Ontogenetic and anatomic variation in mineralization of the wing skeleton of the Mexican free-tailed bat, *Tadarida brasiensis*

**Helen M. Papadimitriou**, Sharon M. Swartz* and T. H. Kunz*

*Department of Biology, Boston University, Boston, MA. USA

**Department of Ecology and Evolutionary Biology, Brown University, Providence, RI. USA

(Accepted 20 July 1995)

(With 1 plate and 4 figures in the text)

We examined patterns of variation in the mineral content of the wing skeleton of the Mexican free-tailed bat, *Tadarida brasiensis*. We ashed humeri, radius, metacarpals II-V, and phalanges of digit III, and quantified mineralization differences among elements at specific ages, and ontogenetically for each element. The most mineralized elements are the humerus and the radius, followed by the metacarpals, of which the third and fifth are the most mineralized. The proximal and middle phalanges of the third digit exhibit the lowest mineral content, and the distal phalanges have no mineral content according to our ashing protocol. Histological examination shows a thin (< 10 μm) shell of unmineralized osteon surrounding a cartilaginous core in distal phalanges. Mineral content of each bone increases linearly with age during post-natal development, but there are differences in the rate and extent of this increase among the different elements.

The mineralization differences we observed parallel substantially different bone loading patterns found in different parts of the wing in other studies. The humerus and radius are subjected to large torsional loads during flight, while the metacarpals and phalanges experience dorsoventral bending. The high mineral content of the humerus and radius and the low mineral content of the metacarpals and phalanges may resist torsion proximally and promote bending distally. Furthermore, the decrease in mineral content along the wing's proxiomedical axis decreases bone mass disproportionately at the wing tips, where the energetic cost of accelerating and decelerating limb mass is greatest.

**Introduction**

The morphology and composition of bones reflects compromises between maximizing stiffness and strength and minimizing the mass that must be gained, nourished and transported during locomotion. The distinctive capacity for powered flight subjects the limb skeletons of bats to selection pressures which differ from those experienced by terrestrial mammals. The airstream subjects the wing bones of flying vertebrates to considerable forces during flight (Norberg, 1990), producing loading regimes that differ significantly from the typical patterns of bone loading that mammals experience during terrestrial locomotion (Swartz, Bennett & Carrier, 1992).

In a study of the natural patterns of bone strain during free flight in the large megachiropteran bat *Pteropus poliocephalus*, Swartz *et al.* (1992) demonstrated large downstroke shear stresses in the humerus and radius due to torsional loading. This loading regime is unique among vertebrates studied to date, and suggests that the evolution of skeletal design in bats may be...
driven by pressures to resist these loads. Hence, unlike the long bone in the limbs of terrestrial mammals, which must resist primarily bending forces (Alexander, 1981; Biewener et al., 1983; Bertram & Biewener, 1988), the proximal wing bones of bats have experienced selection for structural design well suited to resisting large torsional forces.

The mechanical properties of a bone are determined by tissue organization, material composition, and structural geometry (Carrier, 1983; Currey, 1984). Bones of adult mammals generally show little variation in material properties or tissue-level organization (Currey, 1984); differences among taxa in functional design are generally observed in cross-sectional shape (e.g. Ruff, 1989; Demes & Jungers, 1989, 1993; Halgrimson & Swartz, 1995). Functionally, the most important mechanical properties of bones are their bending and torsional strength, determined by structural geometry and the tensile and shear strengths of the bone material (Currey, 1984; Nordin & Frankel, 1989; Currey, 1990). Stiffness is also required to minimize deformation under load and to permit the transmission of muscle force (Currey, 1984).

