and Rydell 1998), and recently, portable lights were shown to increase the prevalence of eared moths in the diets of bats after only a few days at a location (C. Minnaar, University of Pretoria, South Africa, *pers. comm.*). Many crop pests happen to be eared moths (e.g., *H. zea*), so lights placed in agroecosystems, even temporarily, might increase the predation rates of bats on crop pests and therefore lessen insect herbivory on crops. Undoubtedly, this is an area of research that deserves more attention in the future.

Increasing the foraging of bats over agricultural areas seems like a positive outcome, but we must also recognize that it may have negative effects on the bats. Specifically, foraging over crops may lead to increased exposure to pesticides (O'Shea and Clark 2002), which have wide-ranging impacts on the health and fitness of insectivorous species (O'Shea and Johnston 2009). Although generally phased out of widespread use in many countries, organochlorine pesticides (like DDT) that accumulate in the tissues of animals are still commonly used in some countries and are known to have detrimental effects on the health of bats and bat populations (Clark 1988). Newer pesticides that are more widely used are generally thought to be less detrimental to bats because they do not tend to accumulate in body tissues, although studies to confirm this assumption are lacking (O'Shea and Johnston 2009). Future studies of bats in agroecosystems may provide unique opportunities to develop a better understanding of the health effects of pesticides on insectivorous bats.

24.2.7 The Value of Bats in Developing Countries

The potential value of insectivorous bats in developing countries is often overlooked. Bats may be especially valuable in suppressing crop pests in areas dominated by sustenance agriculture, as well as playing important roles in suppressing arthropod disease vectors. In many parts of Africa, for example, sustenance farmers do not have access to modern pesticides, so it is reasonable to predict that bats may act as a primary suppressor of crop pests. In other areas, the pesticides available may be older, highly toxic chemicals, and thus, the loss of pest suppression by bats might lead to increased use of pesticides with substantial secondary effects. Thus, the relative value of bats may be greater in developing countries than in more developed regions, because even though the economic value of crops produced in developing countries is considerably less, their marginal value as food can be enormously greater (http://www.data.worldbank.org/indicator/NV.AGR.TOTL.ZS). Unfortunately, we know of no research estimating the impact of bats on crop pest populations in developing regions [but see Williams-Guillen et al. (2008) for an example in an agroforestry ecosystem in Mexico]. Because the public perception of bats in developing regions is often even worse than in developed countries, and because the greatest bat diversity occurs in developing regions, information on pest control services of bats in these areas may do more for bat conservation than many of the more direct programs already implemented.

24.3 Conclusions

The conservation of bats has taken on a newfound importance in light of WNS and high mortality at wind power facilities. Changing the public perception of bats is one of the most important ways that researchers can contribute to bat conservation efforts (Fenton 1997), and one of the easiest ways to change those perceptions is to attach tangible values to the ecosystem services provided by bats. Surprisingly, insect suppression by bats—one of their most obvious benefits to humans—is severely understudied (Boyles et al. 2011; Cleveland et al. 2006; Federico et al. 2008b). We do not wish to suggest that this research will be quick or easy, but it is important to the advancement of bat conservation.

Acknowledgments We would like to thank Scott Pedersen and Rick Adams for inviting us to write this chapter. Any use of trade product or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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Chapter 25
Bat Conservation: Past, Present and Future

Paul A. Racey

Abstract The critical event by which bat conservation is progressed in a country is the establishment of a dedicated NGO. This allows long-term conservation projects to be carried out and provides a mechanism for recruiting and harnessing the enthusiasm of volunteers. Such organisations, whose aim is to conserve bats, have been established in most European countries, as well as in those comprising North, Central and South America; Australasia and parts of Asia. The rest of the world—most of Africa, parts of South and Southeast Asia, all of Central Asia and much of the Russian Federation—remains a conservation void so far as bats are concerned, although there are individuals working in isolation in some of the countries concerned. The opportunities that have facilitated the development of bat conservation organisations are reviewed in the hope that they may now be pursued in some of the countries where such conservation is non-existent.

