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The Evolution and Development of Chiropteran Flight

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The Evolution and Development of Chiropteran Flight

Intro

Flying is thought of as a reserved skill used by very few animals throughout history. These animals may be the birds outside your window, the pterodactyl in a children's book, or the insects buzzing about on a hot day. There are also thousands of bats flapping around in the dark all around the world. Exploring the sky, bats are the only mammal capable of true powered flight (Gunnel and Simmons 2005).

Bats are an extraordinarily diverse group of mammals. Chiroptera, the order name for this group of mammals, has over one thousand species (Hill, 2018). In fact, the number may be closer to 1,400 species (K. Ineson pers. comm.). In the past several years, organizations and conservation scientists, such as Dr. Winnifred Frick, have worked on genomic sequencing and documenting species of bat. From the recent genomic data and documentation, many species have been newly identified, raising the number from one thousand to almost 1,400 (Teeling et al. 2018).

There are many categories that set apart these species, from size to coloring, and from habitat to diet. The smallest extant bat is the Kittie's hog-nosed bat weighing in at two grams. Some of the largest bats, the group labeled flying foxes, have wingspans greater than two meters (Norton 2011). Chiroptera often inhabit trees in warmer climates, and roost in caves in colder climates. Some bats migrate long distances, some use torpor and hibernation. Bats are mainly known to be insectivores or frugivores, however there are some species that have been

documented eating small fish and amphibians (Jones and Rayner 1988). Few species commonly known as vampire bats are sanguinivorous, obtaining their nutrition from blood. Unique dentition allows these small mammals to obtain nutrition from larger mammals directly (Davis et al. 2010).

All of these species of bats have one important characteristic (of many) in common: powered flight. These mammals have experienced physiological and morphological changes over millions of years to reach the ability of powered flight seen today. Powered flight is how the bats propel themselves into the air as their main mode of locomotion (Jones and Rayner 1988). They do not glide or fall gracefully as other mammals such as a flying squirrel do. Bats have unique ways to conserve energy, along with wings in place of typical forelimbs, granting the ability to fly while staying efficient (Panyutina et al. 2013). As documented by fossils from the eocene era, about 56 million years ago, this transition from land to air has progressed slowly, allowing the mammals to experience diversification for every environment (Gunnel and Simmons 2005). Every aspect of chiropteran life and behavior has now been tailored to accommodate flight.

Understanding the diversity of this mammalian group is imperative to grasp the ecological challenges bats face while flying. Chiroptera, commonly recognized as bats, are an order of mammals. It is the second largest order to rodentia. Bats were once thought to be closely related to mice due to their small furry appearance. However, Chiroptera are their own group of mammals, genetically distant from rodents despite their convergent appearance (Gunnel and Simmons 2005). Being a mammal, bats are viviparous, endothermic, have hair, and are lactogenic among many other mammalian qualities.

Viviparity is the ability to give birth to live young. Most bats carry a pup in the womb for several months before birth, and watch over the pup for about a year before maturity (Nowak and Walker 1994). Chiroptera of all sizes tend to have one or two pups at a time, about once a year in the summer months when food is most abundant (Hill and Smith 1984). The pups hold onto the torso of the mother, able to nurse as needed and stay safe. Mother bats of some species fly with their pups which weigh up to one third of their body weight (Rowe (a) 2020). Caring for young while expending energy on flight is a delicate balance that chiropteran species manage, a challenge unique to the order. Bats are also lactogenic, as mentioned above. This is the classic mammalian trait of producing milk. By contrast, birds are oviparous. They lay eggs, and then care for the young once hatched. Bats may fly similarly to birds, but house a whole set of different skills. As mammals, the bat species expend more energy caring for and rearing their young than non-mammalian animals (Rowe (b) 2020).

It's good to mention that bats are essential pollinators and pest control, around the globe. One example of the capabilities of these small mammals is that the little brown bat (*Myotis lucifugus*) can ingest up to one thousand mosquitos in one night. The large impact of a colony of these small bats, about one hundred bats, adds up to 100,000 mosquitoes gone from the environment in one night. Other frugivore bats search for nectar and fruits of plants for their nightly meals. In doing so the bats pick up pollen from one flower, and transfer it to others. This process is important for flowering plants which rely on external help to sexually reproduce.

Making up about one fourth of mammalian diversity bats are the only ones capable of flight. Many questions remain about the behavior and evolution of these mammals as there is very little fossil documentation (Simmons and Geisler 1998). This paper will explore the

“*how*”s of mammalian flight through the mechanisms of flying; the development of the wing and other important morphological traits; and the evolutionary path which brought these mammals to flight.

