Morphogenesis in Bat Wings: Linking Development, Evolution and Ecology

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Introduction

The evolution of powered flight in mammals required specific developmental shifts from an ancestral limb morphology to one adapted for flight. Through studies of comparative morphogenesis, investigators have quantified points and rates of divergence providing important insights into how wings evolved in mammals. Herein I compare growth, development and skeletogenesis of forelimbs between bats and the more ancestral state provided by the rat (Rattus norvegicus) and quantify growth trajectories that illustrate morphological divergence both developmentally and evolutionarily. In addition, I discuss how wing shape is controlled during morphogenesis by applying multivariate analyses of wing bones and wing membranes and discuss how flight dynamics are stabilized during flight ontogeny. Further, I discuss the development of flight in bats in relation to the ontogenetic niche and how juveniles effect populational foraging patterns. In addition, I provide a hypothetical ontogenetic landscape model that predicts how and when selection is most intense during juvenile morphogenesis and test this model with data from a population of the little brown bat, Myotis lucifugus.

Key Words
Growth • Development • Wings • Functional matrix • Compensatory growth • Ecomorphology • Ontogenetic niche • Bats

Abstract
The evolution of powered flight in mammals required specific developmental shifts from an ancestral limb morphology to one adapted for flight. Through studies of comparative morphogenesis, investigators have quantified points and rates of divergence providing important insights into how wings evolved in mammals. Herein I compare growth, development and skeletogenesis of forelimbs between bats and the more ancestral state provided by the rat (Rattus norvegicus) and quantify growth trajectories that illustrate morphological divergence both developmentally and evolutionarily. In addition, I discuss how wing shape is controlled during morphogenesis by applying multivariate analyses of wing bones and wing membranes and discuss how flight dynamics are stabilized during flight ontogeny. Further, I discuss the development of flight in bats in relation to the ontogenetic niche and how juveniles effect populational foraging patterns. In addition, I provide a hypothetical ontogenetic landscape model that predicts how and when selection is most intense during juvenile morphogenesis and test this model with data from a population of the little brown bat, Myotis lucifugus. Copyright © 2007 S. Karger AG, Basel
Herein I outline some of the work that has uncovered developmental patterns and has given evolutionary insight into how wings evolved in mammals and why studies of morphogenesis are crucial in formulating adaptive functional scenarios for key evolutionary innovations such as bat wings, thus far approached mostly from a purely adult-state perspective [Hill and Smith, 1984; Speakman, 1999, 2001]. In addition, I discuss how wing morphogenesis may be stabilized during flight ontogeny and how newly volant young influence populational foraging patterns. Lastly, I integrate functional morphology, ontogeny and ecology into an ontogenetic landscape model that predicts times of highest selection pressures on juveniles in their first year.

**Prenatal Development of the Wing in Bats**

Of the series of ontogenetic shifts occurring prenatally to establish a winged mammal, the most obvious is elongation of the forearm (fig. 1) and manus (fig. 2), bones that are destined to provide structural support for a flight membrane. More subtly, fusions between the ulna and radius, both proximally and distally during prenatal development, begin to structurally stabilize the wing against future unwarranted rotation during flight (fig. 3). In addition, diachronic growth asymmetry between the ulna and radius is exaggerated relative to that of most nonvolant mammals (fig. 4) and in insectivorous species of the family Vespertilionidae, the ulnar shaft morphs into ligamentous tissue that may serve to facilitate recoiling of the curved radial bones during the upstroke, helping generate forward thrust [Adams, 1992b].

The wing membrane of bats is composed of an elastic, muscular tissue, variably cambered to produce the lift required for flight. Developmental analysis shows that the flight membrane results from a lack of interdigital tissue cell death that normally occurs in most mammals. Studies of prenatal growth and development of the wing

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**Fig. 1.** Illustration of comparative growth of the forearm in the Norway rat, *Rattus norvegicus*, and the little brown bat, *Myotis lucifugus* (from Adams [1992a]). Shifts in diametrical growth during development result in a small-diameter ulna in bats relative to what is observed in the more ancestral rat ulna. In addition, in *M. lucifugus*, histology showed that about 50% of the ulnar diaphysis reconstitutes into a ligament that may be an adaptation for powered flight.

