AN ABSTRACT OF THE THESIS OF

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Title: Phylogenetic relationships derived from neonatal development in *Peromyscus maniculatus, Sigmodon hispidus*, and *Mus musculus*

Abstract approved: [redacted]

The relationship among many closely related rodent species is not completely understood because it is primarily based upon morphological characters, which vary only slightly among species. The accepted phylogenetic relationship between *Sigmodon hispidus, Peromyscus maniculatus,* and *Mus musculus* indicates that *S. hispidus* and *P. maniculatus* are more closely related to each other than either are to *M. musculus*. The purpose of my research was to study the developmental patterns of the three species of mice; *S. hispidus, P. maniculatus*, and *M. musculus* and attempt to distinguish any similarities in ontogenic patterns that could be used to support or reject the known phylogenetic relationships. Laboratory-born neonates were collected from day 1 through day 21 after birth for each species. The neonates' tissues were cleared and the bones stained with an Alizarin Red S bone staining technique. The range of days of first appearance of calcification in the epiphyses, complete fusion between the epiphyses and the diaphysis, and complete calcification of the diaphysis of the long bones of the limbs were earlier in *S. hispidus* and *P.maniculatus* than in *M. musculus*. The rates of decrease in the lengths of the epiphyseal plates and the rates of calcification of the diaphysis were faster in *S. hispidus* and *P. maniculatus* than in *M. musculus*. On the other hand, the rates

of growth of the cranial bones and the rates of increase in the overall length of the long bones were fastest in *M. musculus*, with *P. maniculatus* being the slowest. The similarity in the rates and timing of developmental events between *S. hispidus* and *P. maniculatus*, compared to *M. musculus*, supports the known phylogenetic relationship.

PHYLOGENETIC RELATIONSHIPS DERIVED FROM NEONATAL DEVELOPMENT IN *PEROMYSCUS MANICULATUS*, *SIGMODON HISPIDUS*, AND *MUS MUSCULUS*

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PREFACE

This thesis was written in the style required by *Evolution*.

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INTRODUCTION

The theory of evolution via natural selection is often referred to as the greatest unifying theory of biology. However, the fatal weakness of early biologists was their attempts to explain evolution by single-factor definitions (Keller and Lloyd, 1992). The term evolution commonly refers to a change in a species over time. However, evolution did not always carry this specific set of meanings. In actuality, the term evolution in and of itself is regarded as an evolved product (Keller and Lloyd, 1992). History provides ever-changing definitions and theories surrounding evolution that range from a description of embryological development to its bridging function in the principle of recapitulation (Keller and Lloyd, 1992). However, breakthroughs in the field of evolutionary biology have broadened the scope of evolution to be much more inclusive and to encompass a larger range of evolutionary topics. Genetics, morphology, biogeography, systematics, paleontology, embryology, physiology, ecology, and other branches of biology all have illustrated some special aspect of evolution and have contributed to the total explanation where specialized fields have failed (Mayr, 1970). By expanding the concise summary of the complex natural phenomena of evolution we are able to see its implications for defining phylogenetic relationships among organisms (Hanson, 1977).

All organisms have the capability to evolve, however, the rates of evolution and the timing of divergence varies between different orders, species and even between regions of genomes and genes (Fieldhouse et al., 1997). One of the best examples of rate variation is the two to ten times faster rate of genomic evolutionary changes found in the

Order Rodentia as compared to most mammal species. The rapid divergence exhibited in the Order Rodentia provides the scientific community with an excellent avenue to explore evolutionary events in a short amount of time in an attempt to explain phylogenetic relationships (Hall, 1992). This rationale is the primary reason I chose to work with three species of mice; *Peromyscus maniculatus, Sigmodon hispidus*, and *Mus musculus*. Rodentia provides closely related species that inhabit a wide variety of habitats and experience many different environmental conditions. However, the phylogenies of most rodent species are not completely understood because morphological characters often do not differ greatly between species. Generally, the only morphological variables that have been used in constructing phylogenies are dental, cranial, soft body parts, size, and color (Fieldhouse et al., 1997). Therefore, if all species of the Order Rodentia are morphologically similar, what could be gained by the study of morphological characters and what benefit would these data have on unraveling the mysteries of phylogenetic relationships? Morphological studies, although seemingly primitive in light of advances in molecular techniques, offer many distinct advantages over molecular data. For example, it is unlikely that the phylogenetic information preserved in the fossils of extinct taxa will ever be recovered through molecular techniques. However, morphological studies of the same fossils are at least capable of identifying gross similarities between the extant taxa and their phylogenetic relationships to their potential descendents (Grande and Rieppel, 1994). Many present day morphologists believe that a rich amount of morphological data has yet to be fully uncovered, which could be useful as distinguishing phylogenetic characters. Morphologists today are employing morphological data into phylogenetic analyses in primarily two ways: (1) mapping their data onto presently

accepted cladograms generated from data based on other characters to assess congruence and (2) creating new cladograms to answer questions of phylogenetic relationships by adding their data to existing data sets such as molcular data and systematic data. The second approach is commonly referred to as the "total information" or "total evidence" approach and is championed by Kluge (Grande and Rieppel, 1994). Kluge believes that data from diverse sources such as morphological, molecular, and others should be compiled into one data set for phylogenetic analysis. Systematists believe that any points of noncongruence between an existing cladogram and the newly generated cladogram are areas that require further study (Grande and Rieppel, 1994). I propose that to increase the validity of morphological characters one should not simply examine the adult form, but should investigate the actual developmental process from the neonate to the exhibited adult morphology. Developmental mechanisms provide insight about the interactions and correlations between characters and are useful in identifying species unique characters for phylogenetic analyses (Salthe, 1993). Developmental studies encompass and integrate a myriad of morphological data such as in the analysis of teratology, experimental manipulations of the actual developmental processes themselves, and the ability to provide detailed analysis of intra- and interspecific variations in the developmental process under investigation (Salthe, 1993). Development is defined in terms of ontogeny as regulated growth in an ordered sequence of events resulting in differentiation and increasing complexity in an individual or lineage (Lincoln et al., 1998). The study of ontogeny is an invaluable means for deciphering polarity of characters to establish systematic relationships among taxa (Alberach, 1985). Developmental mechanisms can also be used to identify individual characters for future

use in phylogenetic analyses (Grande and Rieppel, 1994). In my study, I chose to focus on the process of bone development and calcification in a comparative tradition as viewed as a sequence of stages in a temporal order process. A comparative approach is a key element in analyzing evolutionary relationships. There are many distinctive advantages of utilizing a comparative method. Comparisons may potentially assess differences in developmental patterns to determine phylogenetic relatedness and/or diversity. A second advantage of a comparative approach is the potential to provide detailed phylogenetically based information for use in testing overall evolutionary relationships based on other characters (Hanken, 1993). Phylogenetic analyses of comparative developmental processes are key in the analysis of form because both development and character state comparisons are important in the determination of homologies (Grande and Rieppel, 1994). To be able to compare different species' ontogenies in this manner one must be able to recognize any homologies among the ontogenies of the study species (Alberach, 1985). Homologous characters are defined as traits that share a common evolutionary transformation from the same ancestral character states (Lincoln et al., 1998). In morphological data or comparative developmental studies, one must assume homology between the characters being compared. It is similarities in derived homologous traits that are indicative of phylogenetic relatedness and yet accepted phylogenies are used in the determination of homology (Funk and Brooks, 1996). However, the determination of homology in the analysis of morphological characters is simplified to no more than the recognition of similarities broadly distributed among taxa (Grande and Rieppel, 1994). In my comparative developmental study, I assume homology among the bones of all three study species as

the basic construction plan of the tetrapod limb has not changes since the Devonian period (Carter et al., 1991). The forelimb consists of a series of parallel bones, the radius and ulna, and a single upper arm bone, the humerus, and an array of hand bones. The hindlimb also consists of a set of parallel bones, the tibia and fibula, a single upper leg bone, the femur, and a set of foot bones. The reoccurrence of the unaltered limb plan is reinforced by the appearance of similar endochondral ossification and calcification patterns (Carter et al., 1991). This logic provides the rationale for my research in defining the developmental patterns of each of the three species of mice *S. hispidus, P. maniculatus*, and *M. musculus* and identifying any species' unique ontogenic patterns that may lend insight into accepted phylogenetic relationships among the three species.