Ancestral Chiroptera & Flight

The fossil record is a key source of evolution, especially when specific fossils are found with intermediate morphs; the linking physical forms which show the transition from one form to another. One example is the fossil Tiktaalik, the transitional fish discovered in 2004. Tiktaalik is so important due to the front limb structures. This fossil showed how the fish moved from the water onto land, having stronger forelimb structures and other key features (Clack 2009). This species identified a pectoral girdle which was removed from the cranium, and able to support a more upright posture. It demonstrated the transitional state by providing physical evidence that a species was able to make the transition from water to land. Before Tiktaalik, there was only evidence for aquatic animals, and terrestrial animals some time later. Scientists knew that terrestrial animals had marine ancestors, but finding Tiktaalik solidified the knowledge by showing the intermediate form which linked the two (Shubin et al. 2006).

Along with the fossil record, genomic studies have supported evolution by mapping the relationships between different organisms. Understanding what genes are shared between organisms allows scientists to visualize which traits are ancestral, being shared by many organisms, and those genes which are more recent or specific to certain types of organisms. For instance, genomics have led to the phylogenetic tree of mammals, dating back 188 million years ago to the last common ancestor, a shrew like small species (Upham et al. 2019). This tree shows

how species that look nothing alike, are in fact related and often linked by a common ancestor (Figure 5). Chiroptera were once thought to be closely related to Dermoptera, the flying squirrels, on the left cladogram due to behavioral and morphological similarities (Figure 5). They both are aerial and have skin membraned wings. But genetically, Chiroptera and Dermoptera are separated by many other orders of mammals, sharing only ancestral traits in their genome (Figure 5). Chiroptera are more closely related to Carnivora than they are to Dermoptera. The phylogenetic cladogram is widely accepted, where the morphological cladogram is less accepted (Amador et al. 2018). Phylogenetic analysis has given us this information, which scientists a century ago could not have anticipated by sight alone (Teeling et al. 2018). Morphological similarities have several alternative reasons than from evolution of a common ancestor, such as morphological trait convergence (Pettigrew 1991). This is to say that similar ecological pressures can lead to similar physical and behavior traits separately. Flying squirrels and bats may have taken to the sky to avoid predation or to better capture prey insects (Smith 1976). This is one reason why the phylogenetic tree is utilized more than the morphological tree. For example, bat and flying squirrels both have evolved to incorporate flying or gliding, but genetically these clades are separated, suggesting that the flying manifested independently in each clade (Pettigrew 1991).

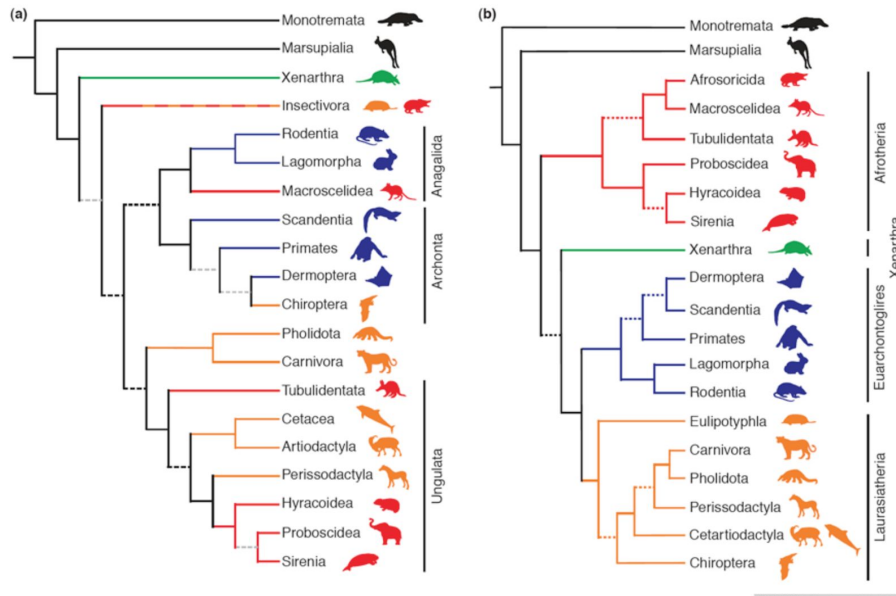


Figure 5: Two cladograms showing different interpretations of the evolution of mammals. (a) The left shows relation based on physical characteristics, while (b) the right is based on genetic ties.

