THE COMPARATIVE OSTEOLOGY OF POSTNATAL PEROMYSCUS LEUCOPUS AND PEROMYSCUS MANICULATUS BAIRDI

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
GORDON L. KIRKLAND, JR.
1968

THESIS

THE COMPARATIVE OSTEOLOGY OF POSTNATAL *PEROMYSCUS LEUCOPUS* AND *PEROMYSCUS MANICULATUS BAIRDI*

By

Gordon L. Kirkland, Jr.

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1968

ACKNOWLEDGEMENTS

Although it is impossible to acknowledge individually each person who has aided in the preparation of this manuscript, certain ones are worthy of recognition on the basis of their indispensible help. These persons include: my wife, Carol, who has been a laboratory assistant, secretary, statistitian, and loyal supporter; Dr. Edward Price, who while working as a laboratory assistant in the Department of Zoology, selected over three hundred specimens of *Peromyscus* graciously donated by Dr. John A. King from his research colony; and Dr. Rollin H. Baker, who took his valuable time to review this manuscript and to give me encouragement in its preparation as the major professor on my graduate committee. I would also like to thank, in addition to the above, the members of the Department of Zoology at Michigan State University who provided me with the laboratory equipment and space needed to perform this project.

In appreciation to all those formally named in this manuscript and those denied proper recognition, I dedicate this research thesis.

TABLE OF CONTENTS

INTRODUCTION

Initially this research was concerned with the perfection of the Alizarin-KOH clearing and staining technique as a tool for comparing skeletal development in closely related vertebrates. This technique had been of interest since I was introduced to it as an undergraduate at Cornell University in a laboratory methods course taught by Dr. Lowell D. Uhler. At that time, the process was represented as a teaching aid for the exhibition of delicate specimens. This technique has been used for many years in the study of the genetics of bone development in fetal and young rabbits by Crary, Sawin, and Atkinson (1958), Sawin and Crary (1956), Sawin and Trask (1965), and Crary and Sawin (1957). However, it was a paper by Nesslinger (1956) on the bone development of young postnatal Virginia opossums which convinced me of the practicality of using this technique to study normal bone development, as opposed to the study of skeletal aberrations.

Peromyscus leucopus novaboracensis and *Peromyscus maniculatus bairdi* were chosen for this study primarily on the basis of their availability from laboratory stocks.

It might have been wiser to have recognized considerations other than the ease of procurement, since the differences between the species were often so slight as to make differentiation nearly impossible on the basis of skeletal development. Specimens were obtained from the laboratory of Dr. John A. King and ranged in age from 0-28 days. Samples of ten per day were taken for each day from 0-7; every other day 7-21; and every third day from 21-28 days. This choice of samples was decided upon before any analysis was done. As will become evident in the analysis of this material, it would have been advantageous to have made daily observations throught 14 days. Most structures appeared during this time, and the second 14 days of life was devoted mostly to the maturation of the structures which had previously appeared. Maturation consisted of the final replacement of cartilage with bone.

Initially, general observations were made of entire animals at various stages of development. These allowed the selection of structures which had the most potential as "key" bone depositional centers. In most cases, observations were recorded from the first day that at least one individual displayed the beginnings of bone deposition until the day that 100% of the age class showed evidences of bone formation. With this method, I felt that it was possible to pinpoint the time of appearance. Under this system, a lack of ossification could be as important as its presence. The areas chosen for analysis included: the vertebral column, carpals, patella, olecranon and hind limb (femur, tibia-fibula, tarsals).

It was hoped that, with a calendar of events derived from this study, it might be possible to determine the age of young Peromyscus with the use of x-rays, rather than having to kill them to use the KOH-Alizarin method. It should be possible to identify the various bones well enough in an x-ray to place the individual in an age category. This would be helpful if nests of the young were found in the field, and information on the young or the possible breeding condition of the mother was desired. Crary and Sawin (1949) have used x-rays to follow the development of limb bones in rabbits.

METHODS AND MATERIALS

The clearing and staining method which I used in preparing my specimens was adapted from Dr. Lowell T. Uhler's laboratory manual for Biology 105 at Cornell University, and that of Nesslinger (1956). Specimens were stored in 95% alcohol after having the abdominal cavity opened. Each specimen older than one day was skinned either before or after being placed in ½-2% KOH, this being necessary in order that small ossifications could be seen. Otherwise these were obscured by the hair pigments which are not affected by the KOH clearing process. The KOH concentration depended upon the age and condition of the specimen. The solution was changed each day until the long bones were clearly visible through the flesh. Caution was observed with 0-2 day individuals since they tended to disintegrate if left overnight. The cleared specimens were then placed in a fresh solution of KOH to which alizarin stain had been added. The concentration was determined by the color of the solution, lavendar being desired. At this point, Uhler's method was altered by not mixing the Alizarin Red-S with acetic acid. The Alizarin stained the bones a purple to red color with the degree of staining controlled by both the concentration and the length of time exposed.

After the desired color was achieved, the specimens were then transferred to a solution of 1 part glycerine and 1 part water (distilled). They were left in this until they sank to the bottom. At this point, they were placed in jars of 100% glycerine to which a crystal of thymol was added to prevent mold formation.

The prepared specimens were then observed under a zoom-scope with magnifications of from 7-30 x. All observations were qualitative noting the presence or absence of a structure, although some mention was given to whether a structure was poorly, moderately, or well developed. With the constant manipulation which was necessary to make the observations, I found that the zoom-scope allowed a greater degree of freedom than was possible with a normal dissecting scope.

It was possible to locate bones that had not ossified since the cartilage did not clear as easily as muscle tissue and appeared from slightly opaque to transluscent. This was particularly useful when working with the ankles and wrists, making it possible to follow ossification in bones which were fused in later life, as it was possible to note these bones as separate entities early in development.

The specimens proved to be quite resistant to damage. I found that skinning was much easier after the clearing and staining process had been completed. By that time, the skin had become quite soft and easy to remove. Skinning before preparation while the specimens were still hardened from the alcohol proved to be disasterous in several occasions with lost appendages the result.

The species that were used in this study are closely related and are difficult to differentiate as prepared specimens. In the laboratory, these species are easily distinguished with the bi-colored tail and greyer pelage of *P. maniculatus* quite evident. There were, however, certain individuals that were especially difficult to classify. In the field, these animals are more easily distinguished on the basis of habitat preference. *Peromyscus leucopus* prefers the woodland, while *P. maniculatus bairdi* is at home in open fields and is even found on the open sandy beaches that border Lake Michigan (Hamilton, 1939). The differences in habitat preference in these animals should be noted for as I will point out later, I feel that this fact may have a bearing on certain of the differences between these two species. One fact which I will mention at this point is the type of nest utilized by these two species. *Peromyscus leucopus* constructs a nest of leaves and shredded bark usually in a cavity.