There are two types of ossification, intramembranous ossification and endochondral ossification. Intramembranous ossification is the simpler of the two and is the formation of bone directly on or between fibrous membranes. This type of ossification is exhibited in the surface skull bones and the clavicles. The type of ossification, which I have chosen to highlight in my research, is endochondral ossification; the process by which most of the bones of the body, including the long bones and parts of the skull, are formed (Tortora and Anagnostakos, 1987). All three study species of mice follow the same process of endochondral ossification of the long bones. Early in embryonic development a cartilage template of the bone is formed where the future bone will be laid down (Tortora and Anagnostakos, 1987). The embryonic endoskeleton actually attains a high level of complexity before the actual process of ossification begins (Maisey, 1988). The beginning of ossification occurs near the middle of the template when a blood vessel actually penetrates the perichondrium, the membrane

covering the cartilage template. This penetration stimulates cells in the perichondrium to increase in size and become osteoblasts around the middle of the diaphysis, which is the first point where compact bone begins to form (Tortora and Anagnostakos, 1987). As the bone begins to appear, the cartilage cells in the middle of the diaphysis begin to enlarge until they burst causing the pH to become more alkaline resulting in the beginning of calcification at the primary calcification center. As compact bone is laid down, the intracellular cartilage cells are nutrient deprived and begin to degenerate leaving cavities for blood vessels to bidirectionally lengthen and enter each epiphysis which will serve as secondary calcification centers. After the appearance of calcification in the epiphyses, cartilage remains between the epiphysis and the diaphysis as the epiphyseal plate. The epiphyseal plate consists of four zones: (1) the zone of reserve cartilage, (2) the zone of proliferating cartilage, (3) the zone of hypertrophic cartilage, and (4) the zone of calcified matrix (Tortora and Anagnostakos, 1987). The zone of reserve cartilage anchors the epiphyseal plates to the epiphyses and the zone of proliferating cartilage makes new chondrocytes to replace degenerating chondrocytes at the surface of the diaphysis. The zone of hypertrophic cartilage is arranged in columns and is responsible for the lengthening of the epiphyseal plate by constantly enlarging chondrocytes. The zone of calcified matrix becomes the area where the epiphyseal plate attaches to the newly formed bone of the diaphysis. Once the epiphyses and the diaphysis are fused together, the only remainder of the epiphyseal plate on the bone itself is a bony structure called the epiphyseal line (Tortora and Anagnostakos, 1987).

The process of ossification and calcification of bones is extremely detailed and complex. Even though the process itself remains in the same sequential order, I have

chosen to focus on the timing and rates of these developmental events in each of the three species. By examining the rates and timing of these pivotal events in the formation and calcification of the long bones, I am beginning to delve into the issue of heterochrony. Heterochrony is defined as a change in timing or rates of developmental events, for example the dates of the first calcification of a bone (McKinney and McNamara, 1991). Therefore, my research, by investigating and outlining the developmental patterns of ossification and calcification of the long bones of three species of mice will bring to light any heterochronic differences among and within each of the study species; data which could be used in supporting or refuting the known phylogenetic relationships among the three species. I will also be examining the presence of allometric growth as defined as differential growth of body parts (Lincoln et al, 1998).

The objective of this research is multifaceted and was designed is to provide insight into the following questions: (1) is a comparative approach an appropriate and useful tool in this morphological study; (2) do these morphological data support or refute the accepted phylogenetic relationship among the three study species; (3) are there any differences in the timing of developmental events between or among the three species; and (4) are there any apparent species' unique ontogenic patterns?

MATERIALS AND METHODS

MAINTENANCE OF ANIMALS

Peromyscus maniculatus and *Sigmodon hispidus* were collected from northern Lyon County, Kansas. Animals were trapped, using Sherman live traps, from March 1999 to August 1999. Reproductive adults were transported in the Sherman traps to the Emporia State University wild animal lab. A total of 10 male and 8 female *P. maniculatus* and 11 male and 12 female *S. hispidus* were trapped and placed into captivity in the Wild Animal Laboratory at Emporia State University. A total of 10 male and 20 female *Mus musculus* were taken from the Emporia State University domesticated animal laboratory. One male, one female, and any resulting offspring for *P. maniculatus* were housed in 152 mm x 305 mm cages with food and water provided *ad lib*. One male, one female, and any resulting offspring for *S. hispidus* were housed in 229 mm x 457 mm plastic cages with food and water provided *ad lib*. All male and female *M. musculus* were also housed in 152 mm x 305 mm plastic cages; however, they were kept in the domestic animal room at Emporia State University. Cages were lined with a combination of corn cob and wood shavings for bedding. A small handful of polyfil was placed in each cage to be used as nesting material. All animals received a diet of rat/mouse chow and black oil sunflower seeds to ensure a high fat and protein diet to increase the likelihood of breeding. The photoperiod was maintained at 14 L:10 D for the duration of the study. The cages and water bottles were cleaned once a week, and all bedding, food, water, and nesting material were replaced. The water and food supply were checked daily and replenished as needed. The care and use of the animals were in strict accordance with the Federal Regulations and Emporia State University Animal

Care and Use Policy. One neonate per day from any given litter was euthanized with ether up to day 21. After birth , any given day until day 21 was represented by 3 - 5 neonates. The average litter sizes were 5.1 for *P. maniculatus*, 6.4 for *S. hispidus*, and 11.4 for *M. musculus*.

BONE STAINING TECHNIQUE

To examine the developmental patterns and ossification rates of the bones of the three species of mice I used a specialized bone staining protocol described by Ragsdale and Moore (1992). The technique allowed me to view the specimens' skeleton completely articulated. The growth plates were visible as unstained regions in contrast to the calcified portions of bone, which appeared deep purple in color.

The first step of the bone staining is to skin and eviscerate the neonate removing all internal organs including the eyes, esophagus, and trachea. The specimens were then submerged in glass jars filled with a 1% potassium hydroxide solution for two days as an initial step to clear the muscles and tissue to make the bones visible for observation. The samples were then rinsed with water and placed in a working stain (2 ml of 0.4% Alizarin Red S; a stain specific for bones, 200 ml 1.9% potassium hydroxide, and 40 ml distilled water) for two to four days depending on the size of the specimen. The samples were then removed, rinsed with water, and placed in a glycerin-based preservative (100 ml 70% ethanol, 50 ml benzyl alcohol, and 100 ml glycerin) for three hours then transferred into a 46° C water bath for one hour to speed up the preservation process. The specimens were finally placed in sealed 80 mm x 200 mm test tubes containing pure glycerin for final storage (Ragsdale and Moore, 1992).

