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Focus Article

The evolution and development of mammalian flight

Lisa Noelle Cooper,[1] Chris J. Cretekos[2] and Karen E. Sears[3]*

Mammals have evolved a stunning diversity of limb morphologies (e.g., wings, flippers, hands, and paws) that allowed access to a wide range of habitats. Over 50 million years ago, bats (Order Chiroptera) evolved a wing (composed of a thin membrane encasing long digits) and thereby achieved powered flight. Unfortunately, the fossil record currently lacks any transitional fossils between a rodent-like ancestor and a winged bat. To reconstruct how this important evolutionary transition occurred, researchers have begun to employ an evolutionary developmental approach. This approach has revealed some of the embryological and molecular changes that have contributed to the evolution of the bat wing. For example, bat and mouse forelimb morphologies are similar during earliest limb development. Despite this, some key signaling centers for limb development are already divergent in bat and mouse at these early stages. Bat and mouse limb development continues to diverge, such that at later stages many differences are apparent. For example, at these later stages bats redeploy expression of toolkit genes (i.e., *Fgf*, *Shh*, *Bmp*, *Grem*) in a novel expression domain to inhibit apoptosis of the interdigital tissues. When results are taken together, a broad picture of the developmental changes that drove the transition from a hand to a wing over 50 million years ago is beginning to take shape. Moreover, studies seem to suggest that small changes in gene regulation during organogenesis can generate large evolutionary changes in phenotype.

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INTRODUCTION

Although most mammals inhabit terrestrial habitats, one lineage, the bats (Order Chiroptera) underwent an extraordinary limb-to-wing (Figure 1) transition over 50 million years ago.[1,2] As a result of this transition, bats became the only mammals to evolve powered flight, and successfully invade the skies. Inhabiting this novel aerial niche allowed bats to diversify and become one of the most successful mammalian groups.[3] As an indicator of their success, bats today comprise approximately one out of every four known mammalian species.[3,4] Therefore, an essential morphological innovation leading to the bats' success was the evolution of a forelimb wing capable of powered flight.[5–8] Details of how this important morphological transition occurred remain sparse.[9] This is primarily because the earliest bat fossils already possess fully developed wings, and no intermediate fossils capturing this transition have yet been found.[10,11] To overcome this paucity of transitional data, evolutionary developmental (evo-devo) researchers are reconstructing the limb-to-wing transition based on molecular and embryological events occurring in developing bats.[8,12–15]

In keeping with its unique adult morphology, the bat wing is exceptional in its development. As in all mammals, the bat forelimb buds out from the flank. As the limb continues to grow its distal-most end enlarges to form a handplate, also as in other mammals.[14,16] However, at late embryonic and early fetal stages (Carnegie Stages (CS) 15-16), this handplate becomes triangle-shaped.[14] Throughout the remainder of fetal development, the posterior digits (III–V) grow longer

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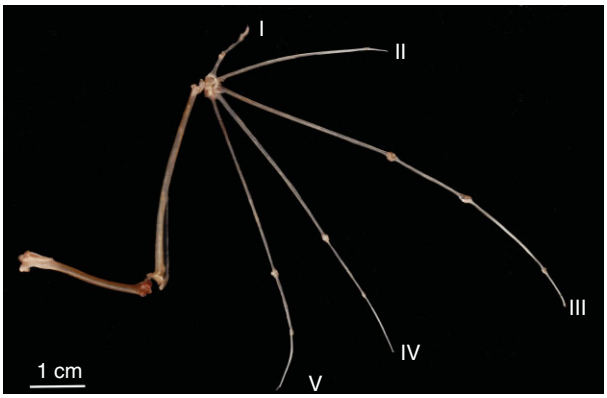

FIGURE 1 | Forelimb skeleton of Seba's short-tailed bat (*Carollia perspicillata*) showing the lengthened digits of the autopod III–V. Scale bar is 1 cm in length.