P. maniculatus does not build as secure a nest. It is usually found under a rock or in a depression (Hamilton, 1943). Burt (1957) states that the nest of *P. lecuopus* is more substantial than that of *P.m. bairdi*. *P.m. bairdi* may, for its nest, seek no shelter other than a partially buried log or plank on a sandy beach, where shelter is at a premium (Hamilton, 1943). With shelter at a premium in areas where *P.m. bairdi* ranges, it is possible that predators and other animals would come across nests of this species more often than those of *P. leucopus*. This would certainly produce differential environmental pressures on these two species.

DESCRIPTION AND ANALYSIS

In this section, each of the major areas will be described and analyzed to determine whether there are any significant differences in bone development between these two closely related species. Because of the type of data collected, it is necessary to use non-parametric statistics in their analysis. Statistical procedures and tables are taken from Siegal (1956).

One difficulty in some of the analysis was that, due to either loss, destruction, or miscounting during collection, not all of the age classes had equal numbers of individuals. In order to run a chi-square test, it is necessary to use equal sample sizes. To allow this, it was assumed that the specimens were examined in random order. Thus, "extra" specimens at the ends of the original data sheets were deleted to provide equal sample sizes.

Olecranon

Although it is not a separate bone, the olecranon is considered in this study because it is a separate center of ossification. This center appears to be distinct from the bone deposition in the shaft of the ulna. Bone deposition is not present in this area at birth and does not commence until seen in four day old individuals. Table 1 and Figure 1 follow the course of the ossification in this structure from 0-7 days.

Table 1.-- Olecranon data.

Days	*P. leucopus*	*P.m. bairdi*
0-3	None (of 36)	None (of 40)
4	None (of 9)	5 (of 11)
5	4 (of 11)	7 (of 10)
6	8 (of 10)	9 (of 10)
7	10 (of 10)	10 (of 10)
Total	22 (of 76)	31 (of 81)

Differences in the degree of ossification in the two species occurred on days 4, 5, and 6 with *P.m. bairdi* developed to a greater extent in each case. When these data are examined using a chi-square test for the total number of individuals ossified during the three days of differences, a value of 3.6839 indicates that no significant difference is present at the .05 level with 2 degrees of difference (Table 2). However, the value is significant between the .10 and .20 levels.

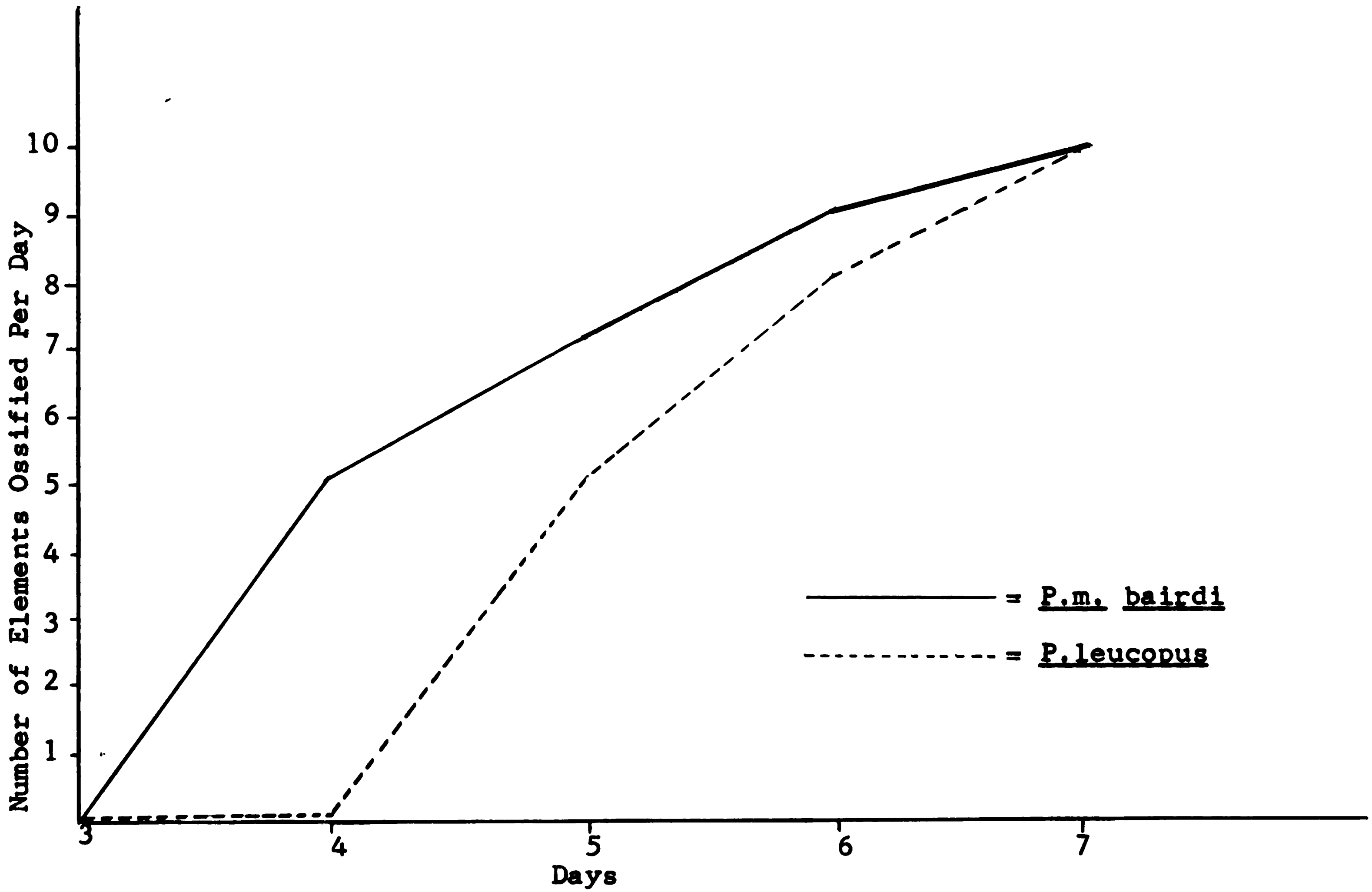

Figure 1.-- Ossification of the olecranon.

Table 2.-- Chi-square analysis of the olecranon data.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
4	0	1.9	5	3.09	5
5	5	4.59	7	7.41	12
6	8	6.5	9	10.5	17
	13	13.0	21	21.0	33

$$\frac{(0 - 1.9)^2}{1.9} + \frac{(5 - 3.1)^2}{3.1} + \frac{(5 - 4.59)^2}{4.59} + \frac{(7 - 7.41)^2}{7.41}$$

$$+ \frac{(8 - 6.5)^2}{6.5} + \frac{(9 - 10.5)^2}{10.5} = x^2$$

$$\frac{3.61}{1.9} + \frac{3.61}{3.1} + \frac{.1681}{4.59} + \frac{.1681}{7.41} + \frac{2.25}{6.5} + \frac{2.25}{10.5} = x^2$$

$$1.9 + 1.1645 + .0366 + .0226 + .346 + .2142 = 3.6839 = x^2$$

Patella

As in other mammals, the patella is present as a sesamoid bone in both species. This structure is late in ossifying, and no bone is deposited until the seventh day. Before this time, the patella can be identified as a shadowy structure representing the unossified tendon lying immediately distal to the condyles of the femur.