25.1 Introduction

Although the discovery of bat echolocation in 1940 and the development of equipment to make bat sound audible to the human ear (Griffin 1941, 1958) opened up huge new opportunities for bat research, it was several decades before those opportunities began to be widely exploited on both sides of the Atlantic (Pye 1973; Fenton and Bell 1981). The 1930s saw the initiation of banding studies in Europe (Bels 1952) and the USA (Hitchcock and Reynolds 1942). As bat research gathered momentum, the first generation of graduate students produced their theses in the

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8_25,

1960s and the First International Bat Research Conference was held in September 1968 in Czechoslovakia but was poorly attended, since the Russians had invaded 10 days earlier. Nevertheless, it marked the beginning of regular international conferences which are held every 3 years. Triennial European Bat Research Symposia (EBRS) also began in Bonn, Germany, in 1981. Bat meetings began in North America in 1970 with the first Southwest Bat Research Symposium in Tucson, Arizona, followed in 1973 by annual North American Bat Research Symposia which are now organised by a registered not-for-profit organisation, the North American Bat Research Society.

Although presentations on bat conservation biology began to appear in the programmes of these scientific meetings, it was a further three decades before the first symposium on bat conservation featured on a conference programme, in Boston in 1995, convened by Dr. Elizabeth Pierson and the present author (Pierson and Racey 1998). This symposium marked the coming of age for bat conservation biology, and much evidence was presented of the threats to bat populations worldwide and what was being done to counter them. Since that time, bat conservation has made huge strides, with the growth of dedicated not-for-profit organisations whose work is underpinned by the work of bat conservation biologists. The aim of this chapter is to outline the history of bat conservation, to review its present state and to make suggestions for future priorities. It is a much expanded version of an earlier article (Racey 2011). It is concerned mainly with the organisation of, and support for, bat conservation. The need for it has been reviewed elsewhere (Jones et al. 2009; Racey and Entwistle 2003).

25.2 International Conservation

25.2.1 IUCN's Bat Specialist Group

In 1975, the Species Survival Commission of the International Union for the Conservation of Nature established a Chiroptera Specialist Group and RE Stebbings became its first chair. When I succeeded him a decade later, my brief was to produce a conservation action plan for Old World fruit bats. This was published in 1992 (Mickleburgh et al. 1992) and was a success in that all 20 of its major recommendations have been actioned, but unsuccessful in that the plight of fruit bats has worsened as hunting them for bushmeat has increased (Mickleburgh et al. 2009). Nevertheless, the plan stimulated a great deal of research as a result of which it now needs updating and is being revised by Tammy Mildenstein and CE Nuevo with the help of the fruit bat research and conservation community.

The Species Survival Commission then requested a 'quick and dirty' action plan for microchiropteran bats. The first plan had dealt with individual fruit bat species, but with so many more species to consider, this was no longer possible, and a different format was adopted. In 2001, Microchiropteran Bats: Global Status Survey and Conservation Action Plan was published containing, inter alia, 20 action plans for diverse and exemplary species (Hutson et al. 2001). When assessing applications for funding, conservation donors increasingly ask which action plan priorities a proposal addresses, and such plans continue to have real influence (Mickleburgh et al. 2002).

A few years ago, the renamed Bat Specialist Group retained its status following an IUCN review of specialist groups but was restructured. Rodrigo Medellin was appointed co-chair with responsibility for the New World, and I retained responsibility for the Old World.

25.2.2 Bat Conservation International

Bat Conservation International (BCI) was founded in 1981 by Merlin Tuttle. The February 1982 issue of the journal Oryx states that at the time, BCI was a group of the UK-based Fauna and Flora Preservation Society (FFPS), which had helped financially in its establishment (Anonymous 1982). The alliance between the two NGOs was short-lived however, mainly because FFPS insisted that BCI members subscribe to both organisations (to include the cost of receiving FFPS's journal Oryx).

One of the first successes of BCI was, together with other organisations and individuals, lobbying for the inclusion of Marianas flying fox *Pteropus mariannus* on Appendix I of the Convention on International Trade in Endangered Species (CITES) and other bats of the genus *Pteropus* on Appendix II. This eventually stopped the international trade of these bats into Guam where they were a prized food of the Chamorro people. BCI helped to protect one of the most important insectivorous bat roosts in Thailand—Khao Chang Pran cave—led the intervention that saved India's Samanar Hills bat caves and convinced the Israeli government to halt the poisoning of the country's bat caves. BCI's film 'The Secret World of Bats' has been screened in over 100 countries and its vampire control video provided the foundation for Latin American bat conservation efforts.

The centre of gravity of BCI's activities has however remained in the USA where it has mounted successful programmes in cave and mine conservation, bats in bridges, artificial roosts known as bat houses and, more recently, bats and wind energy and water for desert bats. It is involved in cave bat conservation in the Philippines and the conservation of migratory species in Latin America and the Caribbean. However, its most important international activities are its Global Grassroots grants programme and its scholarship programme, both of which have been hugely significant in capacity building in the developing world, where a BCI scholarship can be the main source of funding for a postgraduate degree.