FIGURE 2 | Wild bats hanging from the ceiling of a culvert in the island country of Trinidad. Source: Photo by Merla Hubler.

relative to body size,[17] such that the digit I of adults is tiny in comparison[2] and digit II is also short. Also, the interdigital tissues do not undergo complete apoptosis, except between digits I and II, and instead proliferate to form the wing membrane.[8,14] This membrane is partially supported by a novel complex of muscles that has never been found in any terrestrial mammal, but is present in some placental gliders and birds (for a review of the fundamentals of forelimb anatomy see Thewissen and Babcock[2,18]). The wing bones of bats display a unique architecture in which some elements display a low-bone-mass phenotype; proximal elements (i.e., humerus and radius) have enlarged medullary cavities and reduced cortical dimensions, and the distal elements (e.g., metacarpals and phalanges) are predominantly amedullary (for a review of bat wing bone architecture and its biomechanical consequences, see Swartz and Middleton[17,19–21]). The dorsal and ventral surfaces of the wings display a grid-like network of microscopic small domed hairs that are sensitive enough to sense directional alterations in flow, even at low speeds, and therefore provide a means of sensorimotor flight control.[22–24]

Because of their unusual body plan, bats have recently emerged as model organisms for evo-devo studies.[13,25–28] Most research has focused on Seba's short-tailed bat (*Carollia perspicillata*). *Carollia* are small, leaf-nosed bats, agile fliers, breed well in captivity, and females are monoparous, with two cycles of pregnancy per year.[29,30] In the wild, females exhibit seasonal and synchronous births, so embryos of a variety of closely spaced ontogenetic stages can be collected in a single field season.[31,32] During field collection, roosts are found typically in abandoned water tanks, culverts (Figure 2), or homes. Females are collected from these roosts with nets and their embryos are harvested for future research. In collaboration with the University of West Indies, a team of researchers from the United States (University of Illinois, Idaho State University, University of Texas M. D. Anderson Cancer Center, and State University of New York Downstate Medical Center) collected embryonic and fetal *Carollia* twice a year for evo-devo research. In addition to studies on *Carollia*, researchers have investigated aspects of wing development in other bat species (e.g., *Miniopterus*,[12] *Molossus*,[33] *Myotis*, and *Rhinolophus*[34]) collected from the wild.

An important first step in understanding how development has been modified to generate the wing in bats is to determine how bat wing development differs from mouse limb development. Mice are excellent comparative organisms as they are quadrupeds with pawed limbs, have a generalized body plan that is arguably representative of terrestrial mammals, and much is known about their limb development. Key questions to address are the following: (1) what developmental changes have resulted in bats retaining their wing membrane tissues, while mice lack these tissues and have separate digits? (2) how has development been modified such that limb outgrowth is sustained in bats to produce such positive allometry in digits III–V of the forelimb?, and (3) what are the specific gene sequence differences (e.g., mutations in *cis*-regulatory elements) that underlie the unique development of bat limbs?

THE ROLE OF INTERDIGITAL TISSUES IN THE DEVELOPING WING

Bats Inhibit Apoptosis to Retain Interdigital Tissues

Throughout bat and mouse forelimb development, the limb progresses through a standard bud-to-paddle

progression, and an obvious wing develops in the bat only during the mid to late embryonic stages of ontogeny.[14] Bats retain interdigital tissues[8] and continue to lengthen the digits,[5] while mice lose these interdigital tissues via apoptosis, and form separate digits.[35] A study documenting signaling patterns in the developing interdigital tissues of bats and mice found that key genes in the apoptosis pathway,[36] namely the bone morphogenic proteins (*Bmp; Bmp2 and 7*), are expressed in the interdigital tissues of both bats and mice.[8] However, while *Bmps* actively contribute to cell death in the mouse, the genes may be inhibited in bats. The interdigital tissues of bats expressed the *Bmp*-inhibitor, *Grem*, in an expression pattern overlapping that of the *Bmp*s.[8] Bats surprisingly also expressed an anti-apoptosis survival factor in the interdigital tissues, fibroblast growth factor 8 (*Fgf8*). Most vertebrates isolate *Fgf8* expression to the apical ectodermal ridge (AER) during limb outgrowth. However, Weatherbee et al.[8] found that bats expressed *Fgf8* in the interdigital tissues, again in an overlapping expression pattern with *Bmp2/7* and gremlin. The combined effects of gremlin and *Fgf8* likely result in the maintenance of the interdigital tissues and their continued proliferation in the developing bat limb. Taken together, these data demonstrate that alteration of the spatial expression patterns of gremlin and *Fgf8* was an important step toward the development of the wing membrane and thereby powered flight in bats.