The period of partial representation of this structure lasted from 7-13 days. The trend in the appearance of the patella may be seen by noting Table 3 and

Figure 2. The development of the patella is quite similar in the two species. The results of a chi-square test at the .05 level of significance with three degrees of freedom indicate no significant difference between the two species. The chi-square value of .9544 falls between the .70 and .80 levels of significance and indicates a high degree of similarity between the species in the development of the patella.

Table 3.-- Patella data.

Days	*P. leucopus*	*P.m. bairdi*
0-6	None	None
7	None (of 10)	1 (of 10)
9	3 (of 10)	3 (of 11)
11	7 (of 9)	7 (of 10)
13	7 (of 8)	10 (of 10)
Total	17 (of 37)	21 (of 41)

Table 4.-- Chi-square analysis of the patella data.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
7	0	.47	1	.53	1
9	3	2.83	3	3.17	6

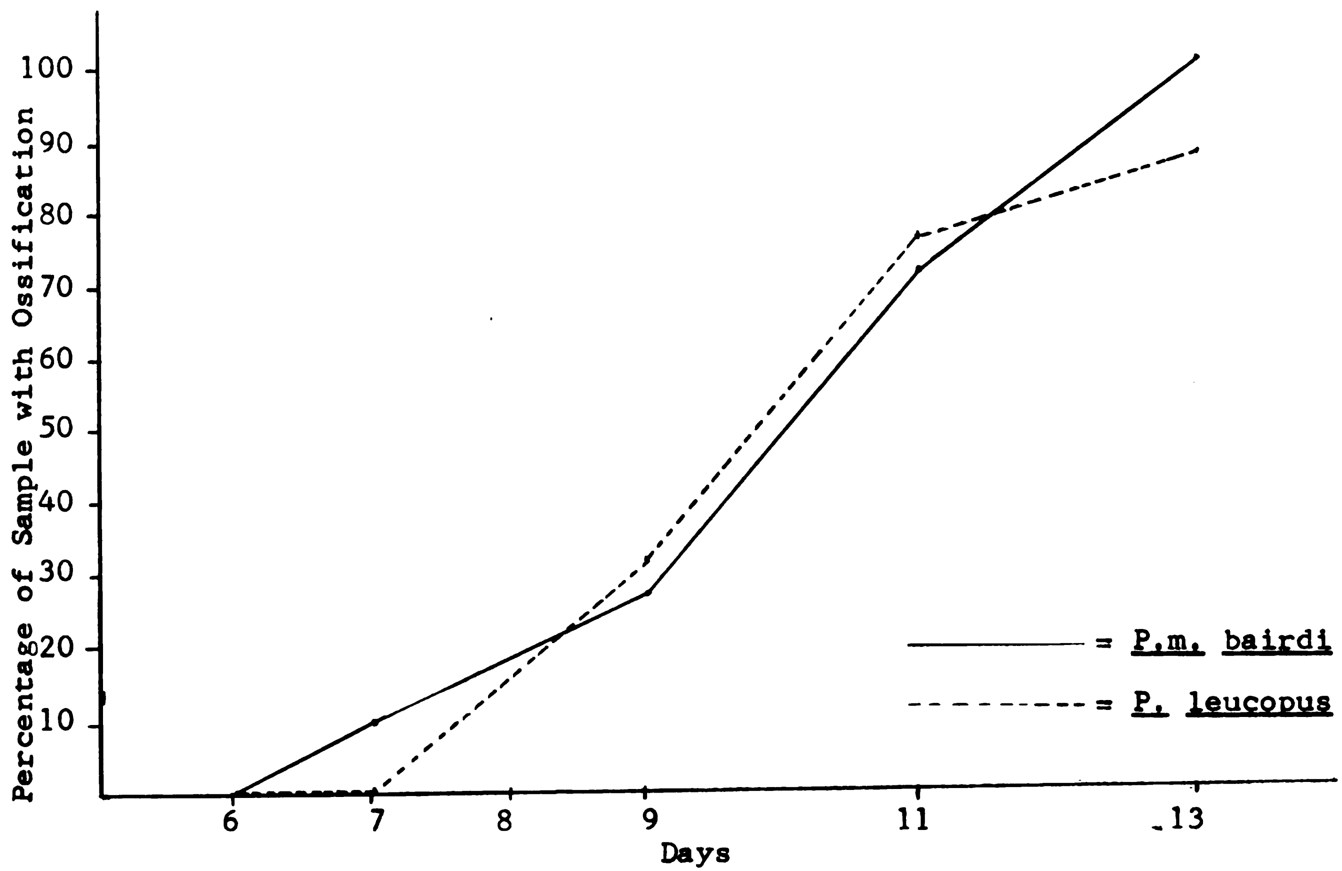

Figure 2.-- Ossification of the patella.

Table 4.-- continued.

Day	Obs.	Exp.	Obs.	Exp.	Total
11	7	6.6	7	7.4	14
13	7	7.1	8	7.9	15
	17	17.0	19	19.0	36

$$\frac{(0-.47)^2}{.47}+\frac{(1-.53)^2}{.53}+\frac{(3-2.83)^2}{2.83}+\frac{(3-3.17)^2}{3.17}$$

$$+\frac{(7-6.6)^2}{6.6}+\frac{(7-7.4)^2}{7.4}+\frac{(7-7.1)^2}{7.1}+\frac{(8-7.9)^2}{7.9}=x^2$$

$$\frac{.47^2}{.47}+\frac{.2209}{.53}+\frac{.0289}{2.83}+\frac{.0289}{3.17}+\frac{.16}{6.6}+\frac{.16}{7.4}+\frac{.01}{7.1}$$

$$+\frac{.01}{7.9} = .9544 = x^2$$

Distal Ossification of the Femur

As is characteristic of all mammalian long bones, the femur of the mouse has a subterminal epiphyseal growth area. Distal to this area of growth, the condyles of the femur calcify and are recognizable as being distinct from the shaft which is also ossifying. Because of its isolated situation, the distal condyles of the femur present a good area of study.

The distal end of the femur appears to be formed in one of three ways. It can appear as a simple arc or line of bone which has no enlargements. It may also form in a bilobed or dumbbell form or it may develop as two separate bony areas which later meet in the midline. There is much variance in size of this structure between individuals.

No ossification of the distal condyles of the femur was noted in individuals through three days, with the exception of one three-day individual in P.m. bairdi in which the condyle may have begun to ossify, as a faint speck of alizarin was present.