MEASUREMENTS

Dial calipers were used to measure the length of each of the long bones; the humerus, radius, ulna, femur, tibia, and fibula. All measurements were taken from the right side of the animal, assuming symmetry between the sides, and were accurate to the nearest 0.05 mm.

To measure the length of the primary calcification center of the diaphysis and the subsequent changes from day to day, I used an ocular micrometer (100 units on the ocular micrometer = 5 mm) mounted in a dissecting scope at 10X magnification and recorded the length of the calcified area of each of the six long bones, all measurements were then converted to millimeters. The calcified area was the darkest stained area of the long bone. I measured from the end of the diaphysis of the long bone to the line on the bone where the dark area and lighter area met (Figure 1). The darker the stained area, the more calcified the region of bone. At the point where a clear dividing line between the calcified and uncalcified areas of the diaphysis was unable to be distinguished, and the diaphysis appeared to be totally calcified I recorded a FC which denoted that the diaphysis was fully calcified.

To measure the calcification of the epiphyses, I recorded the day of first appearance of the secondary calcification centers in the epiphysis at both the proximal and distal ends of each of the long bones. Once the secondary calcification centers appeared I then used an ocular micrometer (80 units on the ocular micrometer = 2 mm) at 20X magnification to measure the length of the epiphyseal plate which I defined as the area between the epiphysis and the diaphysis; all measurements were then converted to millimeters. At the point where a clear dividing line between the diaphysis and the

epiphysis was unable to be distinguished I recorded a FC which denoted that the epiphyseal plate was fully calcified and had completely fused with the diaphysis (Figure 1).

Each neonate was digitally photographed and imported into NIH Imaging software which was used to make three cranial measurements of each neonate skull; greatest length of skull, depth, and width. The greatest length of skull was measured from the tip of the nasal bone to the nuchal crest of the occipital bone. The depth was defined as the length from the angle of the mandible to the crown of the skull and the width of the skull was defined as the distance from the widest part of one parietal bone to the widest part of the other parietal bone. NIH imaging software records distances in pixels with my scale being 14.86 pixels = 10 mm. All measurements were then converted to millimeters and were accurate to the nearest 0.07 millimeter.

DATA ANALYSIS

The averages of the measurements for each day were used in all analyses. The data were analyzed using linear regression to determine the rate of growth per day. Slopes were considered significantly different from zero if $\alpha <$ or $= 0.05$. The Bray-Curtis measure of dissimilarity was used with the rates standardized to unit maxima (Krebs, 1989). A Bray-Curtis dissimilarity measure was used to determine the actual similarity between the three species to justify the grouping or clustering of two of the three species for comparison as a group to the third species. Then a cluster analysis was performed using the unweighted pair-group method using the arithmetic average (UPGMA) (Krebs, 1989). The rates were standardized because faster rates tend to be more heavily weighted in the analysis. The cluster analysis allowed for comparing the

two most similar species as a group to the third (Krebs, 1989). To test for the presence of allometric growth, the ratio of the length of the humerus to the combined length of the humerus and the ulna was calculated for the forelimb as well as the ratio of the length of the femur to the combined length of the femur and the tibia for the hindlimb.

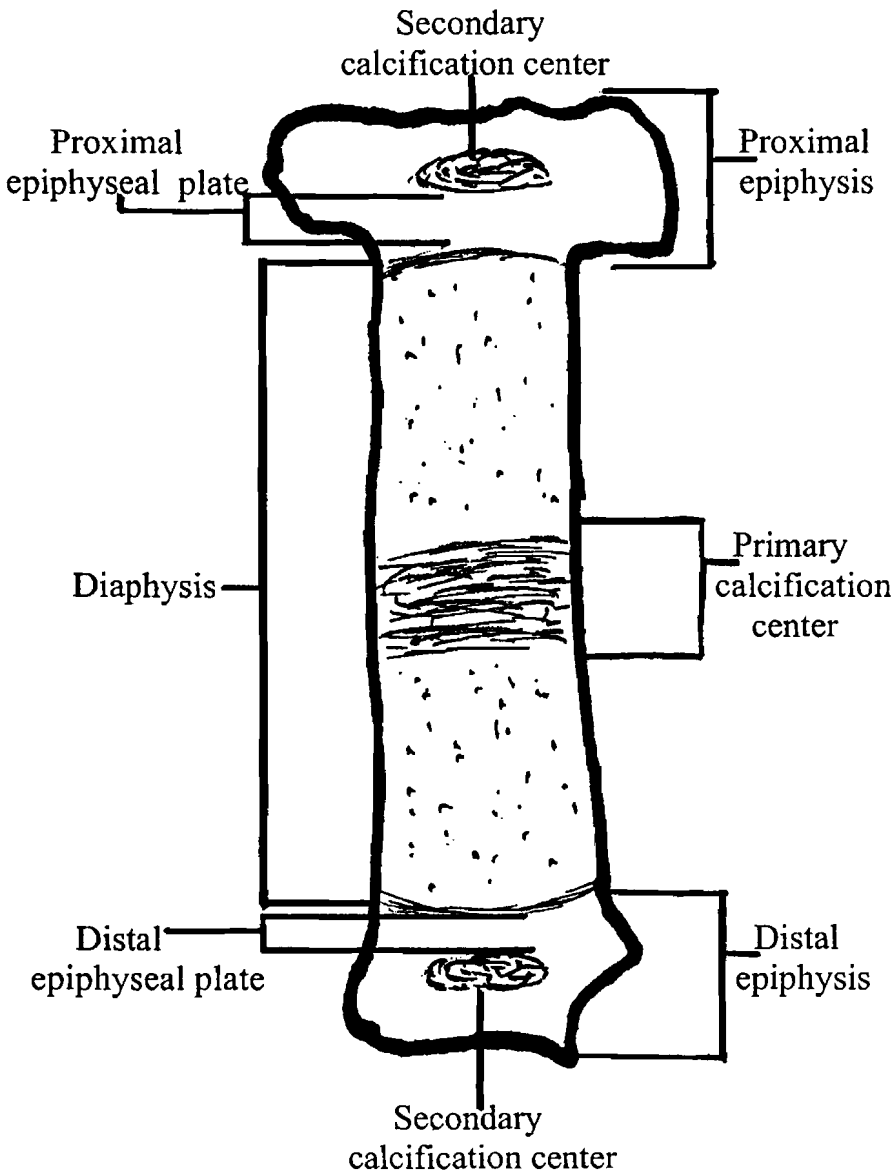

Figure 1. Pictorial representation of locations of long bone measurements.

RESULTS

My cranial data were used as an overall representation of the size of each of the three species. At borth *Sigmodon hispidus* had the largest skull and *Peromyscus maniculatus* and *Mus musculus* were about the same size (Figure 2). The average rate of increase in the greatest length of skull was fastest in *M. musculus*, followed by *P. maniculatus* and S. *hispidus*, which were comparable (Table 1). The rate of increase in the depth of the skull was fastest in *S. hispidus*, intermediate in *M. musculus* and slowest in *P. maniculatus*. The average rate of increase in the width of the skull was fastest in *M. musculus*; followed by *S. hispidus*, with *P. maniculatus* was the slowest.