The strength and stiffness of bone are due both to the properties of its mineral component (Currey, 1984, 1990), largely calcium and phosphate in the form of hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2]\) (Nordin & Frankel, 1989; Ross, Reith & Romrell, 1989), and to its organic matrix of collagen fibres and proteoglycan ground substance. Hydroxyapatite typically accounts for 68–70% of compact cortical bone dry mass (Nordin & Frankel, 1989; Ross, Reith & Romrell, 1989; Currey, 1990). Several studies have shown that, over a certain range of values, increasing mineralization of bone is positively correlated with tensile strength (both yield and failure stresses) and stiffness (Young’s modulus), and negatively correlated with ultimate strain and energy absorbing capacity (Currey, 1969, 1984, 1987, 1988; Borders, Petersen & Orne, 1977; Schaffler & Burr, 1988). At very high values of bone mineral content, energy absorption and strength of bone likewise decrease (Currey, 1969; Currey & Pond, 1989). The variation in mechanical characteristics of bone with increasing mineralization has been effectively exploited during vertebrate skeletal evolution: antlers, bones with a primarily impact absorbing function, have very low mineral content (59%), low Young’s modulus, and high toughness. By contrast, auditory bullae, which experience minimal load and function to provide high acoustic impedance, are very highly mineralized, rock-like, and brittle (Currey, 1969, 1984).

Some aspects of bone development are under direct genetic control, independent of imposed loading. However, the bone structure observed in adults is partially dependent on the mechanical environment both during development and at maturity (Currey, 1984). Studies of dogs, jackrabbits, sheep, captive baboons, axis deer, California gulls, and polar bears have demonstrated an ontogenetic change in the mechanical properties of bone, some of these have also shown that this dependence is linked to the degree of mineralization (Torzilli et al., 1981, 1982; Carrier, 1983; Currey, 1984; Keller et al., 1985; Currey & Pond, 1989; Carrier & Leon, 1990; and Brear, Currey & Pond, 1990). Immature bone is relatively weak, and during post-natal ontogeny: 1) increases in strength and stiffness; and 2) decreases in strain at failure and work of fracture.

The wing skeleton of a growing bat must acquire structural and material characteristics that will enable it to withstand the mechanical pressures of flapping flight. This includes adequate mineralization to confer strength and stiffness, in concert with geometric specializations to resist torsional and or bending stresses. Young microchiropterans are capable of powered flight only when they approach adult linear dimensions (Powers, Kandarian & Kunz, 1991; Kunz & Robson, 1995). Flight musculature, motor programming, and flying skills are poorly developed at birth, mature rapidly, and reach an adult-like state just prior to the onset of flight (Yokoyama & Uchida, 1979; Norberg, 1990; Powers et al., 1991, Kunz & Robson, 1995).
The structural and mechanical characteristics of bone change during growth and maturation, so we ask: do these changes parallel the development of locomotor function in young bats? Do changes reflect the loads to which the bones are subjected? Do they constrain loads that can be applied to the growing skeleton? To address these questions, we investigated the patterns of wing skeleton mineralization during ontogenetic development in an insectivorous bat, Tadarida brasiliensis. This study is the first to examine the ontogeny of bone tissue in any chiropteran. Our analysis is based on a complete ontogenetic series of free-ranging animals, and should reflect the compromises and constraints imposed on these animals more directly than in any captive population. By examining changes in the mineral composition of the maturing wing skeleton, we should be able to draw inferences regarding its mechanical characteristics and gain a better understanding of the events associated with skeletal ontogeny that are of primary importance in the ability to meet the mechanical demands of powered flight.

Materials and methods

Study species

The Mexican free-tailed bat (Tadarida brasiliensis) is an aerial feeding, highly migratory, insectivorous microchiropteran species that dwells primarily in caves (Wilkins, 1989). It is capable of sustained flight for at least 5.3 hours during nightly feeding hours (Kunz, Whitaker & Waidanoli, 1995), and is characterized by high-speed, long-distance flights, averaging up to 50 km nightly (Williams, Ireland & Williams, 1973). Female T. brasiliensis give birth to a single pup after forming maternity colonies in early spring and suckle their young for 6 to 7 weeks (McCacken & Gustin, 1991). After this time, pups become independent of their mothers and begin to engage in foraging flights outside the maternity roost (Kunz & Robson, 1995).