25.2.3 The Lubee Bat Conservancy

The Lubee Foundation Inc. was established as a non-profit in 1989 by Luis F. Bacardi who built an imaginative and successful facility for Old World fruit bats at his private zoo in Gainesville, Florida. The Foundation welcomed visiting researchers who undertook projects on behaviour, nutrition, physiology and welfare, many of which would not have been possible in a conventional zoo and great advances were made. When the decision was made in 2004 to widen its remit to include all plant-visiting bats, it became a truly international organisation. Although the Lubee Foundation has always funded studies on Old World fruit bats in situ, the name 'Foundation' gave the misleading impression that it was primarily a donor organisation, so its name was also changed, to the Lubee Bat Conservancy.

25.3 National and Regional Conservation

25.3.1 United Kingdom

The UK provides a paradigm for national bat conservation. It began in 1975 when two endangered species were given legal protection. Six years later, that protection was extended to all UK bat species and their roosts by the Wildlife and Countryside Act 1981. The UK Mammal Society (with a mixed amateur and professional membership) had, since its foundation, always fostered special interest groups, including one for bats. Now other amateur bat groups became established around the UK, encouraged by AM Hutson, PH Richardson, RE Stebbings and others. The Wildlife and Countryside Act had placed extra burdens on the UK's statutory nature conservation organisation (SNCO)—the Nature Conservancy Council—but no extra resources, and the Council was glad to make use of the enthusiasm and dedication of the new bat groups for visits to householders concerned about resident bats.

In 1984, the oldest conservation organisation in the world, established in 1903 as the Society for the Preservation of the Wild Fauna of the Empire, then known as the Fauna and Flora Preservation Society (FFPS: now Fauna and Flora International or FFI), established 'The Bat Project' with AM Hutson as its first staff member and with a brief to support amateur bat groups. That led to the establishment in 1992 of a fully constituted NGO (non-government organisation or not-for-profit), the Bat Conservation Trust (BCT), which 20 years on has 36 staff supporting over 5,000 members and 100 local groups. An important step function is achieved by the formation of such an NGO, because long-term conservation projects can be undertaken, often by harnessing the enthusiasm and commitment of volunteers. The best UK example is the National Bat Monitoring Project, established in 1996, which detects trends in bat populations mainly by bat detector surveys and, in doing so, creates opportunities for training and outreach. Training workshops are held during Annual Bat Workers Conferences and the best examples of outreach are the hugely popular evening bat walks, often involving holidaymakers eager to extend their days into the evening flight period of bats. The BCT advises the British government on bat population trends, and that in turn is reported to the EU, and perhaps more importantly, it allows the Trust to lever funds to redress downward trends.

The BCT has also helped to establish case law. There is little point in legal protection unless offenders are prosecuted and that has happened in the UK, with the result that the building industry and planning authorities are now fully aware of the needs of bats.

25.3.2 Mainland Europe

In 1985, at the first Conference of Parties to the Convention of Migratory Species of Wild Animals (CMS or the Bonn Convention), John Burton, Executive Secretary of FFPS, who had the previous year established the UK Bat Project, produced an outline concept for a European Bats Agreement. The subsequent publication of The Conservation of European Bats (Stebbings 1988) during the European Year of the Environment underlined the need for such an agreement by showing that although bats were legally protected in most European countries, this meant little because those who persecuted them were not prosecuted. Burton's suggestion eventually led, in 1994, to an intergovernmental Agreement on the Conservation of Populations of European Bats or Eurobats (Hutson 2006). Thirty-three states have now signed the Agreement, of a possible 48 in Greater Europe, with responsibility for 45 bat species. Each year an advisory committee consisting of representatives of range state governments, their SNCOs, NGOs and observers meet to discuss current bat conservation issues and agree resolutions which are considered by the signatory governments when they meet every 4 years. Eurobats is now part of the United Nations Environment Programme, with its headquarters in the UN building in Bonn, Germany. At the first intergovernmental meeting in 1995, an action plan was agreed, the most important aspect of which was the call for the protection of bat foraging sites. Eurobats has established a number of working groups on underground sites, surveillance and monitoring, wind farms and lethal fungal infections, the reports and recommendations of which are freely available. One of the most encouraging features of Eurobats is that an increasing number of range states have NGOs dedicated to bat conservation or have bat conservation as part of their remit. A series of annual European Bat Nights have been successful in raising awareness, and in 2011 Eurobats launched 'Year of the Bat', which became worldwide in 2012 and has been met with similar enthusiasm and engagement.