Interdigital Tissues Likely Drive Outgrowth and Patterning of the Fetal Bat Limb

Most tetrapods isolate *Fgf* and *Shh* signaling to the AER and zone of polarizing activity (ZPA), respectively, and activity of these signaling centers direct limb outgrowth and patterning.[37,38] During the bud and paddle stages of limb development (CS 14; for staging criteria, see Cretekos et al.[14]), bats display enlarged signaling centers, relative to limb size.[12] Compared to mice, the ZPA of bats is extended anteriorly,[12] and the AER is widened in the dorsoventral plane[39] (Figure 3).

After digital condensations have formed (~CS 15-16), this *Shh–Fgf* feedback loop is no longer active in the limbs of mice and bats (e.g., *Miniopterus*, *Carollia*[12]). In bats, this feedback loop is then restarted after metacarpals begin chondrification (~CS 16)[12] (Figure 4). Curiously, instead of *Shh* and *Fgf* expression being isolated to their typical signaling centers, bats express both molecules in the interdigital tissues along with *Bmp*s and *Grem* (see above). Furthermore, these genes are expressed in an anterior–posterior gradient with the greatest concentrations found adjacent to digits III–V. These posterior digits will eventually achieve exceptional lengths, and form the bones supporting the wing. Taken together, these data suggest that bats reinitiate the *Shh–Fgf* feedback loop via novel interdigital expression domains and that this expression pattern is absent in mice. It is likely that this potential redeployment acts to allow for the proliferation of interdigital tissues into more advanced stages of wing membrane development,[12] and it could be that these interdigital tissues act as signaling reservoirs that also direct the extreme lengthening of the posterior digits (see below).

BMP LENGTHENS THE DIGITS IN THE DEVELOPING WING

Bats forelimb autopods display lengthened digits III-V to support the wing membrane. Intriguingly, mesenchymal condensations of these elements are equivalent in length in bats and mice during the incipient phases of limb development (~CS 15).[5] However, during chondrogenesis, the relative length of bat wing metacarpals and phalanges dramatically increases relative to those of mouse via increased rates of chondrocyte proliferation in the growth plate. Sears et al.[5] documented signaling differences between bat and mouse development that at least partially explained these differences in length. During typical endochondral ossification, *Bmp*s are expressed in hypertrophic chondrocytes as well as the osteogenic perichondrium surrounding a developing long bone. Compared to mice, bat expression of *Bmp2* is 30% higher in the perichondrium of the metacarpals. Increased signaling was also documented in the *Bmp* downstream target *phospho-Smad* 1/5/8. Limb culture experiments on cartilaginous metacarpals of bats and mice showed that, when treated with BMP protein *in vitro*, limbs lengthened compared to control limbs. Similar results were found in the cultured limb bones of mice.[40] Taken together, these data demonstrate that alterations in Bmp levels are sufficient to lengthen the metacarpals, and presumably other elements of the autopod of the bat wing. These data offer compelling insights into how novel morphologies evolve. Specifically, these data suggest that relatively small changes in the level of expression of one or a few key genes can dramatically impact limb phenotype.

SEQUENCE IDENTITY AND REGULATION OF LIMB PATTERNING GENES

The developing forelimbs of bats display altered expression patterns of several genes (i.e., *Fgf8*, *HoxD,*