Observations of stationary wing-exercises in young T. brasiliensis showed that juveniles first begin flexing their wings at 24 days of age, and reach a peak in non-flight wing activity at 36 days (Kunz & Robson, 1995). Wing area, wing loading, and aspect ratio reach adult-like values at 32–36 days of age (T. H. Kunz, pers. obs.). At 36 days, juveniles first engage in practice flights within the confines of their roost. They begin to venture outside the cave on foraging flights outside at about 42 days of age (Kunz & Robson, 1995). Because locomotor competence is necessary for foraging bats (Powers et al., 1991), the onset of foraging flights at 42 days and successful weaning a few days later can be considered the successful endpoint of the post-natal developmental period (Kunz & Robson, 1995).

Study specimens

Our study is based on examination of 38 specimens (33 juveniles ranging from 1 to 42 days of age and 5 adults) of T. brasiliensis. These animals were collected during the summers of 1987 and 1988 at Eckert James River Cave, Mason County, Texas as part of a study on post-natal growth and development (Kunz & Robson, 1995). Each animal was killed by cervical dislocation, weighed to the nearest 0.01 g and measured at the time of capture, marked for identification, frozen (−20 °C), and sealed in plastic bags. Each animal was assigned an age using predictive equations based on forearm and total epiphyseal gap measurements (Kunz & Robson, 1995). From one wing of each specimen, we dissected the humerus, radius, metacarpals II–V, and the phalanges of the third digit from soft tissue. The third digit was chosen for detailed analysis because this digit forms the wing's primary proximal to distal axis and plays an important role in conferring rigidity to the leading edge of the wing (Norberg, 1990), and because it appeared to be more developed at birth than the other digits.

After removal of the wing membranes, we carefully cleaned each bone with a soft toothbrush to remove traces of soft tissue. For the humerus and the radius, we used a Hamilton syringe to flush out the marrow. In
the metacarpals and phalanges, the marrow cavities were too small for this protocol, even in adult bats, in some distal wing bones marrow cavities were absent. After the bones were cleaned, we immersed them in acetone for 24 hours to extract fat. After 24 hours, we replaced the acetone, repeated this procedure, and placed the bones in a drying oven for 12 hours at 50-60°C.

**Ash determination**

We weighed each of the dried wing elements using a Cahn 29 Automatic microbalance, and determined their dry mass to the nearest 10 μg. We then determined the ash content of each element by burning all organic material in 2 ml ceramic crucibles in a Sybron Thermolyne 1400 muffle furnace at 600°C for 24 hours (Currier, 1983; Bohr & Schaad, 1985). We dried each element overnight at 60°C before measuring the final ash mass. We calculated bone mineral content or ash fraction as the ratio of the mass of the mineral phase of the bone to the mass of dry bone (Cowan, 1989):

\[
\text{mineral content} = \frac{\text{ash mass}}{\text{dry mass}} \times 100\%
\]

**Statistical analysis**

For comparison with known-age pups, we arbitrarily assigned all adults to an age of 45 days, corresponding to skeletal maturity, as their true ages were unknown and impossible to determine, and because bone mineral content typically shows no significant changes between skeletal maturity and the onset of senescence (Curry & Butler, 1975; Currier, 1983; Brear, Curry & Pond, 1990). Female bats may show decreases in bone mineral content during lactation (Kwecinski, Krook & Wimsatt, 1987), however, our sample included no lactating females. We adjusted percentage data prior to statistical analysis using an arcsin transformation. We used ANOVA to compare the degree of mineralization among bone elements and among age groups. If ANOVA revealed significant differences, we used the *post hoc* Scheffé F-test to determine where these differences occurred. We used least squares linear regression analysis to determine the relationship between mineralization and age; with one exception (mass vs. age), the high correlation coefficients in these regressions were not significantly improved with non-linear regressions or transformations. We employed ANCOVA to compare regression slopes, and multiple regression analysis, with both age and body mass as independent variables, to evaluate the individual and combined effect of these variables on bone mineral content.

**Histology**

We prepared histological sections of the undemineralized third distal phalanges of the left wings of 3 adults after finding that ash values were indistinguishable from zero using the protocol described above. Both longitudinal and transverse sections were cut to 50 μm and were stained with either Trichrome (to permit visualization of collagen) or von Kossa’s safranin specifically to detect hydroxyapatite. This latter protocol allows qualitative detection and localization of bone mineralization at levels far lower than those detectable using measurements of ash content (Page, 1982; Schenk, Olah & Herrmann, 1984).