Four-day individuals were the first to exhibit bone deposition. Three of ten P.m. bairdi had small dots of bones which represented the medial and lateral condyles. None of the P. leucopus showed any signs of bone deposition.

Much the same condition prevailed in the five-day individuals. P. leucopus had no ossifications in nine specimens. On the other hand, P.m. bairdi had four of ten specimens with bone developments. All of these showed an advancement over the four-day individuals in that the two lateral areas of deposition have been joined across the middle with the medial being the larger.

Bone deposition in P. leucopus occurred first in the six-day individuals with two specimens exhibiting minute ossifications while eight lacked ossification. The bone deposition appeared to be at the same stage as that of the four day P.m. bairdi. At the same time, P.m. bairdi had all ten specimens with at least some ossification.

The same discrepency between species was evident in the seven-day individuals with only three of eight *P. leucopus* showing evidence of bone deposition. All ten specimens of the other species were well formed.

The ninth day brought the first instance in which a higher percentage of *P. leucopus* were ossified, with all nine individuals having at least some trace of the distal condyle. In *P.m. bairdi*, eleven of twleve were present. The only individual lacking any development was extremely small.

All eleven day individuals in both species had the distal end of the femur ossified.

Table 5 gives a more graphic representation of the preceeding discussion, which was included only to illucidate my observations of the trends which were noted.

Table 5.-- Distal ossification of the femur data.

Days	*P. leucopus*	*P.m. bairdi*
0-3	None	None
4	None (of 10)	3 (of 10)
5	None (of 10)	4 (of 10)
6	2 (of 10)	10 (of 10)
7	3 (of 8)	10 (of 10)
9	9 (of 9)	11 (of 12)
11	All	All
Total	24 (of 57)	48 (of 62)

Differences in the number of ossified femural condyles occurred in only five of the age classes; 4-9 days. A chi-square test at the .05 level of significance with 4 degrees of freedom was applied to the femur data and produced a value of 11.7609. This was significant at between the .02 and .01 level (Table 6), indicating a significant difference between the two species as to the number of individuals in each age class with ossified femural condyles. Figure 3 suggests that P.m. bairdi had a significantly greater number of individuals with ossified femural condyles than P. leucopus on each day. This figure also suggests that the femural condyles ossify sooner in P.m. bairdi by a factor of about one day.

Table 6.-- Chi-square analysis of the distal condyle of the femur data.

	P. leucopus		P.m. bairdi		
Day	Obs.	Exp.	Obs.	Exp.	Total
4	0	.90	3	2.10	3
5	0	1.20	4	2.80	4
6	2	3.60	10	8.40	12
7	4	4.20	10	9.80	14
9	9	5.1	8	11.9	17
	15	15.0	35	35.0	50

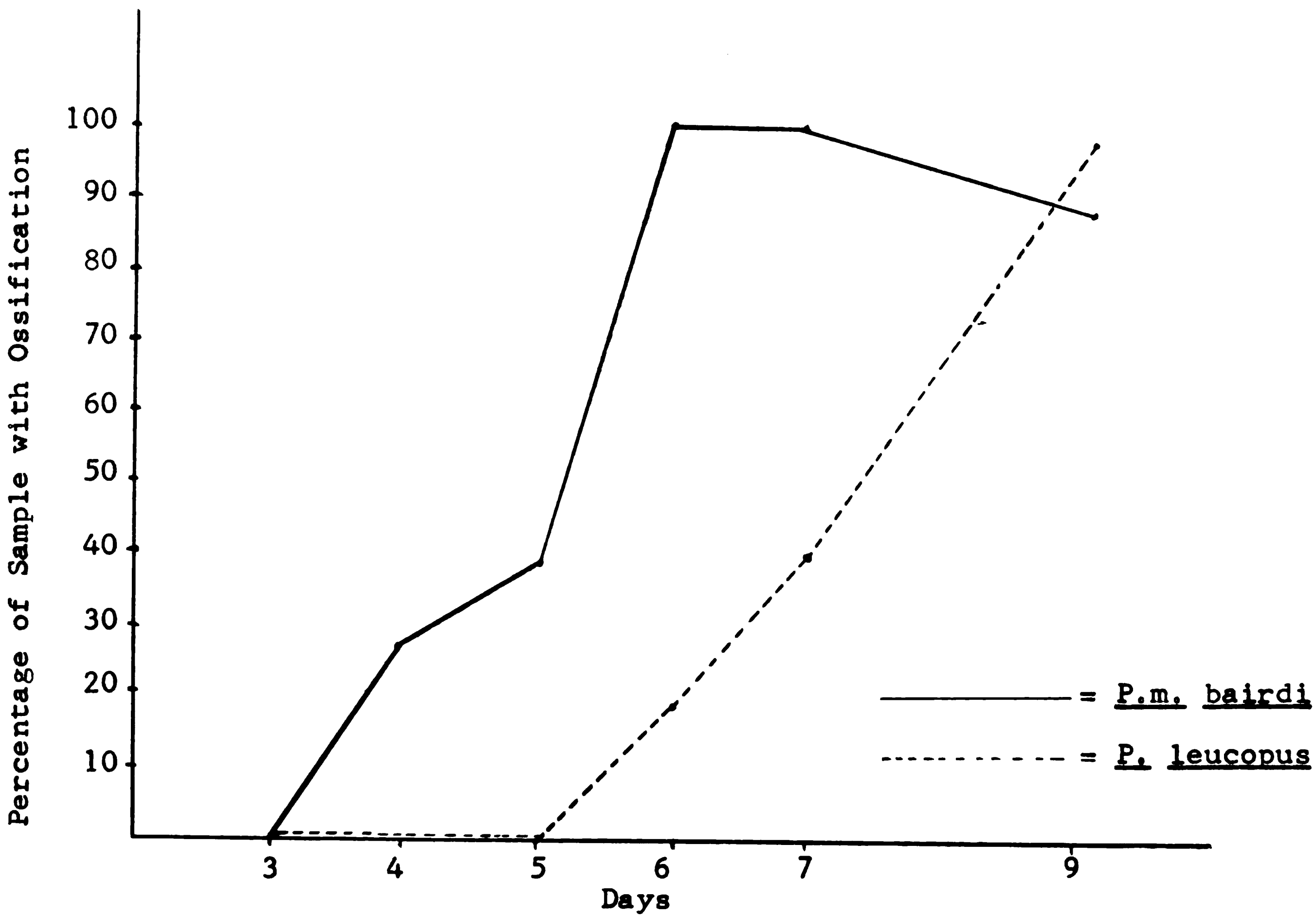

Figure 3.-- Distal condyle of the femur.

