

PRENATAL GROWTH AND DEVELOPMENT IN THE ANGOLAN FREE-TAILED BAT, *MOPS CONDYLURUS* (CHIROPTERA: MOLOSSIDAE)

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We present data on prenatal growth, development, and skeletal ossification for the Angolan free-tailed bat (*Mops condylurus*), a species distributed throughout sub-Saharan Africa. Specimens were measured for crown–rump length (CRL), greatest length of the skull (GLS), forearm length, mass, and wing area. We cleared and differentially stained specimens for cartilage and bone to quantify pattern of skeletogenesis. Significant regressions for general growth trajectories were generated by plotting CRL and fetal mass against GLS. We quantified growth of the forearm, which showed a positive relationship with growth of the skull. Curiously, wing area was highly positively related to fetal mass, suggesting an ecomorphological relationship of wing loading and flight ability being established early in development in this species. Patterns of ossification in this species were more similar to those of phyllostomid and pteropodid bats than they were to vespertilionid bats, to which *M. condylurus* is apparently more closely related.

Key words: Angolan free-tailed bat, bats, development, growth, *Mops condylurus*, skeletogenesis

In this paper, we provide the 1st published account of prenatal growth, development, and skeletal ossification for the Angolan free-tailed bat, *Mops condylurus* (Chiroptera: Molossidae). Understanding developmental patterns provides key insights into the evolution of form and function as well as the origins of key evolutionary innovations (Jablonka and Lamb 1998). Indeed, heterochronic shifts in developmental timing are in large part responsible for the evolutionary diversity of the vertebrate limb (Smith 2003).

The evolution of wings in mammals was undoubtedly governed by alterations in developmental events at the molecular level (Sears et al. 2006), resulting in ontogenetic outcomes that differ from ancestral mammal morphologies (*Acomys*–Joller 1977; *Rattus*, *Sorex*, and *Mus*–Adams 1992a, 1992b, 2000). Gross anatomical differences between bats and other mammals (i.e., overall reduction in bone mass [Allen 1891; Vaughan 1959, 1970; Wassif and Madkour 1963], the scapular–humeral lock [Hermanson and Altenbach 1985], and structures such as the calcar in microchiropteran and the uropatagial spur in megachiropteran bats [Schutt and Altenbach 1997; Schutt and Simmons 1998]) have been long documented and shown to be the product of ontogenetic divergence in prenatal bats (see

Adams and Pedersen [2000] for review; Giannini et al. 2006). Further, a recent study showed that elongation of the forearm, the metacarpals, and the phalanges in bats is apparently the product of changes in the limb formation regulatory gene *BMP2* (Sears et al. 2006).

The intent of this paper is to provide the 1st descriptive analysis of prenatal growth and development of the Angolan free-tailed bat by integrating data from changes in body size, wing area, and skeletogenesis. This species is distributed throughout diverse habitats in sub-Saharan Africa (Koopman 1993; Skinner and Smithers 1990) and much is known of this bat's natural history, especially reproductive biology (Happold and Happold 1989) and thermoregulation (Maloney et al. 1999). *M. condylurus* roosts in hollow trees, rock crevices, and human buildings (Rautenbach 1982). Females in Malawi, Central Africa, were normally monotonous, having 2 births per year, but intervals between pregnancies varied with latitude (Happold and Happold 1989).

To our knowledge, no data have been published on prenatal growth and development for this species. Herein we describe fetal growth in *M. condylurus* using measures of crown–rump length (CRL), greatest length of the skull (GLS), length of the forearm (FAL), and wing area on preserved specimens. In addition, we describe skeletogenesis in whole-mount embryos and compare bone development sequences among species from several families of bats. All specimens were graciously provided by the Harrison Zoological Museum, Seven Oaks, United Kingdom.