(Trends in Ecology and Evolution. 19: 430-438,

<https://research.amnh.org/paleontology/perissodactyl/node/55>)

Though there have been advances in speciation and phylogeny, the evolution of bats remains uncertain and clouded (Shi and Rabosky 2015). With over 1400 species, there are three main clades which house the most species diversity. These clades are Noctilionoidea, Vespertilionoidea, and Emballonuroidea (Upham et al. 2019). Within these clades are about 20 extant families. Using available genetic records and focussing on 29 loci, Shi and Rabosky (2015) found a strikingly high amount of homogeneity among extant bats, despite their wide range of diversity. The chiropteran order has the smallest genome size of any extant mammal, similar in size to birds. This may suggest a streamlining of flight genomics (Teeling et al. 2018). Shi and Rabosky (2015) dated the radiation of speciation to be between 50-15 million years ago, and ever

decelerating since. The conservation of genes within the 29 loci puzzled Shi and Rabosky, as they expected higher heterogeneity among such a large and diverse order of mammals. One reason for this uncertainty may stem from a lack of genetic sampling, estimating only about 65% of extant bats having been recorded (Upham et al. 2019). Recently, the Bat1K Project has united bat conservation efforts into one database. Estimating over 70% of the chiropteran fossil record to be missing, Bat1K has been globally sequencing bat species genome, in an effort to understand more about the chiropteran evolution (Teeling et al. 2018). Having only 14 out of 1300 genome sequences fully documented and readily available for research, the Bat1K Project has a long road ahead of them to solve the mystery of Chiropteran evolution (Teeling et al. 2018).

Much of Chiropteran evolution has been recently represented by a phylogenetic tree (Jones et al. 2007). The backbone of chiropteran phylogeny is widely accepted and established (Amador et al. 2018). Within the order Chiroptera there are two suborders; the Yinpterochiroptera and the Yangochiroptera. These are more commonly referred to as the microbats capable of echolocation and fruit eating megabats incapable of echolocation. Bats within the suborder Yinpterochiroptera are identified by ribosomal genes 12S and 16S. Bats found in the suborder Yangochiroptera are identified by the ribosomal gene 12S (Amador et al. 2018). From these suborders, there are 20 to 21 families, with varying identifying genes (figure 6). Continued research has reclassified some of the families. From there, the abundance of species and outgroups lyes the lack of data for complete classification (Amador et al. 2018). Pettigrew (1991) states that though chiropteran order is said to be monophyletic, there are certain brain characteristics, such as the difference in echolocation abilities, that may suggest

Yinpterochiroptera and Yangochiroptera being polyphyletic, and to have evolved flight and wings convergently. Pettigrew (1991) states this may be possible as both suborders are quadrupeds and have similar ecological stressors in which flight was useful. Though the phylogenetic tree is mainly agreed upon, it's also open to more restructuring as information about species arises through conservation efforts and the Bat1k project.

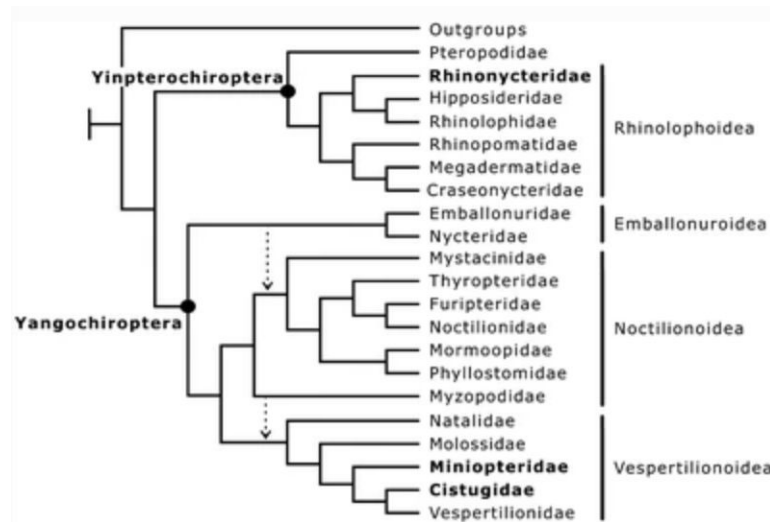


Figure 6: Backbone phylogenetic tree of Chiroptera down to the family level (Amador et al. 2018)

With over one thousand extant species, data collection has been difficult and time consuming. The extinct species are a whole separate task to tackle, as those species are represented by few physical specimens, with a variety of characteristics and dates. It is commonly believed now that chiroptera are a monophyletic group, meaning they have derived from one original bat (Sotero-Caio et al. 2017). However, it is noted that there simply isn't enough evidence yet to support this evolutionary theory (Pettigrew 1991). There are so many differences between species, that it is possible there are more ancestral forms undetected (Jones et al 2007).