**Plate 1 (a)** Representative cross-sectional view of an undemineralized third distal phalanx of an adult *T. brachyurus* (von Kossa’s safranin stained). Pale staining in the outermost cortex of the bone depicts a thin shell of unmineralized collagenous tissue. The remainder of the tissue, darkly stained, appears to be largely cartilaginous, characterized by large, unmineralized, vacuolated cells, and lacks a true medullary cavity. (b) Longitudinal section of an undemineralized third distal phalanx, along with the third distal interphalangeal joint and the distalmost portion of the middle phalanx of a *T. brachyurus* (von Kossa’s safranin stained). The dark staining indicates deposition of calcium phosphate, most likely in the form of hydroxyapatite. Mineral can be seen in the distalmost portion of the middle phalanx, but extends only a short distance into the proximal-most portion of the distal phalanx, attenuating just distal to the level of the DIP joint. The articular surfaces of both bones are capped with unmineralized hyaline cartilage.
Mineralization differed significantly among the elements of the wing skeleton over all ages (ANOVA, $F_{6,258} = 23.48$, $P < 0.001$) (Fig. 1, Tables I and II). According to Scheffé’s post hoc tests (Table I and II), metacarpals as a group did not differ significantly from one another in mineral content, but were less mineralized than the more proximal and more mineralized than the more distal elements. The humerus and radius are statistically similar in mineral content, as are the metacarpals. In the distal phalanges, no bone mineral is detectable by ashing at any age. Hence, mineralization decreases in a proximodistal gradient from the shoulder to the wing tip. Mineralization levels also differ significantly among age classes (< 10 days of age, 11–20 days of age, 21–30 days of age, 31–40 days, adults) (ANOVA, $F_{4,264} = 66.88$, $P < 0.001$) (Table II).

Both age and body mass showed significant correlations with ash content for each bone ($P < 0.001$). Age and mass are highly correlated with one another ($r = 0.926$), and both are highly correlated with all mineral content variables (all $r$ values $> 0.82$). Mineral content of each bone

**Fig. 1** Percentage ash content of wing bones of *Tadarida brasiliensis*. The horizontal line in the approximate centre of each box indicates the mean, the upper and lower bounds of the grey shaded boxes represent one standard error, the white boxes represent two standard errors, and the whiskers indicate the data range. Open circles denote adult values.
showed a more linear relationship to age than mass, and a somewhat higher correlation (Fig. 2). All age mineral content relationships were linear (Figs 3 and 4; Table IV), and in multiple regression analyses, age (all P values < 0.02) but not body mass (all P values > 0.08) showed a significant correlation with ash content for each bone. Thus, calcification of the wing skeleton in young Mexican free-tailed bats occurs in a temporally regular fashion, not strictly dependent on ontogenetic changes in body mass. Variation in mineral content among the elements of the wing is reflected in both ontogenetic rate of mineralization (slopes of the regressions of mineral content on age) and in mineral content values for a given age, as shown by significant differences in
Fig. 2 Changes in body mass in relation to age in *T. brasiliensis*. Mass is a polynomial function of age \( r = -0.005x^2 + 0.397x + 2.612 \) \((r = 0.922\) ). Mineralization of the wing skeleton is more highly correlated with age than mass.