All three species had similar growth rates in both the upper and lower parts of the forelimb (Figure 3). Similarly, the hindlimb showed no differences in the growth rates in the upper and lower parts of the hindlimb (Figure 4). The average rate of increase in the overall length of the long bones was fastest in *M. musculus*, except in femur length, and *P. maniculatus* was the slowest (Table 2).

The range of days of first appearance of calcification was earlier in *S. hispidus* and *P. maniculatus* than in *M. musculus* in both the proximal and distal epiphyses with the exceptions of the epiphyses of the proximal tibia and the proximal fibula (Tables 3 and 4). Calcification did not appear in the epiphysis of the proximal fibula until day 10 in *P. maniculatus.* Calcification appeared earlier in the hindlimb than in the forelimb in *S. hispidus* and at about the same time in *P. maniculatus* and *M. musculus*, however, calcification always appeared in *S. hispidus* first relative to the other two species.

Figure 5 shows the mean increase in the length of the primary calcification center of the diaphysis for the ulna over time, which I chose as the representative of all the long

bones. When the data for all the long bones were analyzed, all the long bones exhibited similar patterns and trends, so to avoid redundancy, I chose to only present the results for the ulna. For all three species, the diaphysis had started to calcify prior to birth in all of the long bones and had begun to lengthen bidirectionally towards the epiphyses. The length of the primary calcification center started out much larger in *S. hispidus* and was the smallest in *M. musculus*. The rates of calcification of the diaphysis of the long bones were faster in *S. hispidus* and *M. musculus* than in *P. maniculatus* in all of the long bones except the tibia and fibula where *M. musculus* was the slowest (Table 5). The rate of increase in the calcification of the diaphysis was always fastest in *S. hispidus* related to the other two species. The range of days of full calcification were earlier in *S. hispidus* and *P. maniculatus* than in *M. musculus*, with *S. hispidus* always being the earliest among the three species (Table 6). The average rate of decrease in the length of both the proximal and distal epiphyseal plates was faster in *S. hispidus* and *P. maniculatus* than in *M. musculus* (Tables 7 and 8). The range of days of fusion between the proximal and distal epiphyses and the diaphysis was earlier in *S. hispidus* and *P. maniculatus* than in *M. musculus* (Table 9 and 10). Complete fusion between the epiphysis and the diaphysis was not observed in the proximal fibula of *P. maniculatus* nor in the distal tibia of *M. musculus*.

The Bray-Curtis measure based on the rates in Tables 1, 2, 5, 7, and 8, indicated that *S. hispidus* and *P. maniculatus* were 10.26% dissimilar, *P. maniculatus* and *M. musculus* were 16.45% dissimilar and *S. hispidus* and *M. musculus* were 11.26% dissimilar. The UPGMA cluster analysis grouped *S. hispidus* and *P. maniculatus*

together and these two then clustered to *M. musculus* with a dissimilarity of 13.85% (Figure 6).

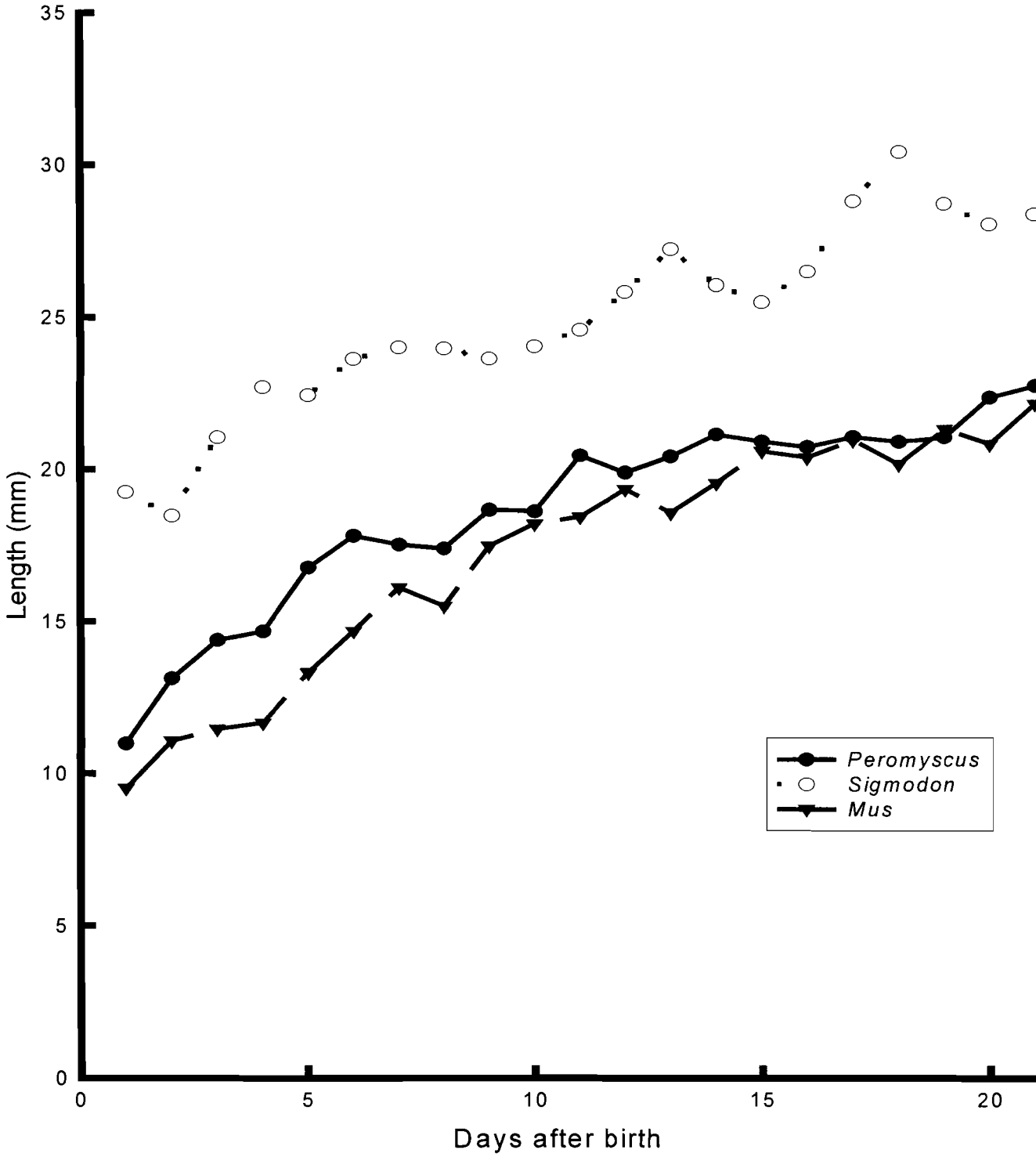

Figure 2. Average greatest length of skull from birth to day 21 for *Mus musculus*, *Peromyscus maniculatus*, and *Sigmodon hispidus*.

Table 1. Average rate of growth for the skulls of *Mus musculus, Peromyscus maniculatus,* and *Sigmodon hispidus* from day 1 to day 21 following birth. All values are given in mm/day.