Table 6.-- continued.

$$\frac{(0 - .90)^2}{.90} + \frac{(3 - 2.10)^2}{2.10} + \frac{(0 - 1.20)^2}{1.20} + \frac{(4 - 2.80)^2}{2.80}$$

$$+ \frac{(2 - 3.60)^2}{3.60} + \frac{(10 - 8.4)^2}{8.4} + \frac{(4 - 4.2)^2}{4.2} + \frac{(10 - 9.8)^2}{9.8}$$

$$+ \frac{(9 - 5.1)^2}{} + \frac{(8 - 11.9)^2}{} = x^2$$

$$\frac{.90^2}{.90} + \frac{.81}{2.10} + \frac{1.20^2}{1.20} + \frac{1.44}{2.80} + \frac{2.56}{3.60} + \frac{2.56}{8.4} + \frac{.04}{4.2}$$

$$+ \frac{.04}{9.8} + \frac{15.21}{5.1} + \frac{15.21}{11.9} = x^2$$

$$.90 + 3.857 + 1.20 + .5142 + .7111 + .3047 + .0095 + .004$$

$$+ 2.9823 + 1.2781 = 11.7609 = x^2$$

Ankle

The development of the ankle shows some interesting differences between the two species. The ankle consists in the fully developed condition of eight elements: four tarsals (#1, 2, 3, 4-5 fused), centrale, tibiale, calcaneus, and astragalus. The astragalus and the calcaneus are the largest and the first to develop.

The bones of the ankle in Peromyscus are essentially the same as those found in the laboratory rat, Rattus (Rowett, 1957). The five metatarsals are met proximally by only four distal tarsal elements, the fourth and fifth being fused. The fused fourth and fifth tarsal lies proximal to the 4th metatarsal and is articulate with the 5th metatarsal on the lateral side by the proximal growth of that metatarsal. The third tarsal is slightly smaller than the fused 4th and 5th and lies immediately proximal to its metatarsal. The second is the smallest tarsal element and occupies the same relative position to its metatarsal as does the 3rd tarsus. The first tarsal element is elongated and extends much further distally than any of the others. The centrale lies just proximal to the second and third tarsal and articulates to the astragalus proximally and the first tarsus laterally.

The astragalus and calcaneus are complex in design and will not be described here. In nearly all cases these are present at birth. The calcaneus appears to be the first ankle element to ossify, while the tibiale is the last element to form, not appearing until about the ninth day.

At birth, the calcaneus was present in all seven P.m. bairdi examined. Six of these also had the astragalus developed (ossified). The situation was nearly the opposite in P. leucopus. Of the nine newborn animals examined, only one had both the astragalus and calcaneus and another had the calcaneus slightly developed.

In one-day old individuals, the earlier appearance of ossification in P.m. bairdi continued. All ten P.m. bairdi had the calcaneus ossified and only one lacked ossification in the astragalus, although some of the latter elements appeared as only a cloud of bone. In *P. leucopus*, two of nine lacked ossification in either bone. Six had both bones ossified and one exhibited only calcaneus ossification. It was not until the two-day individuals that ossification was present in all of the *P. leucopus* with only one of seven lacking an astragalus. The differential size of the astragalus and the calcaneus was noted as the ossified part of the calcaneus was up to 1½-3 times that of the astragalus. Also the first of the tarsal elements was seen in one of the *P.m. bairdi* as a cloud of bone.

The third day was the first time that the calcaneus and astragalus was found in all ten *P. leucopus*. In *P.m. bairdi*, the third day brought on a continued ossification of the tarsal elements with four of nine specimens exhibiting ossification of the first tarsal element. One of these also had the 4-5 fused element present. One individual in P. leucopus exhibited an unusual phenomenon with the 4-5th appearing prior to the first. This situation was never seen in the other species and was only seen one other time in *P. leucopus* (five days). In all other cases, the first tarsal preceeded the 4-5th appearance.

Differences between species continued to be evident in the four day olds. Five of the *P. leucopus* showed at least one tarsus; four lacked any tarsal ossification. On the other hand, P.m. bairdi had only one specimen out of eleven which lacked some tarsal ossification. All the rest had at least two, with five having three.

The sequence of ankle bone ossification might be explored at this point. As stated earlier, the calcaneus appears to be the first bone to ossify with the astragalus appearing shortly afterwards. This occurs either before birth or at the latest by the third day. By the second or third day (fourth in P. leucopus) the tarsal elements begin to appear. Their sequence of ossification is generally the first, the 4-5th, and the third, and finally the second. Some exceptions have been noted previously. The centrale first appears on the fifth day (P.m. bairdi). The tibiale is very late in appearing, the ninth day in both species, at which time it appears only as a small speck of bone barely visible at 30 power.

The disparity between the species was evident in the five-day individuals. All ten P.m. bairdi had at least one tarsal element and only two lacked having at least three. One specimen also possessed a partially ossified centrale. In P. leucopus none of the eleven individuals had any trace of the centrale and only three had as many as three tarsals.

The sixth day found eight of eleven P.m. bairdi lacking only the tibiale. Two individuals lacked both the tibiale and the centrale; while one individual had only the astragalus, calcaneus, and first, third, and 4-5th fused tarsals present. P. leucopus had but two of ten individuals lacking only the tibiale. The rest lacked between three and four bones.

Data collected on the seven-day individuals exhibited the same trend as witnessed earlier. In P.m. bairdi nine of ten specimens lacked only the tibiale, while the tenth was lacking the tibiale and the centrale. P. leucopus had six of ten specimens with only the tibiale absent. The rest lacked the tibiale, the second tarsal and the centrale.

The ninth day brought the first appearance of the tibiale with one of nine P. leucopus and one of ten P.m. bairdi exhibiting all eight ankle bones. Eight of the P.m. bairdi lacked only the tibiale and one lacked the centrale, tibiale, and second tarsal element. Eight P. leucopus lacked only the tibiale.

The appearance of the tibiale was more frequent on the eleventh day. Six of ten P.m. bairdi had all eight ankle bones present. The rest lacked only the tibiale. P. leucopus had six of nine specimens with all the ankle bones present, and the others lacked only the tibiale.

Total ossification was achieved in P.m. bairdi on the thirteenth day. One of eight P. leucopus lacked complete appearance of the ankle bones. Table 7 gives a graphic representation of the progress of the appearance of the ankle elements.

Table 7.-- Ankle data.