The European Union's Directive on the Conservation of natural habitats and of wild fauna and flora (the Habitats Directive) was adopted in 1992 as an EU response to the Berne Convention. It aims to protect some 220 habitats and approximately 1,000 species listed in the Directive's Annexes, including 14 bat species. The Directive led to the establishment of Special Areas of Conservation, which together with the existing Special Protection Areas form a network of protected sites across the European Union referred to as Natura 2000. Article 17 of the Directive requires EU member states to report on the state of their protected areas and species every 6 years. The first complete set of country data was provided in 2007. This process, in which named bat species and sites are protected, marks an important step change in European bat conservation.

The latest European initiative is BatLife Europe which has established an alliance of bat NGOs on the BirdLife model so that the whole will be greater than the sum of the parts. This was first proposed at the EBRS in Le Havre, France, in 2002, and BatLife Europe was finally launched as a free-standing NGO during the EBRS meeting in Vilnius, Lithuania, in 2011, when trustees were appointed and member NGOs and similar organisations enrolled.

25.3.3 United States of America

Although the USA has the same number of bat species as Europe (45), legislation aimed at conserving them is very different. Nine taxa are included in the Endangered Species Act which gives them and their roosts very strong protection. Six of these taxa are on continental USA—four species and a fifth consisting of two subspecies. One is endemic to Hawaii and two to the Marianas islands (although one of these is thought to be extinct). Most of the bat species on continental USA which are listed in the Endangered Species Act roost below ground, so cave and mine management are major issues.

Individual states have their own lists of endangered and threatened species as well as species of special conservation concern. Agencies such as the Forest Service and the Bureau of Land Management also have their own conservation designations for bat species. Although individuals who harm bats considered by the state to be endangered can be prosecuted, the principal role of these designations is to prioritise conservation action, especially mitigation.

In addition to BCI, a grassroots conservation movement has also evolved in the USA consisting of bat working groups—Western, Midwestern and North-East, together with their equivalent in the South-East—the Bat Biodiversity Network. The scale is vast—the Western Bat Working Group encompasses 13 western states, northern Mexico as well as Alberta and British Columbia, but it functions by means of conference calls and meetings of groups and between groups. These working groups influence bat conservation by setting priorities and communicating these to the relevant agencies. The Western Bat Working Group has been particularly involved in the effects of wind energy facilities on bats and the two eastern groups maintain a bat capture data base.

25.3.4 Latin America

The Program for the Conservation of Mexican Bats (PCMM: Programa para la Conservacion de los Murcielagos de Mexico) was established in 1994 to study bats migrating between the USA and Mexico and the ecological services they perform.

At the 14th International Bat Research Conference in Merida, Mexico, in 2007, PCMM launched the Latin American Network for Bat Conservation (RELCOM: Red Latinoamericana para la conservacion de los Murcielagos). At a meeting in 2009 in Costa Rica, RELCOM formulated its strategy and extended its remit to the Caribbean. It is an alliance of NGOs or similar organisations in its 11 member countries, and occasionally individuals, to effect bat conservation. In this respect, it is similar to Eurobats (but without the authority of an intergovernmental agreement) and is growing in influence and effectiveness.

25.3.5 Africa

Africa is largely a void so far as bat conservation is concerned and that is a matter of great concern in view of the large-scale human consumption of fruit bats, mainly in West and Central Africa (Mickleburgh et al. 2009), and their role as vectors of Ebola haemorrhagic fever also in Central Africa (Leroy et al. 2009). In East Africa, caves used as bat roosts are also under threat, mainly from loss of surrounding forests (Metcalfe et al. 2010). There are two bat conservation groups operating in the northern part of South Africa—the Gauteng and Northern Region and the Kwazulu-Natal Bat Interest Groups. Individuals are working on the ecology and conservation biology of bats in several African countries—in Benin, Burkina Faso, Cameroon, the Democratic Republic of Congo, Ghana, Ivory Coast, Kenya, Nigeria, Senegal, South Africa, Swaziland, Tanzania and Uganda—and African Bat Conservation News, a web-based journal produced in South Africa, provides a vehicle for publishing their results.

BCI is presently addressing the lack of bat conservation organisations in mainland Africa, the first step being a capacity building workshop for bat workers from sub-Saharan Africa to be held in Kenya in 2013.