**Fig. 2.** Illustration of metacarpal and digit elongation on cleared and stained fetuses of *Myotis lucifugus* beginning with chondrogenic patterning of the forelimb (a) through postnatal ossification (f) (from Adams [2000]).
membrane are lacking, but Yokoyama and Uchida [2005] reported a decrease in aspect ratio (measured as wing span²/wing area) in the Japanese lesser horseshoe bat, *Rhinolophus cornutus cornutus*, whereas increases in wing area were reported for prenatal Angolan free-tailed bats (*Mops condylurus*) and prenatal wing membrane growth tracked closely with body mass [Wyant and Adams, in press].

**Postnatal Development: The Race to Flight Status**

Newborn young develop quickly and thus have a short window of time to develop flight skills necessary for successful foraging on night-flying insects. Weaning typically occurs within the first 3 weeks after birth, and juveniles must achieve competent flight ability quickly thereafter or perish due to starvation. In most insectivorous species, flight begins at about 21 days postpartum. For vespertilionid bats such as *Myotis lucifugus*, much growth and development occur during the typical 50- to 60-day gestation period, and young are born having about 60% adult mass and 40% wing span [Buchler, 1980; Kunz and Anthony, 1982; Adams, 1997].

As the young grow postnatally, development of the wing is accelerated over growth in body size. At first flight, juvenile *M. lucifugus* showed quite high relative wing loadings that declined significantly between days 1 and 29 of postnatal life, and area of the wing also significantly changed [Powers et al., 1991]. Similar patterns of wing development have been observed in other bat species (*Pipistrellus minus* [Suthakar Isaac and Marimuthu, 1997], *Nycticeius humeralis* [Jones, 1967], *Pipistrellus pipistrellus* [Hughes et al., 1995], *Pipistrellus subflavus*...

Concomitant changes in length of wing bones occur during the early flight stages of development in all bats thus far studied, but specific rates of growth among bones of the forelimb show substantial variation among bat species (fig. 5).

What Orchestrates Morphogenesis of Wing Size and Shape: The Functional Mosaic

Much growth and development of the wing bones and flight membrane occurs during postnatal life. During this time, juveniles conduct wing flapping exercises apparently to ready themselves for first flight. Once on the wing, juvenile survivorship is reliant upon their ability to avoid obstacles and successful foraging on evasive insects. Lengths of digit 3 measured from early-volant to mature-sized juveniles of M. lucifugus captured from a maternity roost in Wyoming [Adams, 1998] ranged from 78.1 to 99.5 mm, representing a 28% increase in length during flight ontogeny. Digit 5 measured from the same individuals ranged from 38.1 to 47.9 mm, representing a 20% increase in length. Juveniles also experience about a 40% increase in wing area during postvolant growth and development. Developing flight skills may be confounded by a rapidly changing airfoil [Adams and Pedersen, 1994] that alters power output and maneuverability. The question then becomes: are there aspects of morphogenesis that serve to functionally stabilize such a rapidly changing dynamic?

Ontogeny of soft and hard tissues of the wing must be synchronized to maintain function. Particularly after first flight, changes due to morphogenesis must be balanced with function allowing for flight competent enough to avoid obstacles and enable forage. Morphogenetic competency, of course, is universal and applies to many aspects of growth and development across taxa, and hence is not peculiar to bats. To this general point, van der Klaauw [1946] and Moss [1962] fashioned an unconventional view of growth and development when they suggested that soft tissues govern size and shape of hard tissues, rather than vice versa. Their model showed that the tensions applied by soft tissues during development determine the fate of skeletal form and function. For example, removal of muscle attachments from a developing humerus will result in an amorphous element, showing little or no aspects of shape or function associated with this bone. They termed the developing mosaic of soft and hard tissues the ‘functional matrix’ and suggested that most aspects of skeletal form and function are derived by interactions with soft tissues.

The underlying imposition of this view is that the shape of life is predominately an epigenetic derivation, driven not by structural genes, but rather by regulatory genes that bring certain tissues together at specific times during development, and it is the tissue interactions that determined the shape of animals [Raff, 1996]. Thus, the evolution of form in vertebrates, especially concerning key innovations and diversity of form, is generated predominately by mutations in regulatory genes and tissue inductions [Müller, 1990]. It is selection on individuals during morphogenesis, governed by epigenetic processes such as reciprocal induction [Oster et al., 1988; Goodwin, 1996], which is one of the foundations of novel evolutionary change. Strikingly to this point, Rowe [1996] presented compelling evidence that the complex changes in cranial morphology during the evolution of mammals from mammal-like reptiles was instigated by selection for larger brain size, rather than direct selection for changes in skull morphology itself. Disarticulation of the masticatory and hearing chains was a consequence of enlarge-
ment of the cranial vault as a result of outward pressure from positive allometry during brain morphogenesis.

