Rick A. Adams • Scott C. Pedersen
Editors

# Bat Evolution, Ecology, and Conservation

Springer

*Editors*
Rick A. Adams
School of Biological Sciences
University of Northern Colorado
Greeley, CO, USA

Scott C. Pedersen
Department of Biology and Microbiology
South Dakota State University
Brookings, SD, USA

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



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

*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*

# 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

L.N. Cooper (✉) • K.E. Sears
Department of Animal Biology and Institute for Genomic Biology,
University of Illinois, Chicago, IL 60607, USA
e-mail: l.noelle.cooper@gmail.com; ksears@life.illinois.edu

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

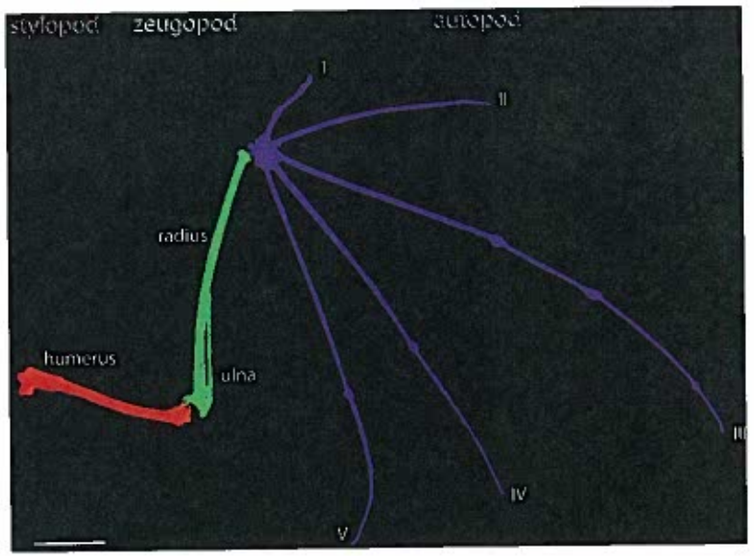


**Fig. 1.1** Bones of the forelimb of bats. Scale bar is 1 cm in length

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

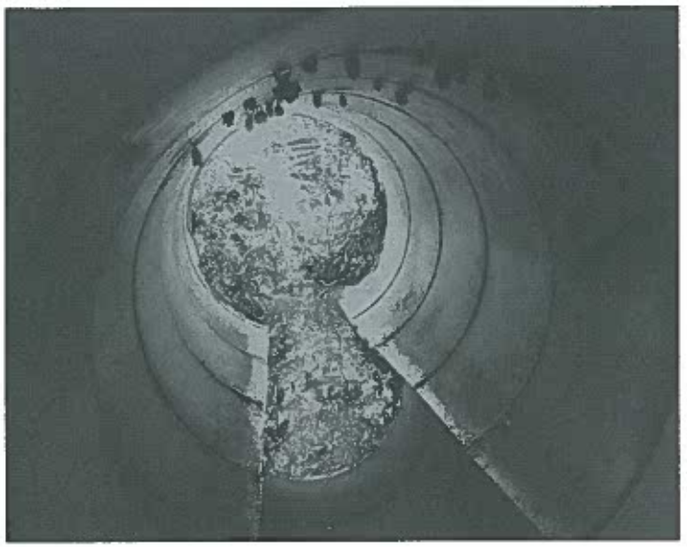

**Fig. 1.2** Bats hanging from the ceiling of a culvert. Photo credit to Merla Hübler

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

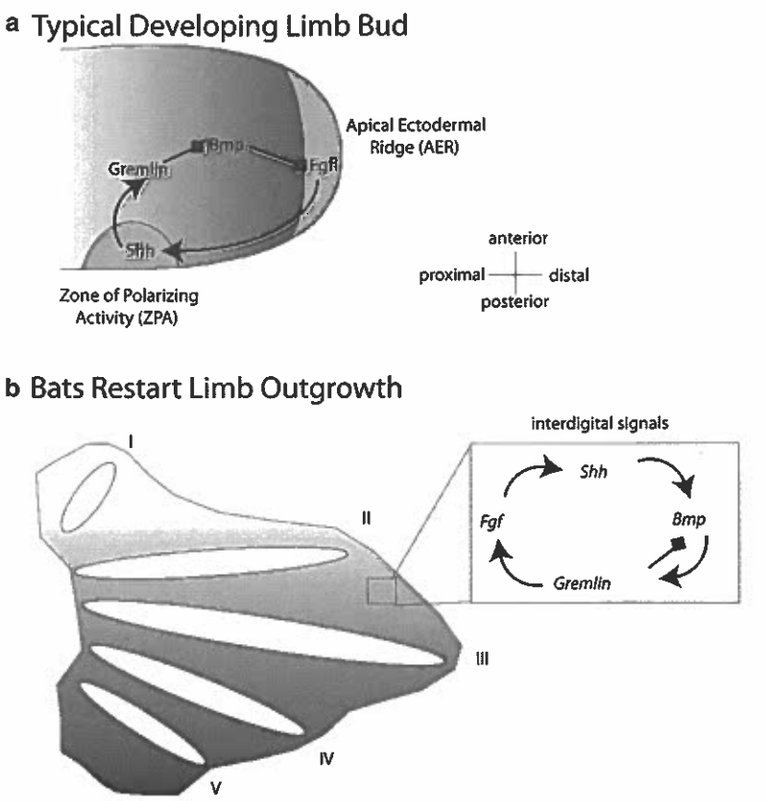


**Fig. 1.3** Schematics of the developing limb of a generalized vertebrate (**a**) and a fetal bat (**b**)

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., proximo-distal, 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

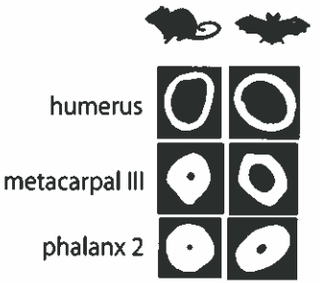


**Fig. 1.4** Results of high resolution micro-CT scans of some forelimb bones of adult bats and mice. Not to scale. Bones sized to show relative dimensions of medullary cavities

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*.

## References

Abbasi AA (2011) Evolution of vertebrate appendicular structures: Insight from genetic and palaeontological data. Dev Dyn 240(5):1005–1016. doi:10.1002/dvdy.22572