Understanding bat evolution is complicated not only by the lack of phylogenetic data, but also by the lack of fossil evidence (Teeling et al. 2018). The chiropteran fossil record goes back 50 million years with very little physical representation of this extensive period (Sears et al 2006). It is estimated that only about 30% of the record has been discovered, with most researchers utilizing 44 specific well preserved fossils (Gunnell and Simmons 2005). Bats do not fossilize well. Their wings are made of a skin membrane, which deteriorates and rarely leaves an imprint in the stone. Many bats are also very small, which means any that did fossilize may have eroded or remain lost.

Though little fossil evidence has been found one component is clear. Bats have had wings and were capable of some form of flight for the past 50 million years. However, no fossil transitional species has been found to suggest how wings began (Geisler and Simmons 1998). The earliest fossil yet from the Eocene era shows striking similarities to modern bat species (Simmons et al. 2008). There are a few key differences in the morphology of *Onychonycteris*, the single preserved member of its species (Figure 7). This bat shows the morphology capable of some powered flight, as the shoulder girdle, clavicle, and rib cage are all similar to those in extant species. However, the limb ratio suggests that this species was not capable of the fully powered flapping we associate with extant bats. Behavior is often determined by patterns seen in other extant species when interpreting fossil morphology. Having much longer hindlimbs, this ancestral species most likely had bouts of gliding and intermittent powered flight to support its large, longer body. Extant species typically have much shorter hind limbs which makes the body more aerodynamic, and in turn makes flight more energetic (Geisler and Simmons 1998). This species also has claws, suggesting it may have spent more time in trees, climbing using these

claws and well developed hindlimbs (Simmons et al. 2008). Extant species of bat only have claws on several digits (Geisler and Simmons 1998). Unfortunately, one fossil can only infer so much information about behavior and movement based on patterns. As more fossils are uncovered, more information will be brought into light about how these ancestral bats behaved compared to extant bats.



Figure 7: Comparison between extinct and extant species of Chiroptera. The left shows *Onychonycteris* fossil, with a body length of 56mm. The right shows a skeletal reconstruction of a common flying fox, with a body length of 300mm.

(Simmons et al. 2008)

Even with the differences, these two individuals are remarkably similar. They are related, but removed by 50 million years of history. It's quite impressive that this Order has stayed so consistent. This may be due simply to the genetic code which allows for the development of the

wing, as it has been shown that bats have incredibly high homogeneity (Teeling et al. 2018, Shi and Rabosky 2015). As it has been conserved for so long, it may be in effect restricting other morphological opportunities and changes, accounting for the decline in diversification over the last 15 million years (Shi and Rabosky 2015). Or maybe it's due to the lack of changes of resources and environment. Without a new stressor or pressure from the environment, many random mutations in a species go unnoticed, as they do not help combat an ecological pressure and further the species (Laland and Sterelny 2006). Bats have been able to be volant in many environments without excessive changes needed to their form. Many microbats roost in caves and hollowed areas, but even with Human development, they have managed to roost in built homes. The environment is different, but is catering to the chiropteran form regardless.

So much information is yet to be discovered about how these mammals came to fly, and have maintained so successfully while being the only true volant mammals on earth. It's known that bats have evolved from a mammalian ancestor. The questions of how their flight began, and how their wings first developed remain unanswered. Without fossil evidence of a transitional mammal, scientists are left to speculate based on patterns and genomics of extant species (Speakman 2001).

Mechanisms

Flight, when referring to mammalian movement, is the ability to purposefully move through the air to reach a destination. There have only been three vertebrate lineages throughout Earth's history to fly, consisting of pterodactyls, birds, and bats (Padian 1984). Pterodactyls soared over the dinosaurs in the late Triassic period (Padian 1984). Some birds migrate

thousands of miles at a time. And there are bats of all sizes zig-zagging through the trees and open air at night.

Flight can be categorized into different forms based on the use of energy around and from the animal. Relying on energy from the environment includes soaring and gliding. Relying on internal metabolic energy is powered flight. Soaring is when an animal uses the wind currents and air pressures or updrafts to cruise at a specific speed and altitude (Rayner 1981). Very little energy is expended during soaring, and it allows animals to continue moving while resting (Hedenström and Bone 1997). Many large birds of prey, such as albatrosses which have long slender wings to catch the wind current, use soaring when looking for a meal. Other more familiar examples may include a seagull riding an air current at the beach. The birds which soar are able to “float” in the air.