Species	Greatest length of skull	Depth	Width
Mus musculus	0.598	0.233	0.345
Peromyscus maniculatus	0.478	0.175	0.177
Sigmodon hispidus	0.476	0.305	0.338

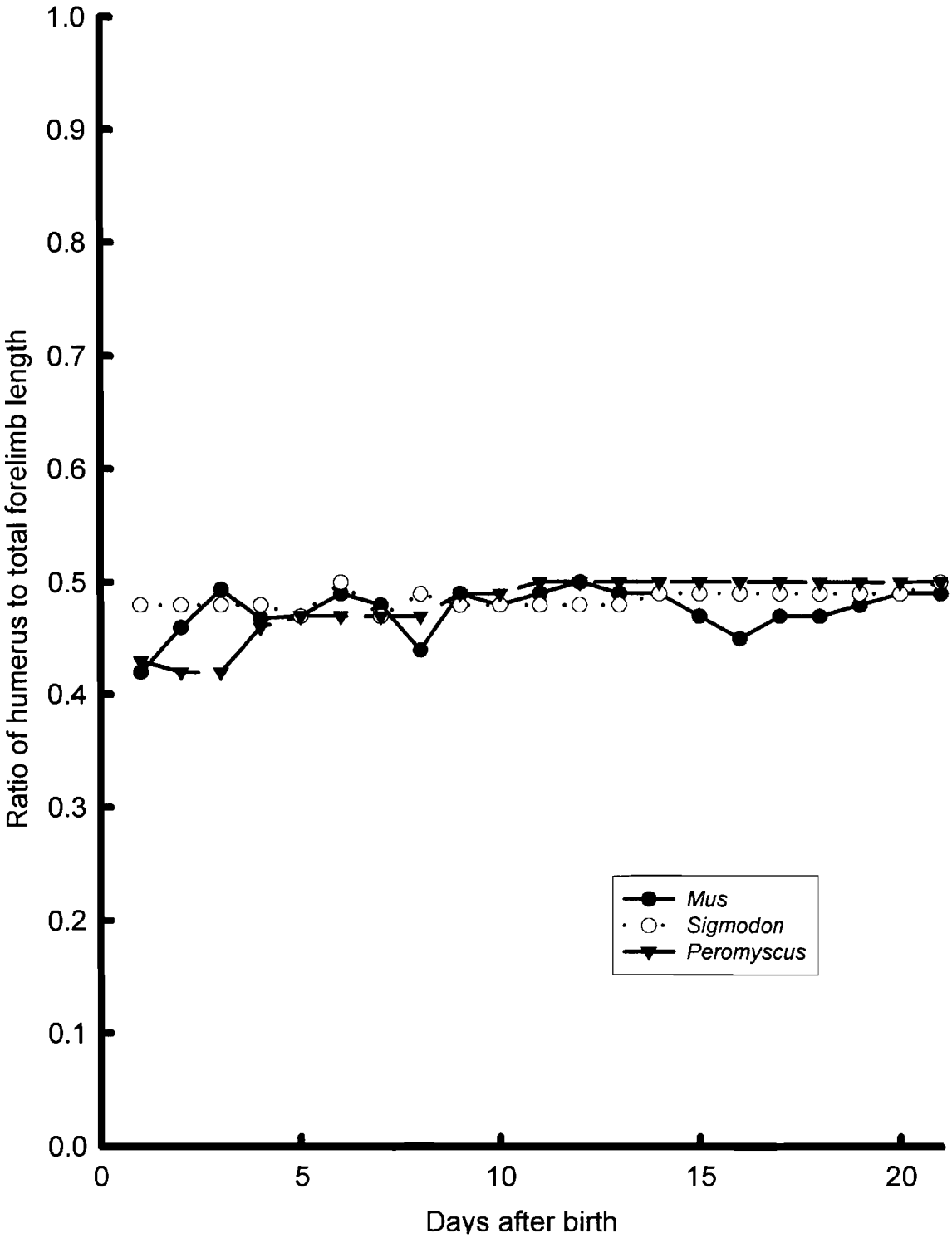

Figure 3. The ratio of the length of the humerus to the combined length of the humerus and the ulna from birth to day 21 for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*.

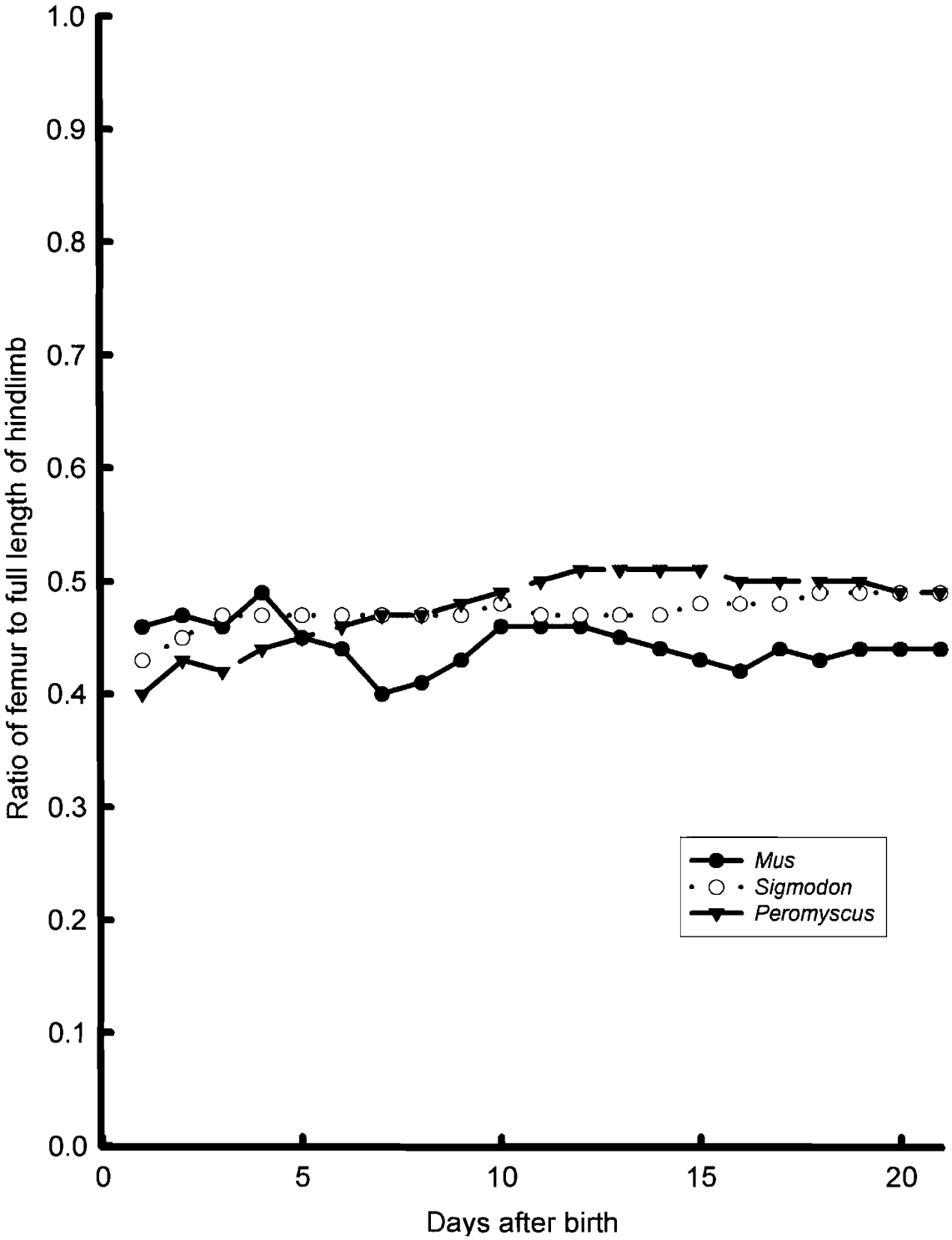

Figure 4. The ratio of the length of the femur to the combined length of the femur and the tibia from birth to day 21 for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus.*

Table 2. Average rate of increase in full length of the long bones from birth to day 21 for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*. All values are given in mm/day.