Fig. 3 Changes in mineral content during post-natal ontogeny for the humerus, radius, metacarpal mean, and mean of digit III proximal and middle phalanges of *T. brasiliensis*. Distal phalanx III shows no detectable mineral using this method. Adults are indicated with uppercase letters.
intercepts for elements with statistically similar slopes. The humerus, which is the most mineralized of the wing bones in *T. brasiliensis*, also exhibits the least rapid ontogenetic rise in mineral content (slope = 0.28, Table IV). The radius has slightly lower ash fractions and a similar rate of increase in mineral content with age (slope = 0.31), but these slopes did not differ significantly (ANCOVA, \( F_{1,22} = 0.842, P > 0.05 \)). Of the five metacarpals examined here, the third had the greatest ash content both at birth and adulthood, and also had the lowest rate of mineralization (slope = 0.38) (Fig. 4). By contrast, the second metacarpal was the least mineralized at birth and adulthood and showed the most rapid increase in mineral content (slope = 0.45). The fourth and fifth metacarpals exhibited similar properties and also showed a relatively high rate of increase in mineral content with age (slope = 0.43 and 0.44, respectively). The differences in slope among the metacarpals were not statistically significant (ANCOVA, \( F_{1,22} = 0.963, P > 0.05 \), but do indicate a possible trend.

The proximal and middle phalanges of the third digit were the least mineralized wing elements at birth other than the non-mineralized distal phalanges. These elements also remained the least mineralized bones through adulthood, and increased in mineral content at a significantly higher rate than average for metacarpals (slope = 0.46). In turn, these elements increased in mineral content at a significantly higher rate than the humerus plus radius (ANCOVA, \( F_{2,20} = 12.01, P < 0.001 \)). The tendency for increased rate of mineralization with decreased total mineralization was statistically significant (Spearman rank correlation = -0.964).

**Histology**

Results from histological preparations permitted a closer examination of the apparent lack of mineral in the third distal phalanx. Trichrome and Van Kossa safranin-stained sections indicate a shell of collagenous material in the distal phalanx, typically less than 10 \( \mu \)m thick, with little or no detectable mineral deposition (Plate I). The collagen-rich tissue surrounds cartilage, characterized by large, unmineralized, vacuolated cells, with little or none of the distinct collagenous extracellular matrix that would be observed in maturing, as yet unmineralized osteoid. In contrast to the long bones of other vertebrates, there is no medullary cavity in this distal-most element (Plate I). In some longitudinal sections, the entire proximal epiphysis is preserved, together with the distal interphalangeal joint and the distal-most portion of the third middle phalanx (Plate I). In these specimens, dark staining areas, presumably hydroxyapatite, are observed in the distal part of the middle phalanx, capped by an articular surface of hyaline cartilage. However, this mineralization continues only a fraction of a millimetre along the length of this distal phalanx.
Figure 3: Changes in mineral content during post-natal ontogeny for MC II-V in T. brasiliensis.

Discussion

During post-natal ontogeny, bones show a progressive increase in stiffness and reduction in toughness (Torzilli et al., 1981, 1982; Carrier, 1983; Currey, 1984; Keller et al., 1985; Currey & Pond, 1989; Carrier & Leon, 1990; Brear et al., 1990). Clear differences have also been observed in the stage at which bones achieve a substantial amount of mineralization. Comparative studies have shown that skeletons of altricial mammal species such as primates (Homo sapiens, Macaca mulatta), bears (Ursus maritimus), and dogs (Canis familiaris) mineralize slowly, whereas those of precocial mammal species, such as axis deer (Axis axis) and black-footed jack rabbits (Lepus californicus), acquire properties similar to those of adults of altricial species soon after birth and subsequently go on to achieve higher levels of mineralization at maturity (Carrier, 1983; Currey & Pond, 1989; Brear et al., 1990).

This pattern of bone mineralization may reflect adaptative variation in locomotor ontogeny (Currey, 1984; Currey & Pond, 1989; Brear et al., 1990). Shortly after birth, precocial mammals, which have relatively stiff bones early in ontogeny, exhibit higher levels of locomotor performance than altricial species. The immature locomotor behavior of altricial mammals typically delays onset of intense skeletal loading, and these species achieve adult levels of bone mineralization much later in life (Carrier, 1983; Currey & Pond, 1989; Brear et al., 1990). The bones of new-born axis deer, for example, are highly mineralized early in development, displaying a relatively high Young’s modulus, and relatively low toughness (Currey & Pond, 1989). The high degree of mineralization should confer a selective advantage since the young must run
with the herd soon after birth. Locomotor proficiency and a high Young's modulus are less important for new-born nidicolous animals such as canids (Torzilli et al., 1981, 1982; Currey & Pond, 1989). The onset of effective locomotor performance in young animals often coincides with the attainment of mineralization levels that approach 88-90% of adult values (Carrier, 1983; Currey & Pond, 1989; Brear et al., 1990). The higher level of mineralization found in adult mammals does not appear to be required for the initiation of effective locomotion.