Adams RA (1992) Comparative skeletogenesis of the forearm of the little brown bat (*Myotis lucifugus*) and the norway rat (*Rattus norvegicus*). J Morphol 214(3):251–260. doi:10.1002/jmor.1052140302

Adams RA (2008) Morphogenesis in bat wings: linking development, evolution and ecology. Cells Tissues Organs 187(1):13–23

Arita HT, Fenton MB (1997) Flight and echolocation in the ecology and evolution of bats. Trends Ecol Evol 12:53–58

Behringer RR, Eakin GS, Renfree MB (2005) Mammalian diversity: gametes, embryos and reproduction. Reproduction, Fertility and Development 18(2):99–107. doi:10.1071/RD05137

Behringer RR, Rasweiler JJ, Chen C-H, Cretekos CJ (2009) Genetic regulation of mammalian diversity. Cold Spring Harb Symp Quant Biol 74:297–302. doi:10.1101/sqb.2009.74.035

Boulet AM, Capecchi MR (2004) Multiple roles of *Hoxa11* and *Hoxd11* in the formation of the mammalian forelimb zeugopod. Development 131:299–309

Carroll SB (2008) Evo-Devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134(1):25–36

Chen Y, Zhao X (1998) Shaping limbs by apoptosis. J Exp Zool 282:691–702

Chen C-H, Cretekos CJ, Rasweiler JJ, Behringer RR (2005a) Hoxd13 expression in the developing limbs of the short-tailed fruit bat, Carollia perspicillata. Evol Dev 7(2):130–141. doi:10.1111/j.1525-142X.2005.05015.x

Chen CH, Cretekos CJ, Rasweiler JJ, Behringer RR (2005b) Hoxd13 expression in the developing limbs of the short-tailed fruit bat, Carollia perspicillata. Evol Dev 7(2):130–141

Cohn MJ, Tickle C (1999) Developmental basis of limblessness and axial patterning in snakes. Nature 399(6735):474–479

Cooper KL, Tabin CJ (2009) Developmental patterning of the limb skeleton. In: Pourquie O (ed) The skeletal system. Cold Spring Harbor Laboratory Press, New York, NY, pp 19–39

Cooper KL, Hu JK-H, ten Berge D, Fernandez-Teran M, Ros MA, Tabin CJ (2011a) Initiation of proximal-distal patterning in the vertebrate limb by signals and growth. Science 332(6033):1083–1086. doi:10.1126/science.1199499