Species	Humerus	Radius	Ulna	Femur	Tibia	Fibula
Mus musculus	0.367	0.343	0.374	0.393	0.522	0.472
Peromyscus maniculatus	0.256	0.234	0.207	0.315	0.215	0.247
Sigmodon hispidus	0.340	0.327	0.268	0.453	0.402	0.357

Table 3. Range of the days of first appearance of calcification in the proximal epiphysis for the long bones of *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*.

Species	Proximal humerus	Proximal radius	Proximal ulna	Proximal femur	Proximal tibia	Proximal fibula
Mus musculus	4 - 6	5 - 7	4 - 6	5 - 6	2 - 4	2 - 4
Peromyscus maniculatus	2 - 4	3 - 5	3 - 4	4 - 6	3 - 5	9 - 11
Sigmodon hispidus	1 - 3	4 - 5	2 - 4	1 - 2	1 - 2	4 - 6

Table 4. Range of the days of first appearance of calcification in the distal epiphysis for the long bones of *Mus musculus*, *Peromyscus maniculatus*, and *Sigmodon hispidus*.

Species	Distal humerus	Distal radius	Distal ulna	Distal femur	Distal tibia	Distal fibula
Mus musculus	4 - 6	5 - 6	4 - 6	2 - 3	3 - 5	6 - 8
Peromyscus maniculatus	2 - 4	3 - 5	3 - 4	2 - 4	4 - 5	3 - 5
Sigmodon hispidus	1 - 3	4 - 6	3 - 5	1 - 3	1 - 2	3 - 5

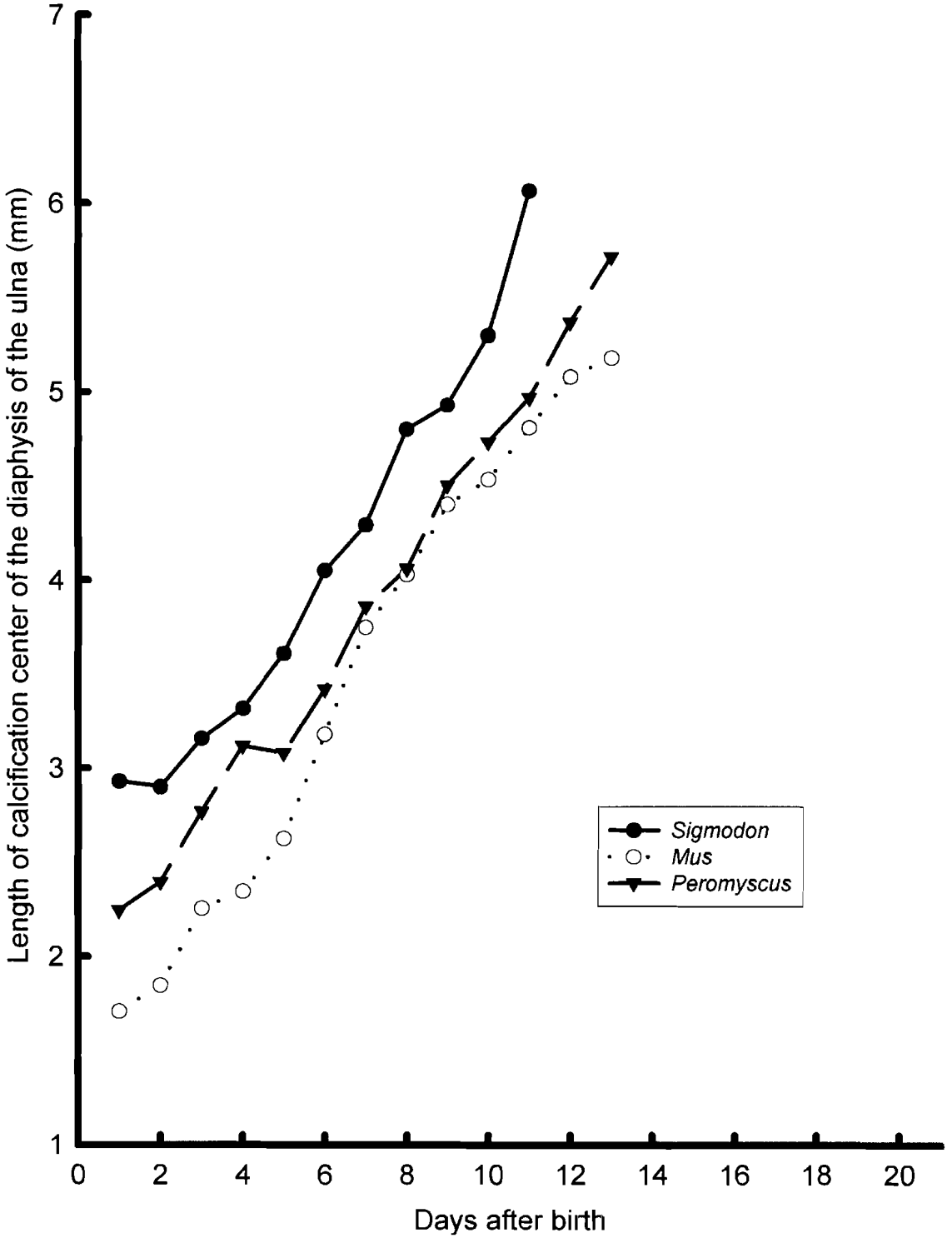

Figure 5. Average length of the calcification of the diaphysis of the ulna from birth until the diaphysis is fully calcified for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*.

Table 5. Mean rate of increase in calcification of the diaphysis for each of the long bones for *Mus musculus*, *Peromyscus maniculatus*, and *Sigmodon hispidus*. All values are given in mm/day.

Species	Humerus	Radius	Ulna	Femur	Tibia	Fibula
Mus musculus	0.334	0.333	0.319	0.311	0.253	0.313
Peromyscus maniculatus	0.273	0.286	0.290	0.305	0.287	0.314
Sigmodon hispidus	0.341	0.342	0.321	0.342	0.296	0.375

Table 6. Range of the days of full calcification of the diaphysis of the long bones for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*. All values are given in mm/day.

Species	Humerus	Radius	Ulna	Femur	Tibia	Fibula
Mus musculus	14 - 16	14 - 15	13 - 15	14 - 15	14 - 15	14 - 16
Peromyscus maniculatus	12 - 14	13 - 15	13 - 15	12 - 14	13 - 15	13 - 15
Sigmodon hispidus	11 - 13	13 - 14	11 - 13	12 - 14	11 - 13	13 - 14

Table 7. Average rate of decrease in length of the proximal epiphyseal plate of the long bones of *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*. All values are given in mm/ day.

Species	Proximal humerus	Proximal radius	Proximal ulna	Proximal femur	Proximal tibia	Proximal fibula
Mus musculus	-0.023	-0.026	-0.022	-0.028	-0.025	-0.029
Peromyscus maniculatus	-0.039	-0.032	-0.034	-0.049	-0.051	-0.050
Sigmodon hispidus	-0.044	-0.030	-0.049	-0.035	-0.035	-0.051

Table 8. Average rate of decrease in length of the distal epiphyseal plate of the long bones of *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*. All values are given in mm/day.