Among mammals, bats are altricial with respect to forelimb development and function; most insectivorous bats do not attain the ability to carry out powered flight until they have reached 90% of adult wing dimensions (Yokoyama & Uchida, 1979; Powers et al., 1991; Kunz & Robson, 1995). In comparison to other bats, T. brasiliensis is exceptionally altricial in this respect. Other bat species of similar or greater body mass achieve flight capacity much sooner in both developmental and chronological age (Kunz & Robson, 1995). The pattern of mineralization in the wing skeleton during post-natal ontogeny in T. brasiliensis is consistent with this highly altricial condition. In T. brasiliensis at birth, the mineral content of the humerus is only 58% of the values observed in the adult humerus. In fact, the proximal and middle phalanges of new-born pups have attained only about 21% of the maximum mineralization that will be achieved in the these elements at adulthood. In precocial species, limb bones have far higher mineral content values at birth and develop proficient locomotion early in life (e.g. 88.5% of adult values for Avus anser and 75.5% for Lepus californicus, Carrier, 1983; Currey & Pond, 1989).

Young T. brasiliensis first engage in practice flights within the confines of their roost at about 36 days of age (Kunz & Robson, 1995). Skeletal mineralization at that time must be adequate to meet the mechanical loads imposed on the wing during these early flights. At 36 days, the ash content of the humerus is 67%, the radius 64%, the metacarpals 58%, and the proximal and middle phalanges 44%. These values range from 75% of the adult mean for the phalanges to 93% of the adult value for the humerus. At 36 days of age, the radius and metacarpals have mineral content of 87 and 90% of the adult mineral content, respectively.

For the humerus, radius, and metacarpals, mineral content changes little from the onset of practice flights to 42 days of age, when young T. brasiliensis begin to engage in sustained nightly foraging flights. This suggests that, although the wing skeleton approaches mature levels of mineralization at a time corresponding to the onset of independent flight, skeletal mineralization is not the limiting factor delaying the onset of effective locomotor ability until 42 days. Muscle maturation, neuromuscular development, or other aspects of skeletal maturation, such as changes in geometry or distribution of bone mass, may be more or equally important in permitting effective flight. Because foraging success in insectivorous bats depends not only on well-developed locomotor skills, but also on echolocation ability (Kunz & Anthony, 1982; Norberg, 1990; Powers et al., 1991; Kunz & Robson, 1995), it may be the maturity of the echolocation system and not locomotor ability per se that prevents young T. brasiliensis from engaging in successful foraging flights before the age of 42 days.

Our results show that the humerus and radius achieve adult-like mineral content earlier in ontogeny than the metacarpals and phalanges. This is consistent with observations of bone development in other insectivorous bats. In Myotis myotis, M. lucifugus, and Rhinolophus hipposideros, ossification of the phalanges follows that of the metacarpals, subsequent to epiphyseal fusion in the humerus and radius, as demonstrated by both the persistence of epiphyseal plates and by ossification sequences, observed in cleared and stained specimens (Kunz & Anthony, 1982; Adams, 1992). It appears that mineralization of the distal phalanges is not
required for locomotor function in *T. brasiensis*, a similar lack of mineral is reported for the second and third distal phalanges of *M. lucifugus* (Adams, 1992).

The mechanism through which the variation in mineralization levels in wing elements of bats is achieved is not clear. Our data show that wing bones of *T. brasiensis* differ not only in mineralization levels but also in post-natal rate of mineralization. However, differences in mineralization rates fail to provide a simple explanation for the observed variation in adults. Rates of mineralization are most rapid in the metacarpals and phalanges, the elements that achieve the lowest adult ash levels. Hence, the adult pattern must be acquired by varying both the rate of bone mineral accretion and the absolute length of the period during which bone mineral will be deposited.