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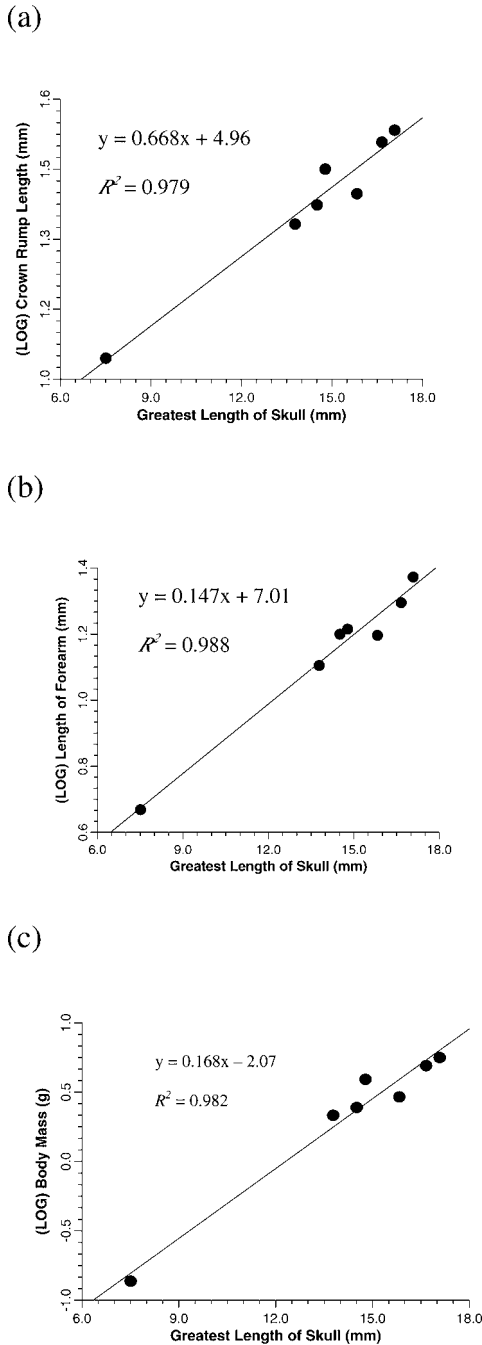


FIG. 1.—Regression analysis of growth in *Mops condylurus*. a) Plot of crown-rump length against greatest length of the skull. b) Plot of forearm length against greatest length of the skull. c) Plot of body mass against greatest length of the skull.

MATERIALS AND METHODS

Specimen collection.—A total of 7 fetuses representing a range (middle to late gestation) of prenatal development were dissected by one of us (RAA) from preserved adult specimens of pregnant females housed in the mammal collections at the Harrison Zoological Museum. All specimens were collected from east African countries of Congo, Tanzania, and Malawi in 1956, 1957, and 1959 and stored in a 75% ethanol solution.

Morphometrics.—Using Mitutoyo digital calipers (Tyler Tool Co., Tylertown, Mississippi), we measured 4 morphological features to establish growth trajectories, including CRL, GLS, and FAL. Each specimen was measured for each variable 3 times to the nearest 0.01 mm and an average was derived. Each fetus was weighed 3 times using an Acculab scale (Acculab, Arvada, Colorado) accurate to 0.001 g and an average was taken. Measurements of mass reflect each specimen’s preserved weight. We soaked each fetus in water for 20–30 min to loosen soft tissue and allow for full extension of the right wing, which was then pinned out in a standardized fashion onto graph paper. A digital photomicrograph was taken using an Olympus SR1000 dissection scope fitted with an Olympus digital camera system (Olympus Inc., Minneapolis, Minnesota). Micrographs were loaded into SPSS Sigma-Scan Pro 4.0 (Aspire Software International, Ashburn, Virginia) measurement software for relative scaling and calculations of wing areas (mm^2).

Statistics.—To generate growth trajectories, multiple regression analysis was performed on log-transformed CRL, FAL, and body mass (g) against GLS. In addition, we regressed wing area (mm^2) against body mass and GLS and percent ossification of the forearm against GLS. All regressions were run using NCSS Statistical Software (NCSS, Kaysville, Utah).

Quantifying ossification.—Specimens were cleared and stained using a procedure published by Hanken and Wassersug (1982) for amphibians and modified slightly for bats (Adams 1992a). We used alcian blue to stain for cartilage and alizarin red to stain for calcium in developing bone of each fetus representing different stages of growth and development. After staining, we cleared specimens in a series of glycerin baths and stored them in 100% glycerin.