	P. leucopus									*P.m. bairdi*								
	Tarsals									Tarsals								
Age	#	1	2	3	4-5	Ast	Cal	Tib	Cent	#	1	2	3	4-5	Ast	Cal	Tib	Cent
0	7	0	0	0	0	1	2	0	0	7	0	0	0	0	6	7	0	0
1	10	0	0	0	0	6	7	0	0	10	0	0	0	0	9	10	0	0
2	7	0	0	0	0	6	7	0	0	7	0	0	0	0	7	7	0	0
3	9	0	0	0	0	9	9	0	0	9	4	0	0	1	9	9	0	0
4	9	4	0	1	0	9	9	0	0	9	8	5	5	7	9	9	0	0
5	10	8	0	2	8	10	10	0	0	10	10	4	8	9	10	10	0	1
6	10	10	2	9	9	10	10	0	2	10	10	9	10	10	10	10	0	9
7	10	10	6	10	10	10	10	0	6	10	10	10	10	10	10	10	0	9
9	9	9	9	9	9	9	9	1	9	9	9	8	9	9	9	9	1	8
11	9	9	9	9	9	9	9	5	9	9	9	9	9	9	9	9	6	9
13	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

The analysis of the ankle was achieved by taking the mean number of ankle bones present in each species from birth to the ninth day and testing with a sign test. The probability of *P. leucopus* having a higher average number of tarsals only once in nine days is .020 and indicates a significant difference (Table 8). This suggests that the ankle of P.m. bairdi develops more rapidly. The figures from Figure 4 indicate that the difference is on the order of magnitude of one day with the percentages of P. leucopus resembling the P.m. bairdi's of the preceeding day.

Table 8.-- Sign test of the ankle data.

Day	P. leucopus	P.m. bairdi	Sign
0	.33	1.81	+
1	1.45	1.9	+
2	1.81	2.0	+
3	2.1	2.56	+
4	3.0	4.09	+
5	4.09	5.78	+
6	5.2	6.63	+
7	6.2	6.90	+
9	7.1	7.0	-

P.m. bairdi = 8

P. leucopus = 1

Wrist

The wrist consists of nine elements in both species. These are arranged in two rows with the distal row composed

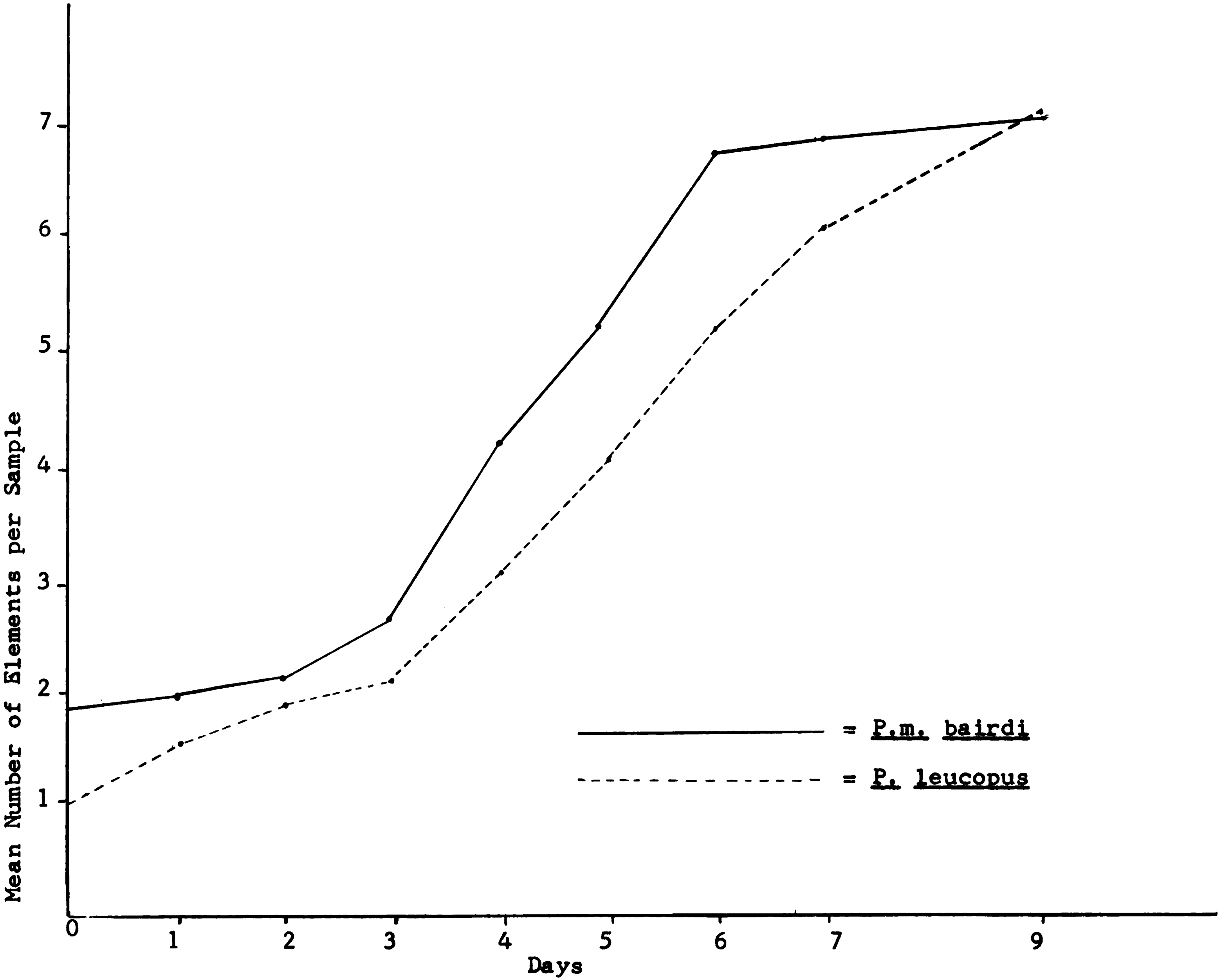

Figure 4.-- Ossification of the ankle.

of four carpels with the lateral representing the fused 4th and 5th carpels. The second transverse row is made up of the five remaining elements: sesamoid, fused radius-intermedium, centrale, ulnare, and pisiform. At birth the wrist exists as an unossified structure and bone deposition does not appear to commence until the third day (two-day olds) when both species exhibit some ossification. The progress of the bone deposition in the wrist may be seen in Table 9.

Table 9.-- Wrist data.

	P. leucopus											*P.m. bairdi*									
	Carpals											Carpals									
Age	#	1	2	3	4-5	C	R-I	U	S	P		#	1	2	3	4-5	C	R-I	U	S	P
2	9	0	0	0	1	1	0	1	0	0		9	0	0	0	2	1	0	1	0	0
3	10	0	0	0	3	3	0	2	0	0		10	1	0	0	7	6	1	6	0	0
4	9	1	1	1	6	6	1	5	3	0		9	5	4	5	8	8	6	7	6	0
5	9	4	5	5	8	5	5	7	6	0		9	9	9	9	9	9	9	9	9	0
6	10	9	9	9	10	10	9	10	9	0		10	10	10	10	10	10	10	10	10	1
7	10	10	10	10	10	10	10	10	10	1		10	10	10	10	10	10	10	10	10	4
9	8	8	8	8	8	8	8	8	8	5		8	8	8	8	8	8	8	8	8	6
11	10	10	10	10	10	10	10	10	10	10		10	10	10	10	10	10	10	10	10	10

The sequence of appearance of the wrist elements is slightly less predictable than that of the ankle bones.