Cooper LN, Armfield BA, Thewissen JGM (2011b) Evolution of the apical ectoderm in the developing vertebrate limb. In: Hallgrimsson B, Hall BK (eds) Epigenetics: linking genotype and phenotype in development and evolution. University of California Press, Berkeley, p 472

Cretekos CJ, Rasweiler JJ, Behringer RR RR (2001) Comparative studies on limb morphogenesis in mice and bats: a functional genetic approach towards a molecular understanding of diversity in organ formation. Reprod Fertil Dev 13(8):691–695

Cretekos CJ, Weatherbee SD, Chen C-H, Badwaik NK, Niswander L, Behringer R, Rasweiler JJ (2005a) Embryonic staging system for the short-tailed fruit bat, *Carollia perspicillata*, a model organism for the mammalian order *Chiroptera*, based upon timed pregnancies in captive-bred animals. Dev Dyn 233(3):721–738



Cretekos CJ, Weatherbee SD, Chen CH, Badwaik NK, Niswander L, Behringer RR, Rasweiler JJ (2005b) Embryonic staging system for the short-tailed fruit bat, Carollia perspicillata, a model organism for the mammalian order Chiroptera, based upon timed pregnancies in captive-bred animals. Dev Dyn 233:721–738

Cretekos CJ, Deng J-M, Green ED, Rasweiler JJ, Behringer RR (2007a) Isolation, genomic structure and developmental expression of Fgf8 in the short-tailed fruit bat, *Carollia perspicillata*. The International Journal Of Developmental Biology 51(4):333–338

Cretekos CJ, Deng JM, Green ED, Rasweiler JJ, Behringer RR (2007b) Isolation, genomic structure and developmental expression of Fgf8 in the short-tailed fruit bat, Carollia perspicillata. Int J Dev Biol 51(4):333–338

Cretekos C, Wang Y, Green E, Martin J, Rasweller J, Behringer R (2008a) Regulatory divergence modifies limb length between mammals. Genes Dev 22(2):141–151

Cretekos CJ, Wang Y, Green ED, Program NCS, Martin JF, Rasweiler JJ, Behringer RR (2008b) Regulatory divergence modifies limb length between mammals. Genes Dev 22(2):141–151. doi:10.1101/gad.1620408

Crossley PH, Minowada G, MacArthur CA, Martin GR (1996) Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell 84(1):127–136

Dahn R, Fallon J (2000) Interdigital regulation of digit identity and homeotic transformation by modulated BMP signaling. Science 289:438–441

Davis CA, Holymard DP, Millen KJ, Joyner AL (1991) Examining pattern formation in the mouse, chicken and frog embryos with an *En*-specific antiserum. Development 111:287–298

Davis MC, Dahn RD, Shubin NH (2007) An autopodial-like pattern of Hox expression in the fins of a basal actinopterygian fish. Nature 447(7143):473–476, http://www.nature.com/nature/journal/v447/n7143/suppinfo/nature05838_S1.html

de Crombrugghe B, Akiyama H (2009) Transcriptional control of chondrocyte differentiation. In: Pourquie O (ed) The skeletal system. Cold Spring Harbor Laboratory Press, New York, NY, pp 147–170

Dealy CN, Roth A, Brown AM, Kosher RA (1993) Wnt-5a and Wnt-7a are expressed in the developing chick limb bud in a manner suggesting roles in pattern formation along the proximodistal and dorsoventral axes. Mech Dev 43:175–186

Duboc V, Logan MPO (2011) Regulation of limb bud initiation and limb-type morphology. Dev Dyn 240(5):1017–1027. doi:10.1002/dvdy.22582

Dumont ER (2010) Bone density and the lightweight skeletons of birds. Proceedings of the Royal Society B: Biological Sciences 277(1691):2193–2198

Eiting T, Gunnell G (2009) Global completeness of the bat fossil record. Journal of Mammalian Evolution 16(3):151–173. doi:10.1007/s10914-009-9118-x

Farnum CE, Tinsley M, Hermanson JW (2008) Forelimb versus hindlimb skeletal development in the big brown bat, *Eptesicus fuscus* functional divergence is reflected in chondrocytic performance in autopodial growth plates. Cells Tissues Organs 187(1):35–47

Fernandez-Teran M, Ros MA (2008) The apical extodermal ridge: morphological aspects and signaling pathways. Int J Dev Biol 52:857–871

Fröbisch NB, Shubin NH (2011) Salamander limb development: integrating genes, morphology, and fossils. Dev Dyn 240(5):1087–1099. doi:10.1002/dvdy.22629