Other than soaring, is the term gliding, which in many cases is very similar. Gliding is often used for short bouts of assisted falling through the air, using the energy from gravitational pull to reach their destination. Gliding requires no propulsion from the animal, and very little energy in general (Thomas and Taylor 2001). The animal can't gain height on its own through gliding. Gliding is up to the wind and air channels that the animal comes into contact with. Similar to someone paragliding or skydiving, the animal is harnessing the wind for its locomotion, but not able to overcome the air's motion itself. Gliding is used by some birds like vultures landing near fallen prey, and some small mammals like sugar gliders or flying squirrels. These animals leap in small bouts from one tree to another, using excess skin to catch the air and make the fall slower and more controlled (Savile 1962). The use of metabolic energy is so low, that alternative vertebrates are also able to glide including some snakes and lizards (Savile 1962).

Lastly, powered flight requires flapping wing beats, controlled by metabolic energy expenditure. Flapping is the use of wings to create thrust, pushing the trunk of the animal both upward and forward (Rayner 1988). In birds, the wings create thrust by a strong push downward below the body, and a controlled relaxation of the wings back above the body. As the bird is in the down stroke of the wingbeat, the force pushes the body upward and forward. As the thrust weakens, the body begins to drop lower, and the wings move back into the optimal position for the downstroke. Birds primarily use this up and down wing motion mixed with soaring to be most efficient. Birds are able to control speed and distance of their flight by controlling the amount of thrust produced by their wings (Marden 1987).

Bats, on the other hand, never glide or soar, no matter the size of the mammal. These mammals use continuous flapping to move throughout the air (Jones and Rayner 1988). The wings of different bat species differ in shape, ability, and power, but all have a similar base form. Continuous flapping gives bats the most control and range of flight, as they are able to manipulate the air in many directions (Panyutina et al 2013). Though this continuous motion requires a high amount of metabolic energy, there are other key points to Chiropteran flight mechanics which allows the continuous motion to be very efficient (Rayner 1988).

In order to fly, bats must possess wings. Bat wings are thin and mobile with a soft membrane, which enables them to grab at and move the air around them in a more flexible motion as their wings are less rigid (Bishop 2008). The wing spans from the tip of the index digit to the body, expanding between all five digits. The length and width of the wing differs slightly among species (Deitz et al. 2006). Bird wings are restricted to one shape with many muscles and feathers to support that shape (Padian 1984). Chiropteran flight requires more motion from the

shoulder girdle than avian flight (Panyutina et al 2013). In contrast to birds which are able to push their bodies straight up into the air, Starting from a rotation of the clavicle, the shoulder is then able to move in a circular motion rather than a linear one (Panyutina et al 2013). The rotation follows through the “elbow” of the bat, bringing the wing in front of the head, under the body, and back around. The motion is similar to that of a swimmer’s butterfly stroke (Figure 1). The wing grabs at the air in front of the bat, and scoops it behind the body.

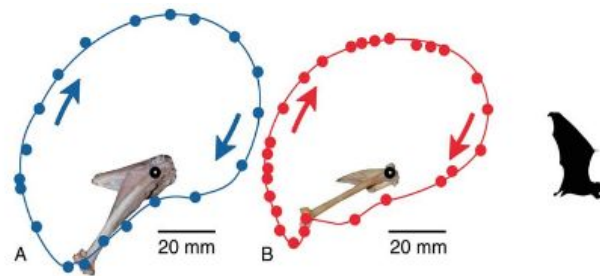


Figure 1: Motion of the elbow trajectories of (A) *R. aegyptiacus* and (B) *H. armiger* (Panyutina et al. 2013)

This motion from the clavicle and scapula is unique to Chiropteran flight. The extent of this shoulder rotation has been determined by a series of ligament connections within the sternoclavicular joint, holding the clavicle in place in relation to the shoulder blade (Panyutina et al 2013). Tighter connections have shown limited mobility and rotation, with more control and less likelihood of disarticulation of the clavicle (Panyutina et al 2013). Looser ligaments allow for a larger rotation. This larger rotation has been seen in more primitive and ancestral bat species via fossils. These primitive species have shown to have had larger wingbeat and may have acquired more speed and distance with each beat, but the wing morphology is less stable due to the looser ligaments in the shoulder (Panyutina et al 2013). More modern species, with the tighter ligaments bounding the clavicle, scapula, and shoulder girdle have more stability in the wing, and therefore have more control of the wing itself (Panyutina et al 2013).