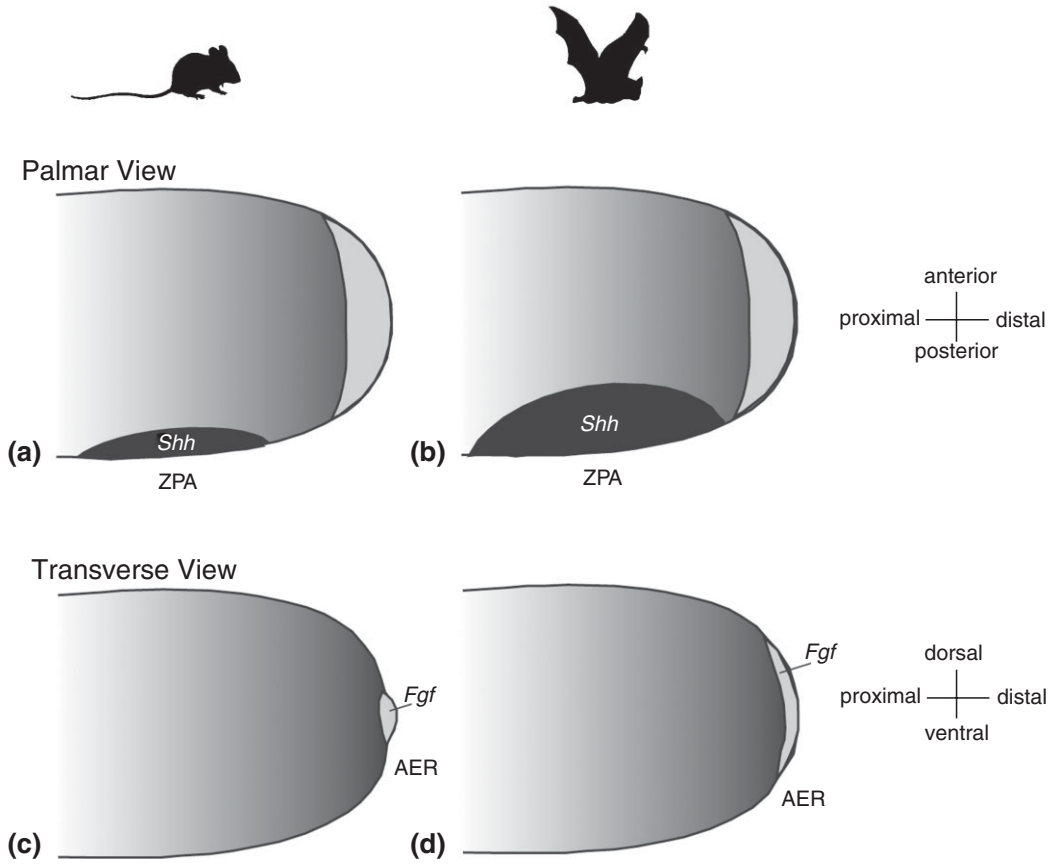

FIGURE 3 | Developing limbs of bats and mice exhibit different sized signaling centers. Bats have a larger zone of polarizing activity (ZPA) (b), compared to mice (a).[12] Bats (d) also have a dorsoventrally wider apical ectodermal ridge (AER) compared to mice (c).[39]

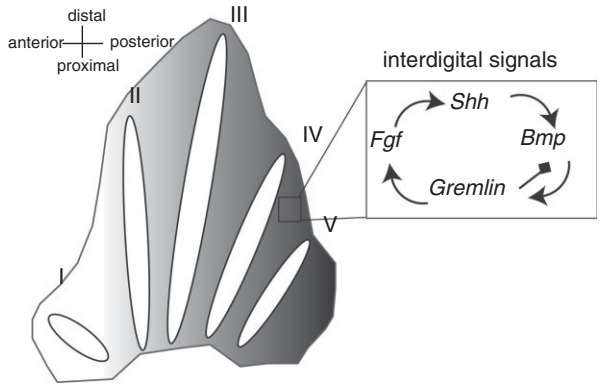

FIGURE 4 | Developing forelimb of a fetal bat displays interdigital signals that allow for the maintenance of interdigital tissues[8] and restarting of the *Fgf–Shh* feedback loop that allows for lengthening of the limb.[12]

Prx1) known to pattern the limb relative to other studied tetrapods (e.g., mouse). However, analyses have indicated that the coding sequences of these differentially expressed genes are highly conserved in bats, humans, and mice.[28,34,39,41] In bats, *Fgf8* expression in the AER displays a dorsoventral expansion in width compared to mice,[39] and during the fetal stages *Fgf8* displays a novel expression domain in the interdigital tissues.[8,12] However, *Fgf8* sequence data indicate the bat coding sequence is 95% identical to that of humans and 94% identical to mice.[39] Similarly, the coding sequence of *HoxD13* in bats is 95% identical to those of humans and mice,[15,34] but expression of *HoxD13* is posteriorly shifted in the bat autopod.[42] In bats, the expression of *Prx1* in the developing wing is expanded in its anterior and posterior domains, and is upregulated in the bat handplate.[41] In contrast, PRX1 proteins shared 99% identity and no evidence suggested this protein differed in its activity between the two taxa.[41] Finding that expression patterns, but not coding sequences, of these genes were unique in bats suggests that evolutionary modifications in regulatory regions are responsible for the unique phenotype of the bat wing.[28]