Ganan Y, Macias D, Duterque-Coquillaud M, Ros MA, Hurle JM (1996) Role of TGF(beta)s and BMPs as signals controlling the position of the digits and the areas of interdigital cell death in the developing chick limb autopod. Development 122:2349–2357

Gilbert SF (2006) Developmental biology, 8th edn. Sinauer Associates, Sunderland, MA

Guimond J-C, Levesque M, Michaud P-L, Berdugo J, Finnson K, Philip A, Roy S (2010) BMP-2 functions independently of SHH signaling and triggers cell condensation and apoptosis in regenerating axolotl limbs. BMC Dev Biol 10(1):15

Gunnell G, Simmons N (2005a) Fossil evidence and the origin of bats. Journal of Mammalian Evolution 12(1):209–246. doi:10.1007/s10914-005-6945-2

Gunnell GF, Simmons NB (2005b) Fossil evidence and the origin of bats. J Mamm Evol 12:209–246
Harfe BD (2011) Keeping up with the zone of polarizing activity: new roles for an old signaling center. Dev Dyn 240(5):915–919. doi:10.1002/dvdy.22597
Harfe BD, Scherz PJ, Nissim S, Tian H, McMahon AP, Tabin CJ (2004) Evidence for an expansion-based temporal *Shh* gradient in specifying vertebrate digit identities. Cell 118(4):517–528
Hedenstrom A, Johansson LC, Wolf M, von Busse R, Winter Y, Spedding GR (2007) Bat flight generates complex aerodynamic tracks. Science 316(5826):894–897. doi:10.1126/science.1142281
Hockman D, Cretekos CJ, Mason MK, Behringer RR, Jacobs DS, Illing N (2008) A second wave of Sonic hedgehog expression during the development of the bat limb. Proc Natl Acad Sci 105(44):16982–16987. doi:10.1073/pnas.0805308105
Hodgkinson VS, Ericsson R, Johanson Z, Joss JMP (2009) The apical ectodermal ridge in the pectoral fin of the Australian lungfish (*Neoceratodus forsteri*): Keeping the fin to limb transition in the fold. Acta Zoologica 90(s1):253–263
Holbrook KA, Odland GF (1978) A collagen and elastic network in the wing of the bat. J Anat 126(1):21–36
Hopyan S, Sharpe J, Yang Y (2011) Budding behaviors: Growth of the limb as a model of morphogenesis. Dev Dyn 240(5):1054–1062. doi:10.1002/dvdy.22601
Jepsen GL (1966) Early Eocene bat from Wyoming. Science 154:1333–1339
Kirkpatrick SJ (1994) Scale effects on the stresses and safety factors in the wing bones of birds and bats. J Exp Biol 190(1):195–215
Kronenberg HM (2003) Developmental regulation of the growth plate. Nature 423:332–336
Kronenberg HM, McMahon AP, Tabin CJ (2009) Growth factors and chondrogenesis. In: Pourquie O (ed) The skeletal system. Cold Spring Harbor Laboratory Press, New York, NY, pp 171–203
Kunz TH, Anthony ELP (1982) Age estimation and post-natal growth in the bat *Myotis lucifugus*. J Mammal 63(1):23–32
Larsson HCE, Heppleston AC, Elsey RM (2010) Pentadactyl ground state of the manus of *Alligator mississippiensis* and insights into the evolution of digital reduction in Archosauria. J Exp Zool B Mol Dev Evol 314B(7):571–579. doi:10.1002/jez.b.21362
Laufer E, Nelson C, Johnson R, Morgan B, Tabin C (1994) Sonic hedgehog and Fgf-4 act through a signaling cascade and feedback loop to integrate growth and patterning of the developing limb bud. Cell 79:993–1003
Laufer E, Pizette S, Zou H, Orozco O, Niswander L (1997) BMP expression in duck interdigital webbing: a reanalysis. Science 278:305
Martin GR (1998) The roles of FGFs in the early development of vertebrate limbs. Genes Dev 12:1571–1586
Merino R, Rodriguez-Leon J, Macias D, Ganan Y, Economides AN, Hurle JM (1999) The BMP antagonist Gremlin regulates outgrowth, chondrogenesis and programmed cell death in the developing limb. Development 126(23):5515–5522
Minina E, Wenzel H, Kreschel C, Karp S, Gaffield W, McMahon A, Vortkamp A (2001) BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. Development 128:4523–4534
Montavon T, J-Fo LG, Kerszberg M, Duboule D (2008) Modeling Hox gene regulation in digits: reverse collinearity and the molecular origin of thumbness. Genes Dev 22(3):346–359. doi:10.1101/gad.1631708
Muijres FT, Johansson LC, Barfield R, Wolf M, Spedding GR, Hedenstrom A (2008) Leading-edge vortex improves lift in slow-flying bats. Science 319(5867):1250–1253. doi:10.1126/science.1153019
Niswander L, Martin G (1993) FGF-4 and BMP-2 have opposite effects on limb growth. Nature 361:68–71
Nolte MJ, Hockman D, Cretekos CJ, Behringer RR, Rasweiler JJ (2009) Embryonic staging system for the black mastiff bat, *Molossus rufus* (Molossidae), correlated with structure-function