With each wingbeat or stroke, the bats are able to displace air efficiently. By moving the wing membrane, similar to how we move our fingers, they control the speed, direction, and elevation changes. Their wings can take on an aerodynamic streamline shape to increase speed or a parachute shape to decrease speed (Stockwell 2001). Along with the rotation of the wing, bats save mechanical energy by specific flight turning patterns. Many bats have been documented to increase elevation before turning sharply. The increase in elevation allows for gravity to increase acceleration on the turn (Jones and Rayner 1988). In short, the bats use their body weight to turn quickly, instead of needing to flap harder and faster. Bats also bank their wings during the turn to create a shorter distance. This banking motion and control of the wings is partially why bats are able to make such extremely sharp, quick turns. Jones and Reyner (1988) were able to document and graphically demonstrate the banking motion and use of elevation prior to turns in one species of bat using camera footage. Below are some models showing this turning behavior and ability. This behavior has not been researched in megachiroptera, the large fruit bats.

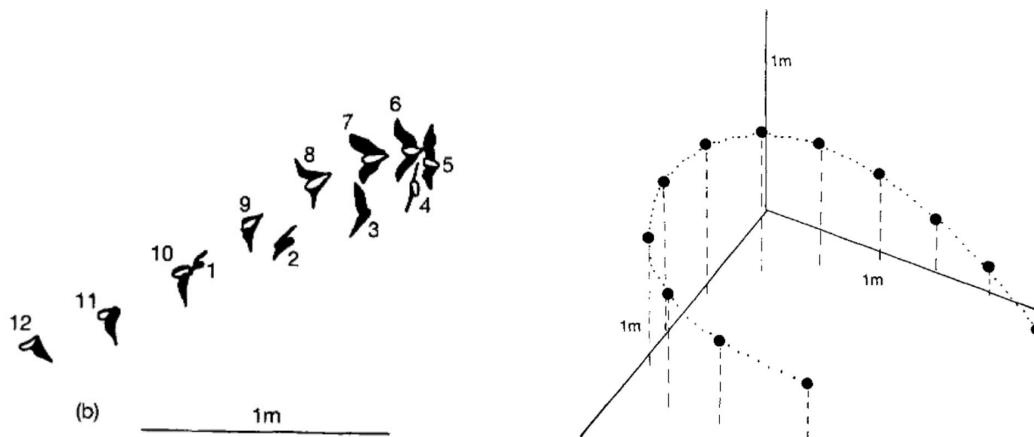


Figure 2: The left model shows an analysis of a typical turn by the species *daubentonii* from position 1 to 12. The right model shows a 3D flight path reconstruction (Jones and Reyner 1988)

The flight patterns and mechanisms highlight the differences between bats and birds. Not only are they far removed on the taxonomic tree, the two exploit the same environment and space very differently. Understanding how chiropteran flight is able to occur, why the clavicle rotates and why the wings are shaped differently than avian wings, requires insight into the morphology and development of the wing as a whole.

Morphology

The chiropteran wing, relatively consistent throughout the whole order, is homologous to the typical mammalian arm and hand. The bones, general form and function of the wing are derived and modified from an ancestral mammal, but the details of that transition and ancestor remain unknown (Gunnel and Simmons 2005). The human arm consists of the shoulder, humerus, radius, ulna, and many small bones which make up the wrist and hand. A bat's wing is made up of these same bones, but modified to create a new shape that better fits the environment. The wing is mainly the bat's fingers extended across a wide area and connected by webbing (Kovalyova 2014). Figure 3 below illustrates how the bones align between humans and bats. Human arms are long, allowing us to hold our young and reach for objects. Bats' wings are light and wide, with the ability to flex as human fingers move independently. The shape of the wing is what enables bats to maneuver effortlessly through the air (Hill 2018).

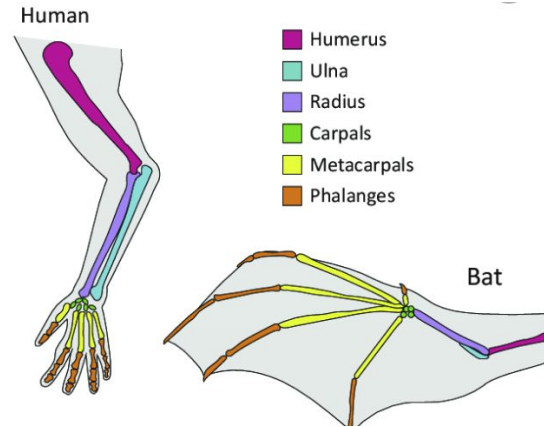


Figure 3: Homologous structures within the forelimb of bats and humans

(Krubitzer & Prescott 2018)

Wing development in place of a typical mammalian forelimb development (like human arms or a dog's front limb) begins early on in utero. The wing's developmental changes consist of three main components: the bone structure, the muscle development, and the skin webbing. The development of bat wings begins with elongation of bones, which allows the muscles and skin to change in response to the bone structure (Hill 2018). Unfortunately, little research has been done on chiropteran embryogenesis, other than looking into the elongation of the forelimb bones and interwebbing (Kovalyova 2014).