The transcription factors *Prx-1* and *-2* have been shown to cooperatively regulate limb morphogenesis through *TNF, Runx, and Osterix*.[43] Compared to mice, *Prx1* expression is upregulated in the developing

perichondrium of bat wing bones (i.e., zeugopod and autopod), possibly as a result of sequence alterations in a known *Prx1* limb-specific enhancer element. Using a novel interspecies enhancer swap approach to test this hypothesis, researchers genetically replaced the limb-specific *Prx1* enhancer in mice with its bat homolog.[41] In the resulting mutant mice, expression of the endogenous *Prx1* gene was upregulated in the developing forelimb skeleton. Furthermore, the limbs of the mutant mice displayed a modest, but significant lengthening relative to those of wild-type mice siblings, showing that the regulatory sequence differences between bat and mouse in a single enhancer in a single gene are sufficient to produce a measurable change in morphology.[41] Further functional testing is needed to pinpoint the specific sequence changes that have led to the elongation of bat wing digits.[30] Once these factors are determined, it may be possible to genetically engineer mice with more dramatic digit elongations, and thereby gain important insights into genetic mechanisms that drove the bat limb's transition from hand to wing.

Researchers have also investigated the possible role of changes to a *HoxD13* limb enhancer, global control region (GCR), in bat development.[34] 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. Because *HoxD* expression is known to affect *Bmp* signaling during limb development,[44,45] it could be that regulatory alterations to *HoxD13* via GCR can at least partially explain the upregulated *Bmp* signals in the developing metacarpals documented by Sears et al.[5] that affect skeletal element length.

CONCLUSION

Taken together, analyses of gene expression and sequence data have demonstrated that bats are a compelling and accessible example of how minor alterations to the spatial and temporal expression of genes that pattern the tetrapod limb (i.e., *Shh*, *Bmp*, *Fgf*, gremlin, *HoxD13*) can ultimately lead to exceptional phenotypes. Intriguingly, those coding sequences comparisons (i.e., *Fgf8* and *HoxD13*) have shown a high level of conservation between bats, mice, and humans. Therefore, the documented variation in expression of these genes is instead likely the result of changes in the regulation of these genes. Such a regulatory alteration has already been implicated as a driver of the differences in *HoxD13* expression between bat and mouse,[33] and many more will likely be identified as research in this exciting new model organism progresses. Bats therefore represent an excellent model organism in which to investigate the multiple hierarchies of genetic mechanisms acting during organogenesis to shape the development of novel structures.

A number of compelling questions regarding bat wing development are the subject of ongoing research by the authors. First, no studies have elucidated the genetic modifications that establish the enlarged AER and ZPA signaling centers in the bat wing, compared to mouse. A complete understanding of these modifications is critical to our understanding of how the bat wing has achieved its unique morphology. Second, bone architectural and biomechanical analyses of bat forelimb bones have documented differences in the bat bone cross-sectional geometries and the presence of a low-bone-mass phenotype that is consistent with disease states, termed osteopenia and osteoporosis, in humans. Few studies have addressed the molecular differences between the developing bones of bats and mice. Finally, multiple studies of vertebrate body growth have shown that modules of the developing body can have correlated development as well as correlated evolutionary changes. Changes in the chiropteran body plan offer an exceptional opportunity to explore modular growth and its evolutionary history. As a result of this ongoing research, studies in bat development will continue to illuminate the developmental basis of morphological specialization, and thereby advance the fields of evolutionary and developmental biology.