relationships in the adult. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology 292(2):155–168. doi:10.1002/ar.20835
Norberg UM (1972) Functional osteology and myology of the wing of the dog-faced bat *Rousettus aegyptiacus* (É. Geoffroy) (Mammalia, Chiroptera). Zoomorphology 73(1):1–44. doi:10.1007/bf00418146
Norberg UM, Rayner JMV (1987) Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Phil Trans R Soc B 316:335–427
Pajni-Underwood S, Wilson C, Elder C, Mishina Y, Lewandoski M (2007) BMP signals control limb bud interdigital programmed cell death by regulating FGF signaling. Development 134:2359–2368
Papadimitriou HM, Swartz SM, Kunz TH (1996) Ontogenetic and anatomic variation in mineralization of the wing skeleton of the Mexican free-tailed bat, *Tadarida brasiliensis*. J Zool 240(3):411–426. doi:10.1111/j.1469-7998.1996.tb05295.x
Pivkin IV, Hueso E, Weinstein R, Laidlaw DH, Swartz S, Karniadakis GE (2005) Simulation and visualization of air flow around bat wings during flight. Proceedings of International Conference on Computational Science 351:689–694
Pizette S, Niswander L (2000) BMPs are required at two steps of limb chondrogenesis: formation of prechondrogenic condensations and their differentiation into chondrocytes. Dev Biol 219:237–249
Ray R, Capecchi M (2008) An examination of the Chiropteran HoxD locus from an evolutionary perspective. Evol Dev 10(6):657–670. doi:10.1111/j.1525-142X.2008.00279.x
Reno PL, McCollum MA, Cohn MJ, Meindl RS, Hamrick M, Lovejoy CO (2008) Patterns of correlation and covariation of anthropoid distal forelimb segments correspond to Hoxd expression territories. J Exp Zool B Mol Dev Evol 310B(3):240–258. doi:10.1002/jez.b.21207
Richardson MK (1999) Vertebrate evolution: the developmental origins of adult variation. Bioessays 21:604–613
Rosello-Diez A, Ros MA, Torres M (2011) Diffusible signals, not autonomous mechanisms, determine the main proximodistal limb subdivision. Science 332(6033):1086–1088. doi:10.1126/science.1199489
Saunders JJW (1998a) The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. 1948. J Exp Zool 282(6):628–668
Saunders JW (1998b) Apical ectodermal ridge in retrospect. J Exp Zool 282(6):669–676
Sears KE (2004) Constraints on the morphological evolution of marsupial shoulder girdles. Evolution 58(10):2353–2370
Sears KE (2005) Role of development in the evolution of the scapula of the giant sthenurine kangaroos (Macropodidae: Sthenurinae). J Morphol 265(2):226–236
Sears KE (2008) Molecular determinants of bat wing development. Cells Tissues Organs 187:6–12
Sears KE, Behringer RR, Rasweiler JJ, Niswander LA (2006) Development of bat flight: morphologic and molecular evolution of bat wing digits. Proc Natl Acad Sci USA 103(17):6581–6586
Shapiro MD (2002) Developmental morphology of limb reduction in *Hemiergis* (Squamata; Scincidae): chondrogenesis, osteogenesis, and heterochrony. J Morphol 254:211–231
Shapiro MD, Hanken J, Rosenthal N (2003) Developmental basis of evolutionary digit loss in the Australian lizard Hemiergis. J Exp Zool 297B:48–56
Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, Jonsson B, Schluter D, Kingsley DM (2004) Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. Nature 428(April)
Sick S, Reinker S, Timmer J, Schlake T (2006) WNT and DKK determine hair follicle spacing through a reaction–diffusion mechanism. Science 314(5804):1447–1450. doi:10.1126/science.1130088
Sterbing-D'Angelo S, Chadha M, Chiu C, Falk B, Xian W, Barcelo J, Zook JM, Moss CF (2011) Bat wing sensors support flight control. Proc Natl Acad Sci USA 108(27):11291–11296. doi:10.1073/pnas.1018740108