Developmental decisions within the womb are controlled by gene activation and proteins. The genes are able to tell cells directions and a blueprint of the design. The proteins deliver the stepwise instructions to neighboring cells, initiating the plan into action (Cretokos et al. 2008). In all vertebrates, such as a mouse, cells in the forelimb start off by creating cartilage in the desired shape and direction. The growth is regulated by apoptosis, or purposeful cell death. These cells create a stopping point by producing bone morphogenic protein (bmp), which signals to nearby

cells to halt the growth process once the limb bone is at an adequate length for the mouse embryo (Weatherbee et al. 2006). Ducks, on the other hand, use alternative proteins like *msx* to inhibit apoptosis between digits, creating longer forelimbs and webbing (Weatherbee et al. 2006). But bat wings look very different from duck wings. A look into these genes and protein expression shows just how the cells are able to form a thin membraned wing from the same bones that create a human arm. Though the use of *bmps* and *msx* in bat wing development is still not fully understood and other proteins may also be at play, several experiments have shown how these proteins are capable of modifying the wing development (Sears et al 2006).

Bats have a unique expression of the *Fgf8* gene and Gremlin inhibitors, allowing the forelimbs to both be elongated and webbed. Between the digits of a bat wing, cells have a high expression of the *Fgf8* genes, which has been shown to strongly inhibit apoptosis, and differs from the expression in other vertebrates (Eckalbar et al. 2016). Weatherbee et al. (2016) describe embryonic tests between ducks, bats, and mice to contrast the regulation of these proteins in response to the forelimb development. From these tests, researchers were able to manipulate those animals into having “incorrect” forelimbs. When the gene expressions were altered by decreasing *fgf8* expression and increasing *bmp* expression in a developing bat forelimb, the forelimb had significantly less webbing and elongation in the wing. Eckalbar et al. shows that the forelimbs become more visible as wings, with the digits lengthening and chirality lost, during specific stages of embryonic development in figure 4 below when these genes are more active. The bones grow longer, and webbing is kept between them. The webbing in some species researched (*Nyctalus noctula* and *Myotis blythii*) consists of a one row layer of epithelial cells over undifferentiated mesenchymal cells (Kovalyova 2014). The wing is very thin skin, different

from the thick highly vasculated wing membrane found in other gliding mammals (Kovalyova 2014).

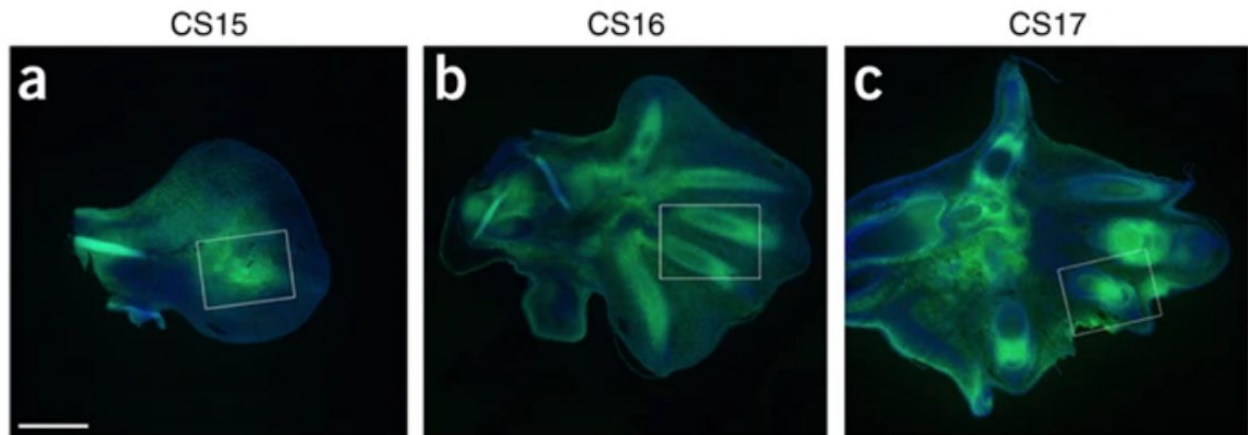


Figure 4: Forelimb development during stages CS15-17 of *Miniopterus natalensis*, showing the progression of the wing shape. (Ecklebar et al. 2016)