The only African NGOs working for bat conservation are on the islands of the Western Indian Ocean—the Comores, Madagascar, Mauritius and Pemba (Tanzania). In the latter, the work of the government Department of Commercial Crops, Fruit and Forestry, with the support of FFI, has resulted in the quadrupling of numbers of the critically endangered *Pteropus voeltzkowi* to about 20,000 individuals (Robinson et al. 2010) and it is now regarded as vulnerable. The work of the Mauritian Wildlife Foundation with its partner institutions has resulted in recovery of the population of *Pteropus rodricensis*, also originally critically endangered, from very few individuals in the 1970s to 10,000 today (Cheke and Hume 2008). Action Comores was established to do the same for *Pteropus livingstonii*, the most endangered of the Western Indian Ocean *Pteropus*, and was initially successful when regular expeditions from the UK and the USA visited the Comores to provide training in monitoring and to initiate education programmes (Jones et al. 2009). Western influence and financial support has now waned and bat conservation has lost momentum. In Madagascar, successful capacity building projects run by the University of Aberdeen and funded by the Darwin Initiative led to the creation of Madagasikara Voakajy, which is involved in the conservation biology of bats (Racey et al. 2009) and other vertebrates and in establishing protected areas. Another Malagasy association, Vahatra, was established by Steven Goodman in 2007 and evolved from the Ecological Training Program established by WWF in 1991. It carries out research on Malagasy vertebrates including bats with a strong emphasis on taxonomy but including ecology and conservation.

25.3.6 Asia

In 1999, after a Conservation and Management Planning (CAMP) workshop revealed the need, Sally Walker established the Chiroptera Conservation and Information Network for South Asia (CCINSA) as one of the activities of the Zoo Outreach Organisation (ZOO) which she founded. This provides a mechanism for bat workers (from researchers to educationalists) to establish contact and seek advice about planned projects and funding mechanisms. It also organises workshops in the countries of South Asia on how to study bats. As a teacher on most of these workshops, I have often found it difficult to measure success. Nepal in 2007 was an exception. The country had just emerged from a Maoist insurgency during which travel was very difficult, but there was so much pent-up energy and enthusiasm from the participants that within a short while, they had established two organisations involved in bat conservation—Small Mammal Conservation and Research Foundation (2009) and Natural Resources Research and Conservation Centre (2010) in addition to the already existing Bat Friends. One of the results of this activity is the publication of Bats of Nepal (Acharya et al. 2010).

PteroCount was established in 2005 by Sanjay Molur, current executive director of ZOO, and Shahroukh Mistry, as part of a citizen science programme. It has recruited 80 such citizens to monitor *Pteropus* colonies throughout South Asia. In 2002, Tammy Mildenstein and Sam Stier established Bat Count Philippines, focussed initially and successfully on an endangered population of *Acerodon jubatus* which co-roosted with the more abundant *Pteropus vampyrus* on Luzon Island (Jones et al. 2009). However, this is another example of a project losing its momentum after western influence and funding wanes. This reflects the need for capacity building, which has been addressed, particularly in the area of taxonomy and systematics, by the UK's Harrison Institute in Thailand and Myanmar. From the recently established Natural History Museum in Prince of Songkla University, Hat Yai, Southern Thailand, it is now extending this work to graduate students from Cambodia, Laos PDR and Vietnam.

The need for capacity building was also revealed at the first Southeast Asian Bat Research Conference in 2007 when Tigga Kingston established the Southeast Asian Bat Conservation Research Unit (SEABCRU) which awards grants and training fellowships. This activity is now being strengthened and consolidated with the aid of a 5-year National Science Foundation grant to establish a Research Coordination Network (RCN) to promote international communication and stimulate collaboration. There are four main subject areas: taxonomy and systematics, response of forest-dependent bats to landscape change, cave bat diversity and conservation and flying fox distribution and population ecology (Kingston 2010).

These two initiatives—of the Harrison Institute and SEABCRU—will be sustained because their leaders are permanently employed and are not dependent on 'soft' money for their own salaries, so they can provide continuity of leadership and fund raising. Lastly, there is a volunteer group about 500 strong in Japan, the Bat Study and Conservation Group (Koumori-No Kai) which holds an annual bat festival in different parts of the country.

25.3.7 Australasia

The Australasian Bat Society has about 300 members and organises regular conferences, prepares position statements about conservation issues, trains members in field work and liaises with government. Australian bat workers have done much to attempt to solve the problems, such as defoliation of trees, caused by colonies of flying foxes moving into cities, partly as a result of harassment in orchards, loss of natural habitat and as they search for food in times of drought. Australians have also led the way in mitigating conflicts between commercial fruit growers and fruit bats, mainly by the use of protective netting and the development of sound systems as deterrents.