Stopper GF, Wagner GP (2005) Of chicken wings and frog legs: a smorgasbord of evolutionary variation in mechanisms of tetrapod limb development. Dev Biol 288:21–39
Sun X, Mariani FV, Martin GR (2002) Functions of FGF signalling from the apical ectodermal ridge in limb development. Nature 418:501–508
Swartz SM (1997) Allometric patterning in the limb skeleton of bats: implications for the mechanics and energetics of powered flight. J Morphol 234(3):277–294
Swartz SM, Middleton KM (2008) Biomechanics of the bat limb skeleton: scaling, material properties and mechanics. Cells Tissues Organs 187(1):59–84
Swartz SM, Bennett MB, Carrier DR (1992) Wing bone stresses in free flying bats and the evolution of skeletal design for flight. Nature 359(6397):726–729
Swartz SM, Groves MS, Kim HD, Walsh WR (1996) Mechancial properties of bat wing membrane skin. J Zool 239(2):357–378. doi:10.1111/j.1469-7998.1996.tb05455.x
Tamura K, Nomura N, Seki R, Yonei-Tamura S, Yokoyama H (2011) Embryological evidence identifies wing digits in birds as digits 1, 2, and 3. Science 331(6018):753–757. doi:10.1126/science.1198229
Teeling EC (2000) Molecular evidence regarding the origin of echolocation and flight in bats. Nature 403:188–192
Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ (2005) A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307(5709):580–584. doi:10.1126/science.1105113
Thewissen JGM, Babcock SK (1992) The origin of flight in bats. Bioscience 42(5):340–345
Thewissen JGM, Cohn MJ, Stevens LS, Bajpai S, Heyning J, Horton WE (2006) Developmental basis for hind-limb loss in dolphins and origin of the cetacean bodyplan. Proc Natl Acad Sci USA 103(22):8414–8418
Watts P, Mitchell EJ, Swartz SM (2001) A computational model for estimating the mechanics of horizontal flapping flight in bats. J Exp Biol 204(16):2873–2898
Weatherbee SD, Behringer RR, Rasweiler JJ, Niswander LA (2006) Interdigital webbing retention in bat wings illustrates genetic changes underlying amniote limb diversification. Proc Natl Acad Sci USA 103(41):15103–15107
Wellik D, Sun X, Boekhoff-Falk G (2011) John F. Fallon, PhD: fifty years of excellence in limb research and counting. Dev Dyn 240(5):909–914. doi:10.1002/dvdy.22594
Welten M, Pavlovska G, Chen Y, Teruoka Y, Fisher M, Bangs F, Towers M, Tickle C (2011) 3D expression patterns of cell cycle genes in the developing chick wing and comparison with expression patterns of genes implicated in digit specification. Dev Dyn 240(5):1278–1288. doi:10.1002/dvdy.22633
Wyant KA, Adams RA (2007) Prenatal growth and development in the Angolan free-tailed bat, *Mops condylurus* (Chiroptera: Molossidae). J Mammal 88(5):1248–1251
Yokouchi Y, Sakiyama J, Kameda T, Iba H, Suzuki A, Ueno N, Kuroiwa A (1996) BMP-2/-4 mediate programmed cell death in chicken limb buds. Development 122:3725–3734
Zakany J, Kmita M, Duboule D (2004) A dual role for Hox genes in limb anterior-posterior asymmetry. Science 304(5677):1669–1672. doi:10.1126/science.1096049
Zhu J, Mackem S (2011) Analysis of mutants with altered Shh activity and posterior digit loss supports a biphasic model for shh function as a morphogen and mitogen. Dev Dyn 240(5):1303–1310. doi:10.1002/dvdy.22637
Zou H, Niswander L (1996) Requirement for BMP signaling in interdigital apoptosis and scale formation. Science 272:738–741

# 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?*

R.A. Adams (✉)
School of Biological Sciences, University of Northern Colorado, Campus Box 92, 501 20th Street, Greeley, CO 80301, USA
e-mail: rick.adams@unco.edu

J.B. Shaw
Division of Math/Science, University of Science and Arts of Oklahoma, Chickasha, OK 73018, USA
e-mail: Jason.Shaw@unco.edu

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