Species	Distal humerus	Distal radius	Distal ulna	Distal femur	Distal tibia	Distal fibula
Mus musculus	-0.019	-0.022	-0.022	-0.020	-0.017	-0.038
Peromyscus maniculatus	-0.034	-0.046	-0.032	-0.050	-0.042	-0.037
Sigmodon hispidus	-0.042	-0.047	-0.041	-0.047	-0.017	-0.043

Table 9. Range of the days of complete fusion between the proximal epiphysis and the diaphysis for the long bones of *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus*.

Species	Proximal humerus	Proximal radius	Proximal ulna	Proximal femur	Proximal tibia	Proximal fibula
Mus musculus	20 - 21	17 - 19	18 - 20	15 - 17	16 - 18	18 - 19
Peromyscus maniculatus	16 - 17	17 - 18	17 - 19	15 - 17	12 - 14	21+
Sigmodon hispidus	12 - 14	12 - 14	14 - 16	16 - 17	11 - 13	15 - 17

Table 10. Range of the days of complete fusion between the distal epiphysis and the diaphysis for the long bones of *Mus musculus*, *Peromyscus maniculatus*, and *Sigmodon hispidus*.

Species	Distal humerus	Distal radius	Distal ulna	Distal femur	Distal tibia	Distal fibula
Mus musculus	19 - 21	15 - 16	13 - 15	16 - 18	21+	16 - 18
Peromyscus maniculatus	17 - 19	13 - 15	14 - 16	13 - 14	15 - 17	17 - 18
Sigmodon hispidus	13 - 14	12 - 14	13 - 14	12 - 14	14 - 15	15 - 17

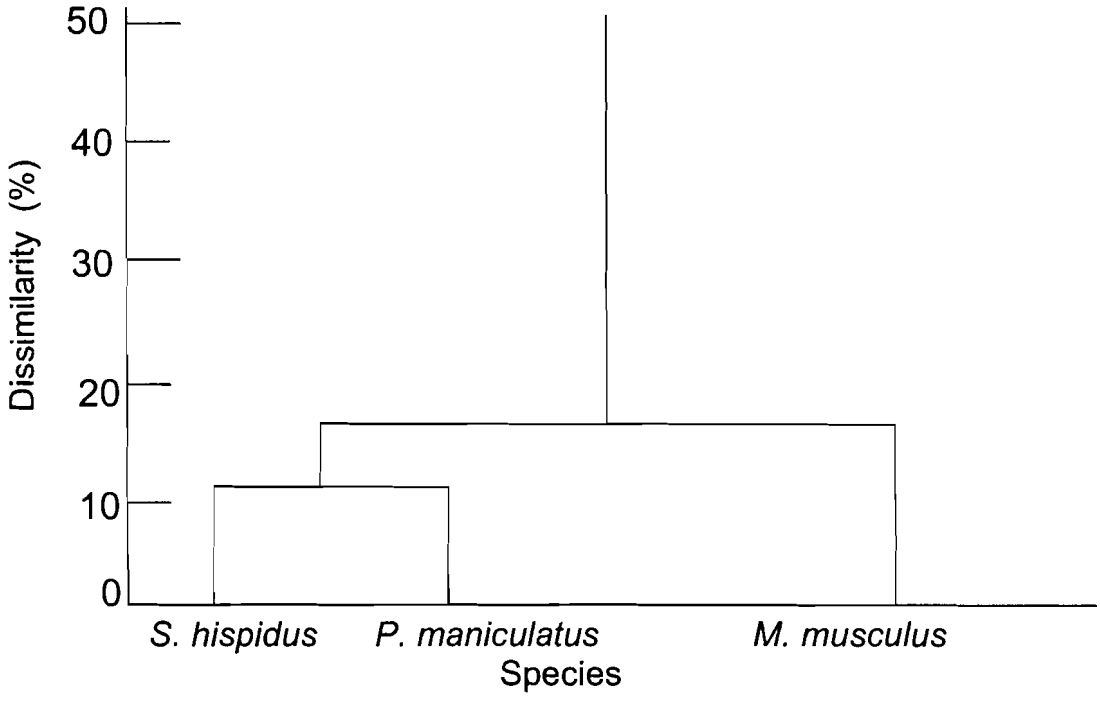

Figure 6. Cluster analysis of the rates of growth using unweighted pair-group methods using the arithmetic average (UPGMA) for *Mus musculus, Peromyscus maniculatus*, and *Sigmodon hispidus.*

DISCUSSION

The ideas behind evolutionary biology attempt to address several poignant questions such as why is there such diversity between organisms and by what mechanisms or adaptations did these unique animals reach these different morphological levels that allow them to be well adapted to their respective environments? There are several methods and sources of information to aid in answering some of these evolutionary questions. One source of phylogenetic information is the fossil record. The fossil record offers a glimpse into extant taxa which is invaluable in the quest to find common ancestors to link extinct taxa to present day taxa (Freeman and Herron, 1998). Data from the fossil record can trace the evolutionary pathway from a primitive condition to the modified condition, however, the fossil record contains many gaps and one must be cognizant of the limits to which fossil data may be interpreted and applied to present day data. The traceable changes and modifications from a common ancestor, as defined by the fossil record, to the present taxa provide an evolutionary history, which defines the phylogeny (Freeman and Herron, 1998).

The first question addressed in this study was a comparative method an appropriate and useful tool in analyzing these morphological characters? By understanding the accepted phylogenetic relationship among the three study species, I was able to control for any effects of shared ancestry (Grande and Rieppel, 1994). The developmental process of long bone ossification of the three species was determined to be a phylogenetically informative trait to study because the characters being examined were homologous as they were derived from a common ancestor (Freeman and Herron,

1998). Therefore, by meeting the assumptions of homology; similarity in location, composition, and development, I was able to compare across the three species and extrapolate any species' patterns which could potentially support or refute the accepted phylogeny (Freeman and Herron, 1998). Overall, a comparative method, under the assumption of homology, was a useful and appropriate tool in this morphological study.

The second question addressed by this research was do these data support the accepted phylogenetic relationship between the three species of rodents? The relationship, as presently accepted, states that *Sigmodon hispidus* and *Peromyscus maniculatus* are more closely related to each other than either is to *Mus musculus* (Eisenberg, 1981). Therefore, one would expect that the developmental patterns of *S. hispidus* and *P. maniculatus* would be more similar to each other than either would be to *M. musculus*. Most of the data did support the accepted phylogenetic relationship between the three species. The range of days of first appearance of calcification in the epiphyses, complete fusion of the epiphyses and the diaphysis, and full calcification of the diaphysis of the long bones was much earlier in *S. hispidus* and *P. maniculatus*, with *M. musculus* being later. The same pattern was also observed with regard to the rates of calcification of the diaphysis of the long bones as well as the rates of decrease in the length of the epiphyseal plates. Therefore, it can be concluded that *S. hispidus* and *P. maniculatus* have more similar long bone developmental patterns than either do to *M. musculus* and these data support the known phylogeny. The Bray-Curtis measure of dissimilarity performed on all of the rates as a whole reinforced my original hypothesis that these data do show that *S. hispidus* and *P. maniculatus* are more closely related to each other, with regard to developmental patterns and rates, than either were to *M.*

musculus, hence supporting the known phylogeny of these three rodent species. It is clear that any areas of noncongruence must be targeted as areas of further research and analysis. However, some aspects of my data do not appear to support the understood phylogeny. For example, the average rates of growth of the cranial bones were faster in *M. musculus* and *S. hispidus*, compared to *P. maniculatus* being the slowest; therefore *M. musculus* and *S. hispidus* exhibit more similar patterns of cranial development. However, it is important to note that the ossification of the cranial bones is by the process of intramembraneous ossification, not endochondral ossification as seen in the long bones (Tortora and Anagnostakos, 1987) . Therefore, the faster rates of growth of the cranial bones of *M. musculus* suggest that this species may have evolved to be more efficient at intramembraneous ossification, as opposed to endochondral ossification. Another example of noncongruence between my data set and the accepted phylogeny was the rates of growth in the overall length of the long bones. The average rate of increase in the full length of the long bones was fastest in *M. musculus*, followed closely by *S. hispidus*, with *P. maniculatus* always being the slowest. Therefore, *M. musculus* and *S. hispidus* exhibited closer developmental rates hence not supporting the known phylogeny.