A long and exhausting battle in the 1980s raged to protect ghost bats, *Macroderma gigas*, and little bent-wing bats, *Miniopterus australis*, from losing their cave roosts to a cement company, but was resolved after many years by creating a reserve, and now the cement company is one of the protectors.

The Ku-ring-gai Bat Colony Committee was established in 1985 to protect the flying foxes roosting in the Sydney suburb of Gordon. It became incorporated in 1998 and changed its name to the Ku-ring-gai Bat Conservation Society and now has a wider remit.

25.4 The Conservation Void

This brief review of global bat conservation has revealed its strengths in Europe and North America and the fact that it is growing in strength in Central and South America and parts of Asia and in Australasia. But half the world remains a conservation void so far as bats are concerned: most of Africa, all of the Middle East, much of the Russian Federation and all of the former Russian republics, together with most of Asia including China, Mongolia and Tibet.

So what can be done to resolve this? There is clearly a pressing need for capacity building, not only of individuals, but also of institutions and particularly NGOs. Some of the countries concerned have wildlife NGOs which could be encouraged to include bats in their remit, as has happened in Europe. Some have governments which discourage or prohibit the formation of NGOs which they regard as opening the door to western influence. In such cases, the focus must be on building the capacity of individuals, often within the university system. This has happened successfully in Myanmar, for example, as a result of the activities of the Harrison Institute, in collaboration with the universities of Yangon and Mandalay (Bates et al. 2000).

25.5 Funding Opportunities

The Conservation Leadership Programme (CLP) evolved from the BP Conservation Awards which in turn grew out of the British universities' tradition of student expeditions during the long summer vacation, funding for which was often provided by BP. The company enlisted the help of two British-based NGOs, FFI and BirdLife to administer the awards. When it took over the American company Amoco in 2000, and acquired greater than 50 % American assets, BP globalised the scheme and recruited the American-based NGOs Conservation International and the Wildlife Conservation Society to join the consortium which administers the awards. In its various forms, this very successful programme has funded many projects on bats. Each year, one representative from each winning team is sponsored to attend a training course.

The UK's Tropical Biology Association (TBA) organises month-long courses in Tanzania, Uganda and Madagascar for an equal mix of first-world students who pay and developing-world students for whom funding is provided. In addition, separate courses in grant and paper writing are held. It is also associated with an annual Student Conservation Conference in Cambridge, UK, where the TBA is based, and sister conferences at the American Museum of Natural History in New York and in Bangalore, India. Students now compete for month-long internships following the Cambridge conference. An important feature of both the CLP and the TBA is their alumnus schemes which maintain contact with their graduates and the CLP also provides a year-long subscription to FFI's journal Oryx.

Applicants for Rufford Small Grants can now receive three successive grants—enough for a Ph.D. in the developing world—and projects on bats are often funded. BCI scholarships, mentioned earlier, often provide crucial funding for master's and doctoral students in the developing world. The Russell E Train Education for Nature program, administered by the Worldwide Fund for Nature, provides fellowships for applicants from Africa, Asia and Latin America.

The prestigious Whitley awards are for established academics and conservationists and have helped three bat conservationists—Daniela Hamidovic (Croatia), Rodrigo Medellin (Mexico) and Igor Prokofiev (Russian Federation) to develop NGOs. A particularly relevant initiative launched in 2010 is the master's course in Conservation Leadership in Cambridge UK which aims specifically to develop the leadership qualities required by directors of NGOs. In Europe, several NGOs have received substantial funding from the EU LIFE Programme for major bat conservation projects. The European Projects Initiative of Eurobats also funds applicants from the 68 range states of those bat species occurring in Europe, which include the countries of North Africa and the Middle East.

Originally, tenured UK academics and staff of UK NGOs could apply to the Darwin Initiative, which was the British government's response to the United Nations Conference on Environment and Development in Rio in 1992 and in particular the Convention on Biological Diversity. It aimed to assist those countries rich in biodiversity and poor in resources to preserve their biodiversity by the transfer of British postdoctoral expertise. It has supported Aberdeen University projects in Madagascar which led to the establishment of the NGO Madagasikara Voakajy, the Harrison Institute's capacity building in bat taxonomy and systematics in SE Asia and Bristol University's establishment of bat conservation in southern China. The Darwin Initiative has evolved over the last 20 years and is now open to applicants worldwide and seeks to combine poverty alleviation and livelihoods with the preservation of biodiversity.