Along with the use of proteins such as Fgf8 and Gremlin, the Sonic Hedgehog (shh) protein is used by chiroptera in the feedback loop for developing anterior and posterior patterning. Two species of bat were shown to express shh in early development, causing the widening and loss of symmetry in the forelimb, making the forelimb more sprawled (Hockman et al., 2008). This allows the limb and digits to become more wing-like than human hand-like, as the digits are spaced further apart. In later development, shh is initiated once again in order to restart the lengthening feedback loop. The feedback loop itself is that which consists of the gremlin and Fgf8 proteins referenced earlier (Sears et al 2006). This “second wave” of the sonic hedgehog loop is what some researchers believe is responsible for the unique elongation of the wing (Hockman et al., 2008). It's interesting to note that the development of the hindlimb is separate from the forelimb. The hindlimb is not elongated, and thus does not need the same

expression of proteins as the forelimb. The hind limbs have been seen to more closely mimic those of a mouse (Cretokos et al., 2007). In a sense, the hindlimb may have experienced less divergence from the ancestral mammal than the forelimb. Unfortunately, there are no intermediate species discovered which can help bridge the understanding of how bats have this unique gene and protein expression (Gunnel and Simmons 2005).

Another gene, *Prx1*, may have an impact on the development of forelimb bones between mice and bats. *Prx 1* is shown to be present in both developing mammals. The key difference is that in bats, the gene is upregulated later in development at the digits (Cretokos et al., 2007). This adds to the elongation of the digits. When mutant mice were created to express the bat's gene, the results were inconclusive, as the mutant mice did not express elongated forearms or digits as expected. Statistical analysis confirmed that the gene itself is not responsible for elongation of the bones, but the specific mutated enhancer of the gene found in bats is responsible. The mice mutated with the enhancer were found to have elongated forelimbs and digits, but those with the gene deleted expressed normal growth (Cretokos et al., 2007).

For any trait in an organism, there are many components involved. For bat wing development, there are at least three proteins and several genes which create the perfect blueprint; *bmp*, *fgf8*, *Prx 1*, Gremlin Inhibitor, and the Sonic Hedgehog protein. A small change, or one protein without the other, would result in a different form of forelimb, as seen in experiments done by Weatherbee et al., Cretokos et al., and Hockman et al. Over 50 million years, bat wings have barely changed. This suggests that the genes and proteins involved have also been conserved; however there is no definitive proof as the transitional forms have yet to be

discovered (Sears et al. 2006). All of these genes working together creates the bone structure of the wing.

Kovalyova (2014) states how so little is known about bat embryogenesis. This includes the development of the shoulder girdle. As explained in the former section, the shoulder girdle is responsible for the propulsion of the wing. As the shoulder girdle and clavicle rotate, the wing moves the air around (Panyutina et al 2013). By studying and observing the development of the shoulder girdle, there may be more answers to the evolution of flight as a whole, and what set apart this mammalian order from the ancestral form.

Conclusion

The only truly volant mammals on Earth have an elusive evolutionary history. With only about 30% of the fossil record documented, many of the early forms are unrepresented (Gunnell and Simmons 2005). The earliest fossils depict fully winged mammals (Simmons et al. 2008). As of now, the transitional mammal between a quadruped and bats has not been documented. Going as far back as 50 million years ago, bats have developed wings and complex shoulder girdles giving them the ability to fly as efficiently as they do (Sotero-Caio et al. 2017). Managing to control speed and direction with ease, bats owe their wing development to a series of genes and proteins like the Prx1 gene and bmp protein (Cretokos et al., 2007). These many proteins elongate the forelimbs and keep interdigit webbing (Kovalyova 2014). The wings rotate from the clavicle, propelling the bats through the air (Panyutina et al 2013). With over a thousand extant species documented, the Bat1k project and other conservationists are working to understand the

speciation of this enormous order, and hope to understand more about the morphological differences (Teeling et al. 2018).

Many aspects of chiropteran wing development and evolution are currently unanswered. Having little genetic variation among the species may be a consequence of specific genes associated with the wing and flight (Sears et al. 2006). Without more research into bat embryogenesis, the development of the shoulder girdle can not be fully understood (Kovalyova 2014). For a small animal to go from land or trees to the air is not an easy feat (Pettigrew 1991). Bats were able to do so seemingly out of the blue 50 million years ago. In time, perhaps a transitional species will show just how these wings formed in the first place.

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