Selection for larger brain size literally split the connection between the jaw and the inner ear of mammal-like reptiles. Cascading changes of the selective pressure for larger brain size thereby supported simplification of the mammalian dentary and co-optation of ‘freed’ jaw bones for forming ear ossicles. Consequently, the modern construct of the mammalian skull is both proximately (developmentally) and ultimately (evolutionarily) a matter of soft tissue’s push and pull [Rowe, 1996].

Throughout the literature, it appears commonly assumed that wing morphogenesis and evolution are driven by elongation of forearm and manus bones that push the form of the wing membrane [Sears, 2008, this issue]. However, this may be backwards. A dissertation by Tumilson [1990] quantified an interesting pattern of what appeared to be serial compensatory growth of developing finger bones in adult plecotine bats. Adams [1998] quantified morphogenesis of this apparent pattern by grouping juvenile *M. lucifugus* with similar total wing lengths and quantifying variability in serial bone element lengths contributing to total length and width of the wing among differently sized (age) juveniles. Unexpectedly, even those individuals with exactly similar wing lengths showed disparate measures of serial bone lengths, i.e. those that had shorter forearms, would show compensation by hav-

**Fig. 6.** Illustration of compensatory growth of the forearm, third metacarpal and third digit, during postnatal morphogenesis of *M. lucifugus* in a series of juveniles with similar wing lengths. Those with very similar wing lengths have very different lengths of elements that make up wing length. FA = Forearm; M3 = 3rd metacarpal; P1 = proximal 3rd phalanx; P2 = middle 3rd phalanx; P3 = distal 3rd phalanx (from Adams [1998]).

**Fig. 7.** Plot of summed positive residuals versus summed negative residuals from bivariate plots of an individual’s bone element length of digit 3. As intensity of positive residuals increases, negative residuals decrease indicating compensation among bony elements composing wing length (Pearson’s *r* = 0.78, *p* < 0.05, from Adams [1998]).

**Fig. 8.** Box truss for wing shape design. Seven homologous landmarks (1–7) produce 13 linear trusses (I–XIII) connecting each landmark to 5 others. Distances between landmarks through developmental sizes are used to quantify wing shape changes during development.
and negative residuals are significantly correlated, thus supporting the compensatory hypothesis of limb skeletal growth and development in bats (fig. 7).

Because variation within serially repeating bone elements of the wing was significantly higher than the variation in total wing width and length, bone growth is likely not in control of wing size and shape. It appears simply too sloppy with variation to act as the "orchestrator" of form and function during ontogeny. What then controls wing morphogenesis in bats? Using a box truss analysis (fig. 8) to provide distances between homologous landmarks, the wing membrane shape was shown to be essentially isometric relative to size changes during morphogenesis [Adams, 1998] (fig. 9). Hence, the low variation in wing membrane shape during ontogeny gives compelling evidence that control of morphogenesis rests with soft tissue ontogeny like the functional matrix. The wing bones compensate serially to accommodate the pattern laid down by the wing membrane as it grows. The elastic nature of the wing membrane exerts forces along the long axis of the wing bones controlling form and function [Papadimitriou et al., 1996; Norberg, 1985; Swartz et al., 1996; Swartz and Middleton, 2008, this issue] during flight ontogeny.

Clearly, the adaptive nature of such a developmental mechanism would be to stabilize wing shape during large-scale changes in wing size, thereby invoking a balancing effect allowing for functional flight during juvenile growth stages. As an ontogenetic push for positive brain allometry in mammal-like reptiles was apparently instrumental in setting the stage for reconfiguration of the head leading to true mammals, the ontogenetic tow of the interdigital membrane during ontogeny was perhaps a key innovation in the evolution of flight in mammals.

Fig. 9. Principal component (PC) analysis of box truss network for 7 subadult (SA) and 9 juvenile (J) M. lucifugus. High overlap in shape between PC-2 (12% of variation) and PC-3 (6% of variation) shown by the circle indicates little shape change during morphogenesis relative to changes in wing size (PC-1, 75% of variation) (from Adams [1998]).