Variation in mineralization observed in the present study has important implications for understanding the mechanical properties of the wing skeleton. A number of studies have shown that mechanical properties of compact bone tissue can be predicted from the degree of mineralization (Currey, 1969, 1984, 1987, 1988; Borders et al., 1977; Carter et al., 1981; Schaffer & Burr, 1988). Comparative data indicate that species differences in the rate at which bones acquire age-related differences in mechanical properties are also determined largely by the rate of skeletal mineralization (Brear et al., 1990). We suggest that, as in other species studied, strength and stiffness in the wing elements of *T. brasiensis* should increase as a function of mineralization, while failure strain and work to fracture should decrease, contributing to profound changes in mechanical properties of the wing during the post-natal growth period. In particular, mechanical properties and mineralization are related in a non-linear fashion. If a reduction in mineral content in wing elements is related to flight performance, perhaps as a mechanism for promoting wing up deflection during downstroke, we would expect to find a pattern of distally decreasing mineralization in bats. Preliminary studies on *Pteropus poliocephalus*, an Old World fruit and flower-eating species, and on *Eptesicusfuscus* and *Myotis lucifugus*, two common North American insectivorous species, show the same general mineral distribution reported here. Although details of the pattern do vary (S. Swartz, unpubl.) Direct mechanical testing is needed to evaluate rigorously the mechanical performance consequences of reduced mineralization in bat wing bones and to relate accurately mineral content to material properties and the mechanical conditions under which bones function during flight. From mechanical tests, properties including Young's modulus, yield and ultimate stress and strain, and total work to fracture can be calculated. Only with these tests can the mechanical properties of different wing elements be assessed throughout ontogenetic development.

The variation in mineral content along the proximal to distal axis in the wing of a small insectivorous bat is a notable exception to the overall similarity in mineralization of other mammal bones (Currey, 1984, 1987, 1990). It is particularly striking that the distal phalanx is almost totally lacking in mineral. The large differences between the mineralization of proximal and distal wing elements suggest substantial variation in mechanical loading along the wing axis. *In vivo* bone strain measurements have shown that the humerus and radius of large flying fruit bats (*Pteropus poliocephalus;* 650–800 g) are subjected to large shear stresses due to torsional loads, and that their geometric properties (increased outer diameter and reduced cortical wall thickness in comparison to mammals of comparable size) maximize torsional resistance (Swartz et al., 1992). The proximal wing elements have mineral content similar to that of other mammals, and are, presumably, similarly stiff and rigid. These are the wing elements on which the primary propulsive (e.g., pectoralis, serratus anterior, subscapularis, clavodeltoideus) and upstroke (e.g., biceps brachii) muscles insert. These muscles also define downstroke angle of attack and
help control rotational stability (Vaughan, 1959; Altenbach & Hermanson, 1987; Norberg, 1990). It has also been suggested that the humerus and radius play an important role in stabilizing the wing during downstroke, forming an inflexible brace for the anterior edge of the proximal wing segment that requires great rigidity (Vaughan, 1959).

The geometry of the metacarpals and the phalanges, however, contrasts markedly with that of the proximal elements and with other mammalian limb bones in having greatly increased cortical thickness, sometimes to the extent that the medullary cavity is completely obliterated (Swartz et al., 1992). For a given cross-sectional area, lack of a medullary cavity will minimize moments of inertia, thereby maximizing non-axial stresses for a given applied load. The geometry of distal wing bones, then, is poorly suited to resist torsion, instead promoting an unusually large degree of bending for a particular force. Direct measurements show that these elements experience bending rather than torsional stresses, with higher strains than those reported from any other vertebrate bones (Swartz et al., 1993). We suggest that the difference in the degree of mineralization in these two wing regions also directly reflects the function of the wing skeleton to resist torsion proximally and promote bending distally.