The centrale, 4th-5th carpal, and ulnare appear first. Indications from these three-day old individuals suggest that the 4th and 5th may ossify first on the basis of the one representation of this element in one individual (*P.m. bairdi*), while the centrale is second on the basis of two individuals having both this element and the 4th and 5th. Eight individuals had all three elements out of the total of eleven three-day olds with some bone present. The other six bones make their first appearance almost simultaneously, and thus it is difficult to make a definitive statement as to their sequence of appearance.

The ossification of the wrist apparently occurs at different rates in the two species, as suggested in Figure 5. Only in the first day and eleventh day or in older individuals are the rates of occurrence equal. In each of the intermediate days, a higher average number of wrist elements per individual occurs in *P.m. bairdi*.

Using the sign test (Siegal, 1956), the results indicate that the probability of having $x = 0$ is .008 (Table 10). This value falls below the .05 level of significance chosen for all analyses in this thesis. Therefore, there is apparently a significant difference between the species in the rate of formation of the wrist elements with *P.m. bairdi* having its wrist bones forming sooner than those of *P. leucopus*.

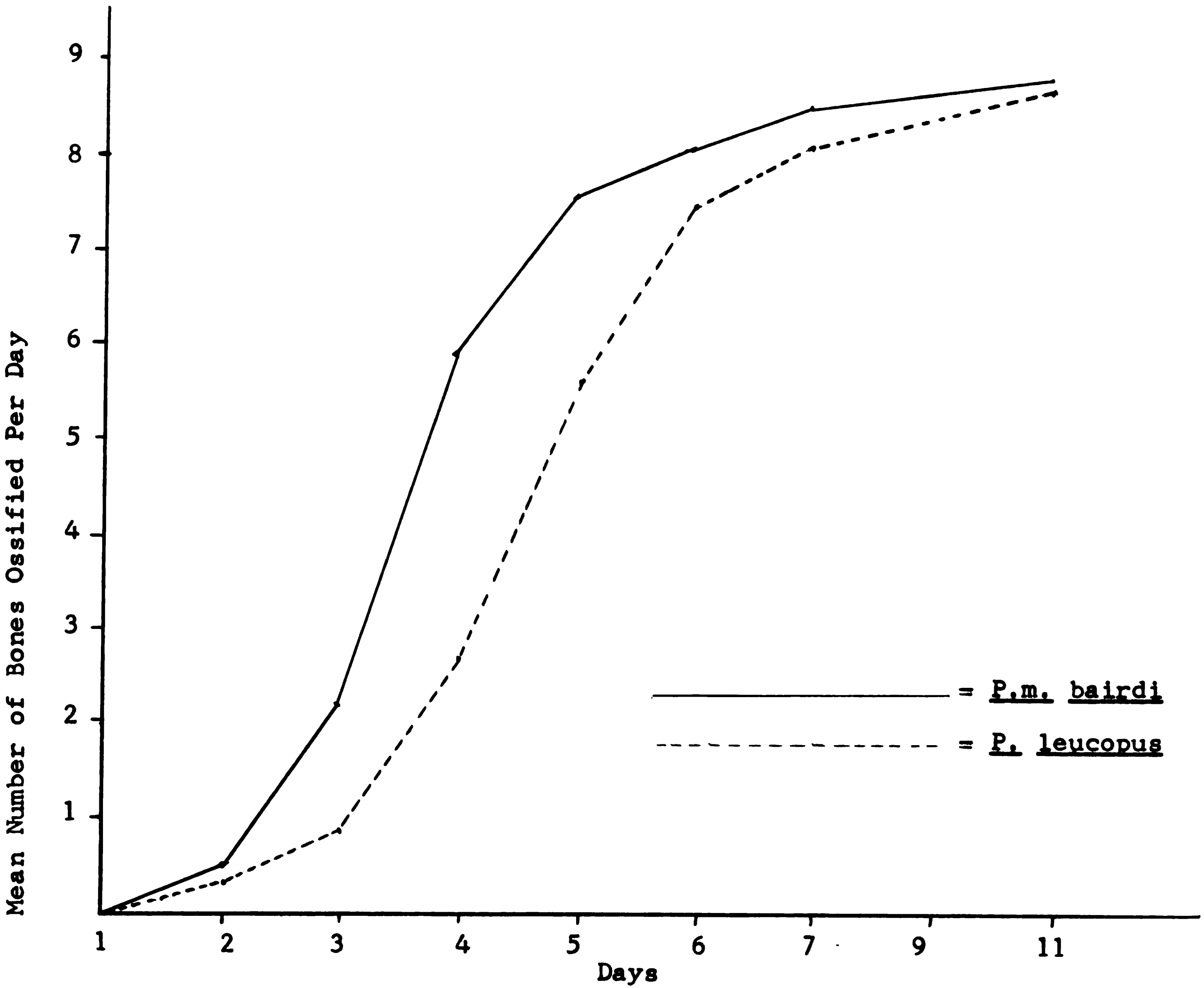

Figure 5.-- Ossification of the wrist.

Table 10.-- Sign test of the wrist data.

Day	*P. leucopus**	*P.m. bairdi**	Sign
2	.33	.50	+
3	.80	2.10	+
4	2.67	5.84	+
5	5.64	7.45	+
6	7.50	8.10	+
7	8.10	8.40	+
9	8.62	8.75	+

P.m. bairdi = 7

P. leucopus = 0

Prob. x = 0 = .008

* These figures indicate the mean number of bones per individual.

Tibia-fibula

The tibia and the fibula are closely appressed bones in many higher vertebrates, so intimately associated in some species as to become fused along part or all of their lengths. Partial fusion of these two bones is found in the genus *Peromyscus*. In this genus, they are twisted so as to fold around one another, apparently due to the twisting of the limbs as they have come to lie under the body in the mammals. The first signs of fusion of the tibia and fibula are a series of net-like strands which bridge the gap between the bones starting approximately 1/3 of the distance from the distal end at the area where the bones first are in close proximity. Growth continues at the ends and as a result fusion occurs last here. Since the fusion takes place on the inner faces, it is often difficult to determine whether fusion has taken place except with close inspection. The progress of the tibia-fibula can be seen in Table 11.

Table 11.-- Fusion of tibia-fibula data.