Fig. 10. Discriminant function analysis of wing bones in relation to macrohabitat clutter: 1 = least cluttered, 2 = semicluttered and 3 = heavy clutter. Factor 1 separation was driven predominately by length of middle phalanx (from Adams [1996]).


Juvenile stages are commonly considered as simply a necessary pathway towards the ultimate goal of adulthood and reproduction. However, stages of juvenile de-
Development are in and of themselves adaptive states [Cop- pinger and Smith, 1990] that affect intraspecific population interactions as well as interspecific community dynamics. In fact, the ontogenetic niche (a term coined by Werner and Gilliam [1984]) is a universal component of a species niche space that had been largely ignored, but has gained ground over recent decades [Takimoto, 2003]. Age- and/or size-dependent segregation within size-structured populations may occur via behavioral and/or morphological differences between adults and juveniles. Ontogenetic niche shifts have been studied extensively in fishes [Amundsen et al., 2003; Jones et al., 2003] and insects [Dopman et al., 2002]. Some species, such as holometabolous insects [Snodgrass, 1954; Chapman, 1969; Wilbur, 1980] and metamorphic amphibians [Bell, 1975; Wassersug and Sperry, 1977; Fox, 1984], provide obvious examples of morphological and ecological segregation between juveniles and adults. However, in most vertebrate animals, the ontogenetic niche is more subtle and thus prone to be overlooked by researchers [Werner and Gilliam, 1984; Polis, 1991; Claessen and Dieckmann, 2002]. Because in most cases juveniles are smaller in body size than their parents [Ellstrand, 1983] and differ allo-metrically, juveniles and adults may show niche distinctions that could clearly categorize them as different species [Polis, 1991]. In addition, recent modeling has shown how niche shifts during ontogeny may act as evolutionary branching points [Claessen and Dieckmann, 2002].

For bats, little is known about the influence of volant juveniles on population and community dynamics. Some insight has come from studies on age-specific dietary preferences, where juveniles significantly differed from adults in insect consumption [Rolseth et al., 1994; Adams, 1997]. Perhaps more interestingly, Buchler [1980] reported temporal displacement among differently aged juveniles and adult M. lucifugus with younger juveniles leaving the roost to forage later than older juveniles and adults. In addition, juveniles appeared to avoid cluttered habitats.

Adams [1996, 1997] was able to analyze the foraging and dietary components of the ontogenetic niche in an almost species-isolated natural population (i.e. only 3 individuals of other species were captured at the site over a 4-year period). In addition, a mosaic of habitat types provided a gradient of flight skill challenges. He divided the habitats into 6 microhabitats based upon level of vegetative clutter relative to known aspects of maneuverability and sonar capability for this species: CI-0 = open areas >3 m above the ground; CI-1 = open areas <3 m off the ground; CI-2 = open areas above shrubs; CI-3 = forested areas with canopy and flight paths >3 m in diameter; CI-4 = forested areas with can-

![Fig. 11. Graph indicating changes in juvenile forearm length and wing span as related to microhabitat clutter. There is a clear pattern of growth that facilitates foraging in microhabitats of greater and greater clutter. Thus, those with improper morphology for navigating more highly cluttered habitats are ‘forbidden’ from such a morphospace in the microhabitat mosaic (from Adams [2000])]
The foraging patterns of class A adults (those captured before juveniles became volant), class B adults (those captured after juveniles had become volant) and juveniles. There was a clear and significant shift of adults who were caught in significantly higher numbers in open areas close to the ground before juveniles began flying, whereas, after juveniles were flying, adults were captured more abundantly foraging in more complex microhabitats, with juveniles restricted to the open microhabitats, mostly CI-0. In the last year of the study, the population density of returning bats was 40% less than in the previous year. When population density was low, adults did not shift their foraging from open areas to the higher clutter habitats, but instead maintained their foraging close to the ground in open areas. Adults nevertheless remained segregated from the youngest juveniles with poorest flight skills via microhabitat separation. Thus, the degree of effect by juvenile foraging patterns was on some level a matter of population density [Adams, 1997]. Curiously, dietary difference between juveniles and adults was more pronounced under low-density conditions when adults did not move to another microhabitat.