RESULTS

Prenatal growth and development.—The specimens represented a clear developmental series from early bone development to just before parturition. Measurements of CRL of fetuses ranged from 11.09 mm to 34.17 mm and CRL was related positively to growth of the skull, GLS ($R^2 = 0.979$, $P < 0.001$; Fig. 1a). Because of the significant regression of CRL with GLS, we used GLS as the independent variable in other plots because hard tissue such as bone allows for more accurate measurements than does more pliable soft tissue such as that measured in CRL. FAL ranged from 4.66 mm to 23.59 mm and was positively related to GLS ($R^2 = 0.988$, $P < 0.001$; Fig. 1b). Measurements of fetal mass ranged from 0.137 g to 5.611 g and also were positively related to GLS, but less closely ($R^2 = 0.982$, $P < 0.001$; Fig. 1c) than was FAL. Growth in area of the wing was positively related to body mass ($R^2 = 0.959$, $P < 0.001$; Fig. 2a), and the relationship was closer than that of area of the wing with GLS (Fig. 2b).

Ossification.—*Mops condylurus* showed extensive ossification of the cranial and postcranial skeleton prenatally. In fact, the skull and mandible were almost completely ossified (cranial sutures are apparent) well before birth (at 44% of parturition GLS). Ossification of the forearm was positively related to

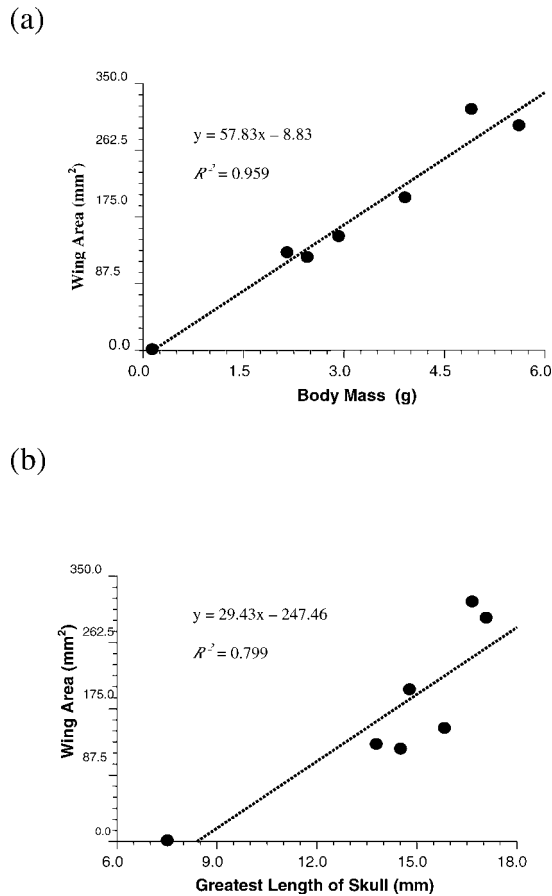


FIG. 2.—Regression analysis of wing area against a) body mass in grams and b) greatest length of the skull.

growth of the skull ($R^2 = 0.77$, $P < 0.05$; Fig. 3), but lagged relative to skull ossification. For our earliest developed specimen (CRL = 11.09 mm, representing 32.4% of parturition size, and GLS = 44% of parturition length), ossification of primary (1°) ossification centers of much of the entire appendicular skeleton had occurred (Table 1). Ossification of secondary (2°) ossification centers of the femur, tibia, and fibula occurred at a CRL of 23.63 mm, which was 69.1% of body size at parturition (GLS = 14.51 mm, 85% of parturition length).

DISCUSSION

Herein we provide the 1st description and analysis of prenatal growth, development, and skeletogenesis in the molossid bat *M. condylurus*. Although some data are available on mineralization of the humerus, radius, and metacarpals in postpartum development of the molossid bat *Tadarida brasiliensis* (Papadimitriou et al. 1996), ours is the 1st account, to our knowledge, on development of this species in whole-mount fetuses. Previous studies have shown wide variation in prenatal growth and development as well as ossification among species of bats. Surprisingly, patterns of development and growth, especially ossification, in *M. condylurus* differed substantially from those observed previously in vespertilionid bats such as the little brown bat (*Myotis lucifugus*, family

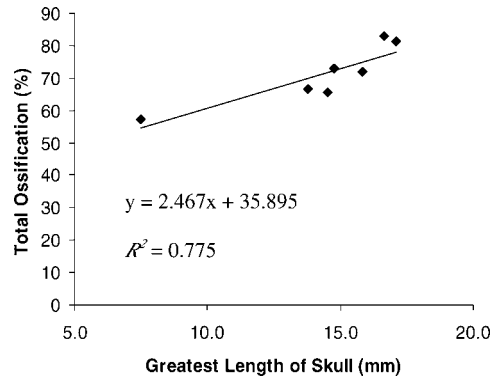


FIG. 3.—Plot of total ossification (defined as lengths of primary [1°] and secondary [2°] ossification centers added together) of the forearm plotted against greatest length of the skull.