No allometric growth differences were observed for the long bones among the three species. All three species had similar growth rates in both the upper and lower bones of the forelimb and hindlimb within each of the individuals and species. Therefore, it can be concluded that the growth rates of the upper and lower long bones for each species were approximately the same.

The final question posed in my research was: are any species-specific developmental patterns present? The first pattern noted was the faster rates of growth of the cranial bones of *M. musculus* in skull length and width as compared to *S. hispidus* and *P. maniculatus*. It is my hypothesis that these faster cranial rates represent a trade-off with regard to the slower rates of development exhibited in the long bones of *M. musculus*. There are several advantages of a faster rate of cranial growth. One advantage is that the brain may increase in size and develop much faster. Another factor may be competition among the individuals within the litter of *M. musculus*. *M. musculus*, compared to the other two species, had a much larger brood size, leading to difficulties vying for suckling advantage. Therefore, the faster the skull fully develops, potentially the more effective the neonate would be at suckling, thereby increasing the individual's food intake leading to an increase in fitness and an increased likelihood of survival. *Sigmodon hispidus* and *P. maniculatus* had smaller litter sizes, which potentially eases some of these competitive pressures, explaining the slower rates of development in the cranium. Another possible explanation could be that *S. hispidus* and *P. maniculatus* give birth to young which are more precocial as compared to *M. musculus* which gives birth to more altricial young. Altricial young are more dependent, have longer weaning times, which could account for the exhibited slower developmental rates of the long bones of *M. musculus*.

Another interesting pattern that surfaced was that *S. hispidus* was always the fastest or earliest related to the other two species with regard to the calcification of the long bones. The faster rates of development may be related to the extensive development exhibited by the *S. hispidus* neonates at birth. The genus *Sigmodon* gives birth to young

that are extremely precocial. *Sigmodon* is unique in that the neonates are born furred and their eyes open within the first 36 hours after birth (Eisenburg, 1981). I found that in *S. hispidus* the length of the calcification center of the diaphysis started out much larger than the other two species which reinforces the exhibited advanced development of the precocial *S. hispidus* neonates. The birth of the precocial young appears to drive these faster rates of development, however gestational studies must be performed to solidify this potential hypothesis.

Peromyscus maniculatus exhibited several developmental patterns. One repetitive pattern among the samples of *P. maniculatus* was the delayed appearance of calcification and no observed fusion between the proximal epiphysis and the diaphysis of the fibula. This pattern may be representative of a potential trade-off in the allocation of calcification energy. The fibula is a non-weight bearing bone and therefore, *P. maniculatus* may be selectively prioritizing the order of bone calcification based on the immediate needs of the animal for survival in the environment. However, to test this hypothesis I would have to compare the lab-reared neonates to field born neonates to determine the effects of environment on developmental patterns.

The exact link between development and evolution has yet to be ascertained due in part to the fundamental differences in the approaches of ontogenists, paleontologists, systematists, and comparative anatomists. However, it is only under the umbrella of phylogeny that one can begin to decipher the origin of ontogenies, assess the true relevance of heterochrony, determine the effects of external factors or pressures on evolution, and analyze the interplay between developmental processes and evolution (Wake et al., 1991). The developmental data presented in this research have proven to be

an effective and appropriate tool in the phylogenetic analysis of the relationship between *S. hispidus, P. maniculatus*, and *M. musculus*. However, in retrospect, to consider the observed differences as convincing and conclusive evidence in the determination of phylogenetic relatedness, I should have provided data from a more distant outgroup such as the Family Sciuridae or the Family Zapodidae.

LITERATURE CITED

Alberach, P. 1985. Problems with the interpretation of developmental sequences. Systematic Zoology. 34 (1): 46 – 58.

Carter, D. R., M. Wong, and T. E. Orr. 1991. Musculoskeletal ontogeny, phylogeny, and functional adaptation. Journal of Biomechanics. 24: 3 - 16.

Eisenberg, J. F. 1981. The mammalian radiations: An analysis of trends in evolution, adaptation, and behavior. The University of Chicago Press. Chicago, IL.

Fieldhouse, D., F. Yasdani, and G. B. Golding. 1997. Substitution rate variation in closely related rodent species. Heredity. 78: 21 – 31.

Freeman, S. and J. C. Herron. 1998. Evolutionary analysis. Prentice Hall. Upper Saddle River, New Jersey.

Funk, V. A. and D. R. Brooks. 1996. Phylogenetic systematics as the basis of comparative biology. Smithsonian Institution Press. Washington, D. C.

Grande, L. and O. Rieppel. 1994. Interpreting the hierarchy of nature: From systematic patterns to evolutionary process theories. Academic Press. San Diego, CA.

Hall, B. K. 1992. Evolutionary developmental biology. Chapman and Hall. London.

Hanken, J. 1993. Model systems versus outgroups: Alternative approaches to the study of head development and evolution. American. Zoologist. 33: 448 – 456.

Hanson, E. D. 1977. The origin and early evolution of animals. Wesleyan University Press. Middletown, Connecticut.

Keller, E. F. and E. A. Lloyd. 1992. Keywords in evolutionary biology. Harvard University Press. Cambridge, Massachusetts.

Krebs, C. J. 1989. Ecological Methodology. Harper & Row. New York, NY.

Lincoln, R., G. Boxshall, and P. Clark. 1998. A dictionary of ecology, evolution, and systematics. Cambridge University Press. Cambridge, Massachusetts.

Maisey, J. G. 1988. Evolutionary biology: volume 22. Plenum Press. New York, NY.

Mayr, E. 1970. Populations, species, and evolution: An abridgement of animal species and evolution. The Belknap Press of Harvard University Press. Cambridge, Massachusetts.

McKinney, M. L. and K. J. McNamara. 1991. Heterochrony: The evolution of ontogeny. Plenum Press. New York, NY.

Ragsdale, V. K. and D. W. Moore. 1992. Preparation of bone-stained mounts of whole animals. Kansas Science Teacher. 8 (2): 3-5.

Salthe, S. N. 1993. Development and evolution: Complexity and change in biology. The MIT Press. Cambridge, Massachusetts.

Tortora, G. J. and N. P. Anagnostakos. 1987. Principles of anatomy and physiology. Harper and Row. New York, NY.

Wake, D. B., P. MaBee, J. Hanken, and G. Wagner. 1991. The unity of evolutionary biology, Volume 1. Dioscorides Press. Portland, Oregon.

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