REFERENCES

1. Gunnell G, Simmons N. Fossil evidence and the origin of bats. *J Mamm Evol* 2005, 12:209–246.
2. Thewissen JGM, Babcock SK. The origin of flight in bats. *BioScience* 1992, 42:340–345.
3. Arita HT, Fenton MB. Flight and echolocation in the ecology and evolution of bats. *Trends Ecol Evol* 1997, 12:53–58.
4. Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ. A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* 2005, 307:580–584.
5. Sears KE, Behringer RR, Rasweiler JJ. Development of bat flight: morphologic and molecular evolution of bat wing digits. *Proc Natl Acad Sci* 2006, 103:6581–6586.

6. Sears KE. Molecular determinants of bat wing development. *Cells Tissues Organs* 2007, 187:6–12.
7. Weatherbee SD. Mammalian limbs take flight. *Dev Cell* 2008, 14:149–150.
8. Weatherbee SD, Behringer RR, Rasweiler JJ, Niswander LA. Interdigital webbing retention in bat wings illustrates genetic changes underlying amniote limb diversification. *Proc Natl Acad Sci* 2006, 103:15103–15107.
9. Eiting T, Gunnell G. Global completeness of the bat fossil record. *J Mamm Evol* 2009, 16:151–173.
10. Gunnell GF, Simmons NB. Fossil evidence and the origin of bats. *J Mamm Evol* 2005, 12:209–246.
11. Jepsen GL. Early Eocene bat from Wyoming. *Science* 1966, 154:1333–1339.
12. Hockman D, Cretekos CJ, Mason MK, Behringer RR, Jacobs DS, Illing N. A second wave of Sonic hedgehog expression during the development of the bat limb. *Proc Natl Acad Sci* 2008, 105:16982–16987.
13. Rasweiler JJ IV, Cretekos CJ, Behringer RR. The short-tailed fruit bat *Carollia perspicillata*: a model for studies in reproduction and development. *Cold Spring Harb Protoc* 2009. 2009:pdb.emo118.
14. Cretekos CJ, Weatherbee SD, Chen C-H, Badwaik NK, Niswander L, Behringer R, Rasweiler JJ I. 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 Dynam* 2005, 233:721–738.
15. Chen CH, Cretekos CJ, Rasweiler JJ, Behringer RR. Hoxd 13 expression in the developing limbs of the short-tailed fruit bat, *Carollia perspicillata*. *Evol Dev* 2005, 7:130–141.
16. Hopyan S, Sharpe J, Yang Y. Budding behaviors: growth of the limb as a model of morphogenesis. *Dev Dyn* 2011, 240:1054–1062.
17. Swartz SM. Allometric patterning in the limb skeleton of bats: implications for the mechanics and energetics of powered flight. *Journal of Morphology* 1997, 234:277–294.
18. Thewissen JGM, Babcock SK. The implications of the propatagial muscles of flying and gliding mammals for archontan systematics. In: Ross DE, ed. *Primates and Their Relatives in Phylogenetic Perspective*. New York, NY: Plenum Press; 1993, 91–109.
19. Swartz SM, Middleton KM. Biomechanics of the bat limb skeleton: scaling, material properties and mechanics. *Cells Tissues Organs* 2008, 187:59–84.
20. Swartz SM, Bennett MB, Carrier DR. Wing bone stresses in free flying bats and the evolution of skeletal design for flight. *Nature* 1992, 359:726–729.
21. Dumont ER. Bone density and the lightweight skeletons of birds. *Proc Roy Soc B: Biol Sci* 2010, 277: 2193–2198.
22. Zook JM. Somatosensory adaptations of flying mammals. In: Kaas JH, Krubitzer LA, eds. *Evolution of Nervous Systems A Comprehensive Reference. Vol. 3 Mammals*. Amsterdam: Elsevier, Academic Press; 2007, 215–226.
23. Sterbing-D'Angelo S, Chadha M, Chiu C, Falk B, Xian W, Barcelo J, Zook JM, Moss CF. Bat wing sensors support flight control. *Proc Natl Acad Sci* 2011, 108: 11291–11296.
24. Zook JM. The neuroethology of touch in bats: cutaneous receptors of the wing. *Neurosci Abstr* 2005, 78:21.
25. Rasweiler JJ IV, Cretekos CJ, Behringer RR. Whole-mount immunohistochemistry of short-tailed fruit bat (*Carollia perspicillata*). *Cold Spring Harb Protoc* 2009, 2009:pdb.prot5167.
26. Rasweiler JJ IV, Cretekos CJ, Behringer RR. Alcian blue/alizarin red staining of cartilage and bone of short-tailed fruit bat (*Carollia perspicillata*). *Cold Spring Harb Protoc* 2009, 2009:pdb.prot5166.
27. Rasweiler JJ IV, Cretekos CJ, Behringer RR. Whole-mount *in situ* hybridization of short-tailed fruit bat (*Carollia perspicillata*) embryos with RNA probes. *Cold Spring Harb Protoc* 2009, 2009:pdb.prot5164.
28. Behringer RR, Rasweiler JJ, Chen C-H, Cretekos CJ. Genetic regulation of mammalian diversity. *Cold Spring Harbor Symposia on Quantitative Biology* 2009, 74:297–302.
29. Cloutier D, Thomas DW. *Carollia perspicillata*. *Mamm Species* 1992, 417:1–9.
30. Cretekos CJ, Rasweiler JJ I, Behringer RR. Comparative studies on limb morphogenesis in mice and bats: a functional genetic approach towards a molecular understanding of diversity in organ formation. *Reprod Fert Develop* 2001, 13:691–695.
31. Fleming TH. *The Short-Tailed Fruit Bat*. Chicago, IL: University of Chicago Press; 1988.
32. Rasweiler JJ IV, Badwaik NK. Improved procedures for maintaining and breeding the short-tailed fruit bat (*Carollia perspicillata*) in a laboratory setting. *Laboratory Animals* 1996, 30:171–181.
33. Nolte MJ, Hockman D, Cretekos CJ, Behringer RR, Rasweiler JJ. 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* 2009, 292:155–168.
34. Ray R, Capecchi M. An examination of the Chiropteran HoxD locus from an evolutionary perspective. *Evol Dev* 2008, 10:657–670.
35. Pajni-Underwood S, Wilson CP, Elder C, Mishina Y, Lewandoski M. BMP signals control limb bud interdigital programmed cell death by regulating FGF signaling. *Development* 2007, 134:2359–2368.
36. Hernandez Martinez R, Covarrubias L. Interdigital cell death function and regulation: new insights on an old programmed cell death model. *Dev Growth Differ* 2011, 53:245–258.