In terrestrial animals, only a limited amount of deflection can be tolerated in the limb skeleton without creating problems in the control of limb position and the generation of propulsive force. This is much less of a problem for a flying animal, provided that the deflection does not fracture or irreversibly deform the bone. Although it is not necessary for wing bones to support the impact loads of ground contact, the wing tips must be flexible and resilient enough to deflect without permanent tissue damage. Reducing mineral content decreases stiffness and increases toughness and resilience. The mineral content of the proximal and middle phalanges in T. brasiensis is similar to that of red deer (Cervus elaphus) antler (59%), the archetypal example of a bone adapted for increased toughness and decreased mass at the expense of stiffness and strength (Currey, 1984). The cartilaginous distal phalanges of T. brasiensis have even lower mineral content, approaching zero, suggesting that this element will no doubt prove to have further reduced stiffness and strength.

The low degree of mineralization of the phalanges in adult T. brasiensis resembles immature bone in other mammalian species (Carrier, 1983; Keller et al., 1985), suggesting that these bones have comparable ability to deform significantly without risk of fracture (Torzilli et al., 1981). This feature is believed to be a mechanism for preventing destructive loading via the absorption of energy (Torzilli et al., 1981). We propose that the ability of the phalanges of bats to deform in the manner of immature bone is advantageous in flight, conferring resilience to the wing and accommodating the large stresses generated by relatively small bending loads in greatly elongated elements.

The combination of reduced mineral content and increased cortical thickness in the distal wing bones of bats could be a mechanism for resisting bending failure. If energy absorbing capacity is critical, as in immature bone, tissue volume will be a primary determinant of functional capacity, and increased cortical thickness will provide increased bone volume without increasing bone diameter in a way that might disrupt air flow over the distal wing. Furthermore, long, slender bones are prone to local buckling instability under axial compression or, on the compressive surface, under bending (Wainwright et al., 1976; Alexander, 1981; Currey & Alexander, 1985). Increased wall thickness increases the threshold at which loads initiate local buckling behaviour, and could therefore allow the bones to withstand increased bending without risk of developing local kinks on the compressive surface of the bone. In general, thick-walled bones are stronger in bending than thin-walled bones if the animal is able to tolerate some permanent deformation (Currey & Alexander, 1985).
The metacarpals and phalanges of bats are more flexible as a result of being less mineralized, but can still confer the necessary rigidity to the wing via specialized ligamentous connections and joint morphology. Keeping the wing steady without involving large muscular forces (Vaughan, 1959, 1970; Norberg, 1969, 1970, 1972). The low degree of mineralization in distal elements is likely to be driven in part by energetic considerations. The mass moment of inertia of the wing is a function of each increment of mass multiplied by the square of its distance from the wing’s centre of rotation at the shoulder. This mass moment of inertia, in turn, is directly proportional to the energetic cost of wing oscillation, and this portion of the energetic cost of locomotion in mammals is between 5 and 25% of the total locomotor energy budget (Fedak, Heglund & Taylor, 1982; Norberg, 1990: Thollesson & Norberg, 1991).

The ability to fly has played an important role in the ability of bats to radiate widely and adopt a number of highly specialized trophic strategies, leading to exploitation of a variety of foraging niches inaccessible to other mammals (Norberg & Rayner, 1987; Norberg, 1990). Powered flight guarantees high agility and fast travel speeds, and it is these characteristics that give bats the ability to locate and acquire food (Norberg & Rayner, 1987). Specializations of material characteristics of limb bones may be a critical element in the integrated structural design of the wing skeleton, and may well have facilitated the acquisition of flight in this unique mammalian order.

We gratefully acknowledge the contributions of Fred Wasserman, April Allgaier, Antonio Alvarez, David Carr, Jennifer Gray, Chuckering, Erika Mitchell, and Antigone Parker to earlier versions of this manuscript. Elizabeth Stockwell provided field assistance. Clinton and Anne Schulze and J. Andrews hospitality, and the Texas Department of Parks and Wildlife permission to collect bats. Anne Schmitt generously provided lab facilities. Histological material was expertly prepared by Paul Montilly, Central Research Laboratories, Rhode Island Hospital. Amy Ritter provided invaluable technical assistance. This research was supported by National Science Foundation grants to SMS (IBN-9119413) and THK (BSR-8700885).

REFERENCES