Adams also documented size-specific ontogenetic niche shifts among juvenile *M. lucifugus* of different body sizes (i.e. age). Discriminant function analysis based upon morphological measures of forearm length, third metacarpal, proximal, middle and distant phalanges of 54 juveniles showed distinctive ecomorphological segregation in habitat use with wing size and flight ability (fig. 10) driven apparently by growth of the middle phalanx (based upon factor loadings) that allowed individuals to shift among uncluttered, semicluttered and cluttered habitats. Thus, there appears to be a ‘forbidden’ interface between ontogeny and functional morphology that imposes constraints (fig. 11) on niche space of juveniles where the range of developmental phenotypes in juveniles is limited and there is an incomplete filling of morphospace [Alberch, 1985; Richardson and Chipman, 2003].

From these data, it is possible to construct an ontogenetic landscape model that integrates proximate patterns with evolutionary outcomes as selection favors specific morphogenetic adaptations among individuals during postnatal growth and development (fig. 12). Bats progress through phases of flight development referred to as flop and flutter stages [Powers et al., 1991]. At first flight, bats enter the ontogenetic landscape within which I have derived 5 adaptive peaks and 5 maladaptive valleys as related to ecological complexity (habitat and diet) that juveniles hypothetically traverse throughout postnatal morphogenesis. Individuals, as they traverse the land-
scape, present and receive unique selective pressures that affect population dynamics. According to the model, most juveniles do not survive and are selected out of the population between adaptive peaks (as illustrated by what Wilson [1985] termed 'darwinian wreckage', shown below the landscape). But those that do survive selection's gauntlet within each maladaptive valley are best adapted in terms of maneuverability and foraging ability at that particular body size (age).

Using data gathered on the ecomorphological development of flight ability in *M. lucifugus*, I tested the model. The data adhere to the basic tenants of the ontogenetic landscape model for both habitat and dietary complexity (fig. 13). In addition, there are discrete habitat and dietary shifts among juveniles differing in size and apparently age that corroborate the predictive model [Adams, 1996]. Juveniles are thereby ecomorphologically segregated according to developmental state and flight ability. As each juvenile approaches a maladaptive zone, there is a high incidence of selection out of the population. The farther an individual makes it along the landscape, the greater the likelihood of survival (depicted by the lessening steepness of the landscape).

**Discussion**

The evolution of wings in mammals is a product of ontogenetic shifts in developmental patterns that led to adaptive flight morphologies. The integration of ontogeny of flight morphology in relation to ecological patterns gives insight into how the evolution of flight may have occurred. The adaptations associated with flight are many and varied and herein I discuss in particular the comparative growth and development of limbs between terrestrial and volant mammals that show clear shifts in the developmental program and resultant divergent morphologies. In addition, it appears that growth and development of wings in bats are a construct of the functional matrix in which soft tissues dictate the fates of hard tissues by epigenetic inductive interactions. The wing membrane fundamentally controls pattern (wing size and shape) during morphogenesis, whereas wing bones are pulled and pushed into conformance and compensate their lengths in serial fashion as dictated by the stresses imposed by the growing elastic wing membrane.

The ecology of flight ontogeny is dynamic and has many facets that have yet to be studied. The main point...
of this section was that the juvenile stage is not simply an ephemeral life stage toward the endpoint of adulthood, but instead has adaptive influence on population and community ecology and represents phases of ecomorphology where selective pressures are most intense and variation provides fodder for the evolution of divergent forms. In bats, flight development of juveniles alters the ecology of the population and in some cases may have strong effects on bat assemblage structure, as has been shown in other animals. I derive an ecomorphological landscape that integrates development, ecology and evolution of wing form in bats. Selective pressures are particularly hard on early juvenile stages of many animal populations, and bats are no exception. For insectivorous bats, the gauntlet squeezes hardest at interfaces between microhabitats of increased clutter or increased dietary diversity of insects consumed, but as juveniles become more agile and competent fliers, the effects of selection weaken and higher survivorship results. Population foraging patterns were altered by the introduction of juveniles into the foraging arena, especially when population density was higher. This pattern has been shown in other animals but is unique in bats in that adults dispersed to different feeding areas, whereas in other species, juveniles typically dispersed to avoid competition with adults.

Understanding ontogenetic patterns has helped give insight into the natural history, ecology and evolution of animal groups and continues to provide a bridge between ephemeral proximate patterns and the long-term ultimate evolution of animal diversity. Nowhere is this more apparent than in bats that today compose almost 25% of all living mammal species and, in some anatomical systems, show as much morphological diversity as observed across all mammal species combined.

References