37. Riddle RD, Johnson RL, Laufer E, Tabin C. Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* 1993, 75:1401–1416.
38. Niswander L, Tickle C, Vogel A, Booth I, Martin GR. FGF-4 replaces the apical ectodermal ridge and directs outgrowth and patterning of the limb. *Cell* 1993, 75:579–587.
39. Cretekos CJ, Deng J-M, Green ED, Rasweiler JJ, Behringer RR. Isolation, genomic structure and developmental expression of Fgf8 in the short-tailed fruit bat, *Carollia perspicillata*. *Int J Dev Biol* 2007, 51:333–338.
40. Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, Vortkamp A. BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. *Development* 2001, 128:4523–4534.
41. Cretekos CJ, Wang Y, Green ED, Program NCS, Martin JF, Rasweiler JJ, Behringer RR. Regulatory divergence modifies limb length between mammals. *Genes Dev* 2008, 22:141–151.
42. Chen C-H, Cretekos CJ, Rasweiler JJ, Behringer RR. Hoxd13 expression in the developing limbs of the short-tailed fruit bat, Carollia perspicillata. *Evol Dev* 2005, 7:130–141.
43. Lu X, Beck GR, Gilbert LC, Camalier CE, Bateman NW, Hood BL, Conrads TP, Kern MJ, You S, Chen H, et al. Identification of the homeobox protein Prx1 (MHox, Prrx-1) as a regulator of osterix expression and mediator of tumor necrosis factor α action in osteoblast differentiation. *J Bone Miner Res* 2011, 26:209–219.
44. Suzuki M, Ueno N, Kuroiwa A. Hox proteins functionally cooperate with the GC Box-binding protein system through distinct domains. *J Biol Chem* 2003, 278:30148–30156.
45. Williams TM, Williams ME, Heaton JH, Gelehrter TD, Innis JW. Group 13 HOX proteins interact with the MH2 domain of R-Smads and modulate Smad transcriptional activation functions independent of HOX DNA-binding capability. *Nucl Acids Res* 2005, 33:4475–4484.