Vespertilionidae; Table 2). Actually, prenatal growth, development, and ossification in *M. condylurus* appear most similar to those observed in phyllostomid bats such as the Jamaican fruit bat (*Artibeus jamaicensis*) and pteropodid bats such as *Rousettus celebensis* (Adams 2000; Table 2). More specifically, the same overall proportion of 1° ossification in the appendicular skeleton we observed in *M. condylurus* occurred much earlier in development relative to that observed in *M. lucifugus* (Table 2). In addition, 2° ossification of the femur, tibia, and fibula began prenatally in *M. condylurus*, whereas in *M. lucifugus* these events occurred postnatally (Adams 1992b). Ossification of the humerus, radius, and ulna of *M. condylurus* had significant 1° ossification centers when GLS was 32.4% of GLS at parturition, whereas in *M. lucifugus*, ossification of these elements had not occurred until the fetus had reached 70% of its parturition GLS (Adams 2000).

TABLE 1.—Elements showing some degree of ossification including the appearance of primary (1°) and secondary (2°) ossification centers relative to crown–rump length (CRL in mm) and greatest length of the skull (GLS in mm) for *Mops condylurus*. Specimen numbers are given in parentheses.

CRL GLS	Elements showing some level of ossification
(18272c) 11.09 7.51	Cranial bones (all), mandible, clavicle, humerus (1°), radius (1°), ulna (1°), metacarpals, femur (1°), tibia (1°), fibula (1°), proximal phalanges (pes), ilium, sacrum, transverse processes (all vertebrae), scapula, ribs
(18272b) 21.49 13.78	Metatarsals; medial and distal phalanges (pes); tarsals (all); proximal, medial, and distal phalanges (manus); sternum (manubrium, body, and xiphoid)
(11271) 23.63 14.51	Carpals, ischium, pubis, femur (distal 2°), tibia (proximal 2°), fibula (proximal 2°)
(18272a) 32.20 16.66	Humerus (proximal and distal 2°), femur (proximal 2°), tibia (distal 2°), fibula (distal 2°)
(18269) 34.17 17.08	Radius (proximal 2°), ilium, ischium, pubis (fully ossified and fused), sternum (fully fused), pes (fully ossified), femur (distal 2°), tibia (fully ossified), fibula (proximal 2°), vertebrae (fully ossified), sternum (fully ossified)

TABLE 2.—Prenatal sequence of formation of primary ossification centers of forearm bones in representative species of a molossid bat (*Mops condylurus*), vespertilionid bat (*Myotis lucifugus*), phyllostomid bat (*Artibeus jamaicensis*), and pteropodid bat (*Rousettus celebensis*) as a percentage of greatest length of the skull at parturition. PN = postnatal.

Bone(s)	<i>M. condylurus</i>	<i>M. lucifugus</i>	<i>A. jamaicensis</i>	<i>R. celebensis</i>
Humerus	32%	70%	32%	33%
Radius	32%	70%	32%	33%
Ulna	32%	70%	32%	33%
Carpus	85%	PN	100%	69%
Metacarpus	44%	84%	44%	69%
Digits	44%	84%	44%	60%

The ossification rate of the forelimb of *M. condylurus* (Table 2) was much higher than that of *M. lucifugus* (Adams 1992a) and was more similar to that quantified for phyllostomid bats such as *A. jamaicensis* (1° ossification centers of the humerus, ulna, and radius at 32% parturition GSL) and pteropodid bats such as *R. celebensis* (1° ossification at 33% parturition GLS—Adams 2000).

Nearly, if not fully, complete ossification of the tibia, sternum, bones of the pes, all vertebrae, and all bones of the pelvic girdle occurred prepartum in *M. condylurus*, whereas these elements were not fully ossified until well into postpartum development in *M. lucifugus*. Indeed, the only member of the pelvic girdle to show some level of prepartum ossification in *M. lucifugus* was the ilium and that event occurred at the last stage (stage 7—Adams 1992b).

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