Apart from BCI's Global Grassroot's programme, there appear to be few funding opportunities in the USA which could lead to the establishment of conservation NGOs for bats, although the NSF's RCN programme has spin-off potential in this respect.

25.6 Limiting Factors

A significant limiting factor in building the capacity of bat conservationists is access to relevant literature. The Internet, and broadband connections in particular, have expanded more rapidly in some parts of the developing world than in the developed world so that publications can be made available. The problem remains with books particularly 'starting point' literature such as the review chapters in Bat Ecology (Kunz and Fenton 2003) and 'how to go about it' literature like Ecological and Behavioural Methods for the Study of Bats (Kunz and Parsons 2009). Such books are not easily accessible where they are most needed in the developing world and in countries of the bat conservation void. What is required to change this is the equivalent of the 'Gratis Books Scheme' operated in the UK by the British Ecological Society and the Natural History Books Service and distributes titles in the Trends in Ecology and Conservation series to graduates who apply. This scheme was the brainchild of William Sutherland, now at Cambridge University, and 3,000 copies of his Conservation Handbook have so far been distributed. Learned societies, NGOs and book distributors in America could adapt this scheme to ensure that key volumes on bat biology, produced in that country, reach the developing world.

Another limiting factor is the difficulty of funding appropriately qualified and experienced (often postdoctoral) conservationists to build the capacity of local NGOs, together with the challenge of funding core NGO expenditure, as opposed to discrete projects.

Conservation biology as a discipline now has a number of prestigious journals and a highly regarded academic society but may not yet be regarded as a mainline career-building pursuit in some institutions. Indeed, many senior academics in bat biology have never embraced it or have done so only after their careers have peaked.

There is also a mismatch between academic and practical conservation or between science and practice, which is reflected in the relevant literature. In 2009, at the Society for Conservation Biology conference in Beijing, a workshop debated this issue and results were presented of a survey of all species-based research papers published in five major conservation journals. Only half presented evidence of implementation of the research findings (Millner-Gulland et al. 2010). Articles in all these journals, except Oryx, were focussed mainly on conservation issues in developed countries. Only 37 % of articles on developing country issues had a corresponding author resident in these countries, compared to 88 % in developed countries.

There is also a need for more evidence-based conservation. Some excellent examples exist. When caves are gated, the number of bats occupying them increases (Pierson 1998). Self-sustaining populations of brown long-eared bats *Plecotus auritus* become established in coniferous monocultures when the forest is well supplied with bat houses (Boyd and Stebbings 1989), and bat houses painted with heat-absorbing black paint attract more bats than those painted with reflective white (Kerth et al. 2001).

Engagement between bat conservationists and politicians has been fruitful in Europe, leading to the total protection of UK bats afforded by the Wildlife and Countryside Act and to the intergovernmental Eurobats Agreement (Hutson 2006). More recently in the USA, bat biologists and conservationists succeeded in securing a congressional hearing on white-nose syndrome, a fungal disease which is decimating bat populations. However, despite repeated attempts, the international conservation community has failed to persuade the Indian government to delist 10 of the 13 species of fruit bats as vermin on the grounds that they are forest bats and there is no evidence that they feed on orchard fruit (Singaravelan et al. 2009). Letters to Indian government ministers, irrespective of the status of the signatory, received no acknowledgement.

25.7 Indicators of Success

How will we know that progress is being made in capacity building? When more first-world biologists invest in building the capacity of bat conservationists in the developing world, when more developing country conservation biologists apply for their own grants and write their own papers, when nationals of developing countries become directors of bat conservation NGOs and when dichotomous taxonomic keys become available for the bats of developing countries (e.g. Srinivasulu et al. 2010). The lack of such keys is a barrier to progress in survey and monitoring. Such free-standing keys did not make their appearance in the UK until the 1980s

(Stebbings 1986; Yalden 1985) and made a significant difference to the assurance with which amateur bat workers became acquainted with their local fauna and could construct distribution maps.

Changes in public attitude towards bats are another indicator of success and are sometimes very tangible. When I began work on bats in the UK in the 1960s, I received frequent telephone calls saying ‘I have bats in my house and I want to get rid of them’. Decades later, that has changed to ‘I have bats in my house. I know they are protected and I don’t want to harm them but I am concerned….’

25.8 Indicators of Failure

The absence of in-country scientists among successive outputs of western research groups working in the developing world suggests a lack of appropriate capacity building. Thirty percent of articles on conservation issues in developing countries in the survey mentioned earlier had no national from the country in question in the author list (Millner-Gulland et al. 2010).

Twenty years after the Convention on Biological Diversity came into force and made it clear that biodiversity belongs to the country in which it occurs, unauthorised specimen collecting still occurs, with the Czech Republic being a major culprit, shamelessly publicising the fact in journal papers reporting the results of surveys and unacknowledged collection of museum specimens (Benda et al. 2006). This contravenes the guidelines of more reputable museums and also smacks of scientific neocolonialism. It is why some countries such as Nepal and Bhutan, which are desperately in need of taxonomic expertise to identify their bat faunas, continue to resist the export of specimens. Others, such as Madagascar, will allow such export for study on condition that the material is returned. During Gerald Durrell’s successful initiatives in breeding endangered tropical species in captivity, he always made it clear that the species concerned belonged to the country of origin which could ask for its repatriation at any time.

25.9 Education and Outreach

Education at all levels is fundamental to achieving the goals of bat conservation, and the NGOs concerned have established education programmes early in their history, including materials for schoolteachers, newsletters for younger members (such as BCT’s The Young Batworker) and bat camps. In the UK, these have developed into special membership schemes for teachers and youth leaders accompanied by education packs and a Young Batworkers Club.

For many years, BCI hosted an annual workshop for schoolteachers after the North American Symposium for Bat Research. It now reaches thousands of schoolchildren through Internet-based distance learning in which BCI educators with live bats and displays run two-way video presentations for multiple classrooms around the USA. A particularly successful initiative was the ‘Bat Trunk’—a box of educational resource materials produced initially by BCI for US schools and then for Latin American ones. The zoo-based Bat Taxon Advisory Group then distributed bat trunks more widely, so they reached Zambia, the Mascarenes and Madagascar (O’Connor et al. 2006). In the USA, BCI still provides travelling Bat Trunks, which now include PowerPoint presentations. BCI has also trained docents working on summer weekends at the Congress Avenue Bridge in Austin, Texas, where the emergence of 1.5 m Mexican free-tailed bats is a popular tourist attraction. In the UK, evening bat walks with bat detectors are popular and European Bat Night attracts increasing numbers of enthusiasts.

In some developing countries, such as Nepal, access to local radio is readily available and this medium has been widely used for educational programmes about bats. Access to schools is also particularly easy in some developing countries, and children experiencing programmes about bat conservation in their schools may then influence the attitudes of their parents. The extent to which this occurs is being investigated with respect to bushmeat consumption in Madagascar.

25.10 The Future

The priority for global bat conservation must be filling the conservation void and establishing it in those countries where it is non-existent, initially by building the capacity of individuals and then by encouraging the formation of NGOs The importance of the latter in establishing long-term conservation projects, often by harnessing the enthusiasm of volunteers, and in education and outreach has been demonstrated repeatedly in many parts of the world. The growth in citizen science also represents an excellent opportunity for bat conservation, particularly with respect to population monitoring using bat detectors.

Great advances have been made in helping members of the public to appreciate the value of bats, not only as major contributors to their countries’ mammalian biodiversity, but also in the ecological services they perform. Much remains to be done in this respect, particularly in those countries in which bats are hunted for food, or their guano mined without regard for the animals that produced it, or where they are implicated in zoonoses. The fact that bat conservation continues to gather momentum globally and has recently been initiated in countries in which it was absent provides grounds for cautious optimism that the global decline in bat populations can be halted.

Acknowledgements This chapter is based on an invited keynote lecture at the 15th International Bat Research Conference in Prague in August 2010, and I would like to thank the organisers for that opportunity. I would also like to thank Luis Aguirre, Pat Brown, Jakob Fahr, Martin Fisher, Suren Gazaryan, Christine Harbusch, Tony Hutson, Richard Jenkins, Dave Johnston, Tom Kunz, Bob Locke, Wanda Markotter, Angie McIntyre, Simon Mickleburgh, Dixie Pierson, Greg Richards, Shirley Thompson and Allyson Walsh for their assistance in its preparation and to Julia Hanmer, Richard Jenkins and Merlin Tuttle for their comments on an earlier draft. The views expressed remain entirely my own.

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R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*,
DOI 10.1007/978-1-4614-7397-8,

Keyword Index

R.A. Adams and S.C. Pedersen (eds.), *Bat Evolution, Ecology, and Conservation*, DOI 10.1007/978-1-4614-7397-8, © Springer Science+Business Media New York 2013