Days old	*P. leucopus*	*P.m. bairdi*
3	None	None
4	None (of 9)	2 (of 10)
5	4 (of 11)	4 (of 10)
6	6 (of 10)	10 (of 10)
7	10 (of 10)	10 (of 10)
Total	20 (of 40)	26 (of 40)

The graph of the fusion of the tibia and fibula (Figure 6) is basically similar to the graph of the appearance of the olecranon (Figure 1). In both, the first appearance of the character in question takes place in four-day individuals and reaches 100% in the seven-day olds. On each of the 4th, 5th, and 6th days, the sample of *P.m. bairdi* has a higher degree of development than that of

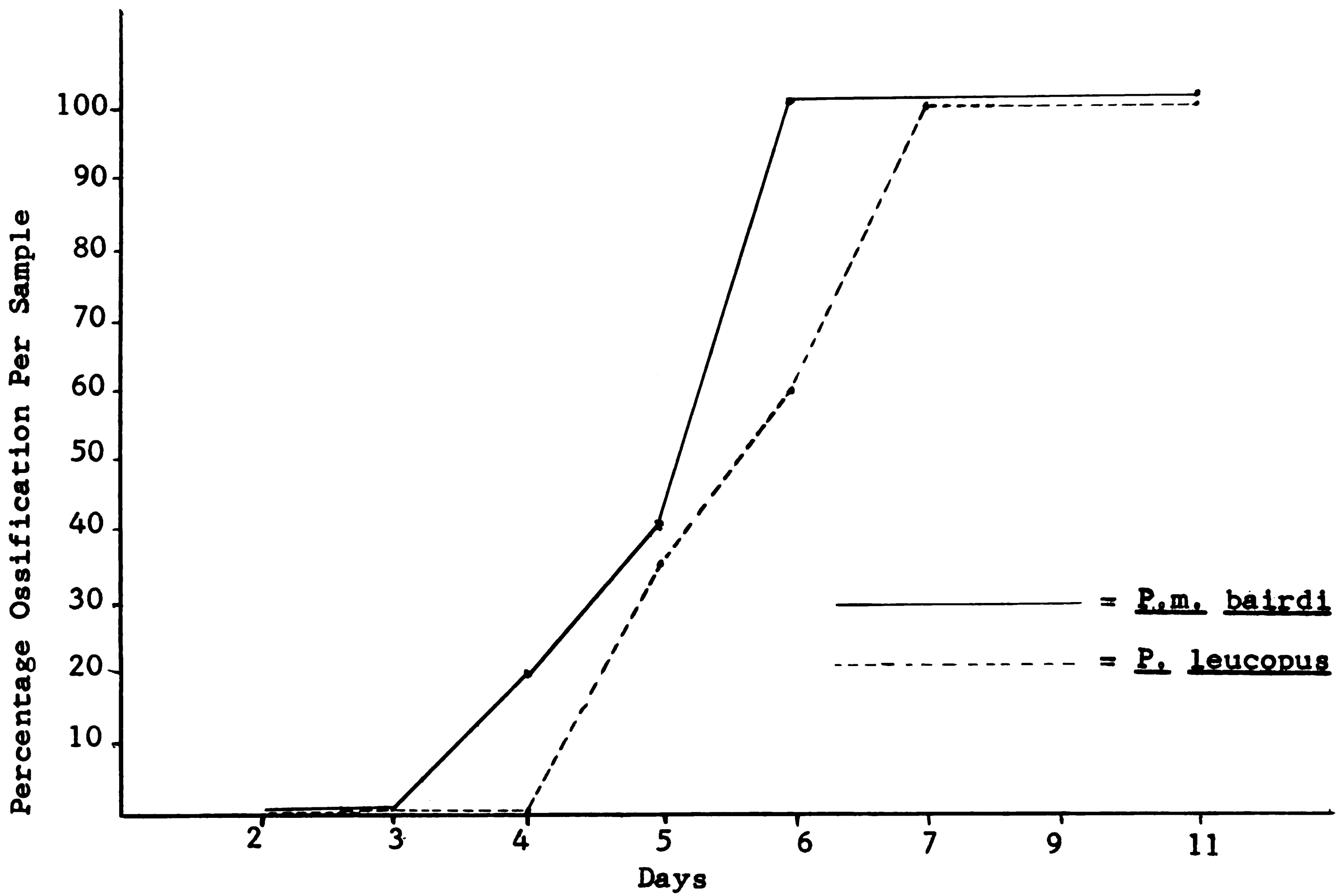

Figure 6.-- Ossification of the tibia-fibula.

P. leucopus.

A chi-square test run on the data for the fusion of the tibia-fibula reveals that with four degrees of freedom the value is 1.707 (Table 12). This figure lies between the .70 and .80 levels of significance. Therefore, it is concluded that there is no significant difference in the rate of appearance of fusion of the tibia and fibula in the two species.

Table 12.-- Chi-square analysis of the tibia-fibula data.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
4	0	.769	2	1.23	2
5	4	3.077	4	4.923	8
6	6	6.154	10	9.846	16
	10	10.0	16	16.0	26

$$\frac{(0 - .769)^2}{.769} + \frac{(2 - 1.23)^2}{1.23} + \frac{(4 - 3.077)^2}{3.077} + \frac{(4 - 4.923)^2}{4.923}$$

$$+ \frac{(6 - 6.159)^2}{6.159} + \frac{(10 - 9.846)^2}{9.846} = x^2$$

$$\frac{.59136}{.769} + \frac{.5929}{1.23} + \frac{.851929}{3.077} + \frac{.851929}{4.923} + \frac{.023716}{6.159}$$

$$+ \frac{.023716}{9.746} = x^2$$

$$.769 + .482 + .2768 + .173 + .00385 + .0024 = 1.707 = x^2$$

Sternal Ossification

The ossification of the sternum is a highly eccentric phenomenon, involving a great deal of individual variation. There are usually six sternal elements (sternebrae) which develop embryologically from paired centers, as documented by Little (1937), and are either partially or entirely fused at birth. Seven true ribs are attached to the sternum along its length. In certain individuals, however, a seventh element appears between the 6th and 7th ribs and separates the 5th and 6th sternebrae. This "extra" element was noted as being present in 28.7% of the *P.m. bairdi* and 14.7% of the *P. leucopus*.

Even though the sternebrae fuse early, they often keep the appearance of having been two distinct rods for some time after birth, up to the third day in some individuals. The first element, the manubrium, is often bifurcated at the anterior end. The xiphisternum, on the other hand, is rarely noted as having developed from two rods and was not divided in any of the 300 specimens used.

The fifth sternal element showed the greatest variation in size and shape. It was usually smaller, often distorted, and frequently sections were missing. There were also instances of co-ossification between various sternal elements.

No statistical analysis was done to compare the number of individuals with or without complete fusion since only three days were involved and on one of these the figures from the two species were identical with only one individual difference on the other two days. It was evident that there was no difference.

The difference between the two species in the development of the 7th sternal element was analyzed to determine if there was a significant difference in the occurrence of the "extra" element. The Wilcoxin Matched Pairs Rank-Sign Test (Siegal, 1956) was chosen to analyze these data with a .05 level of significance. The data indicated that there was a significant difference between the two species (Table 13). The value of 14 with an N = 12 fell exactly at the .05 level.

Table 13.-- Wilcoxin matched pairs rank-sign test of the sternum data.

Day	#	*P. leucopus*	*P.m. bairdi*	Difference	Ranks	Rank with less freq. sign
0	9	0	0	0	0	
1	10	1	0	-1	2	-2
2	9	1	2	+1	2	
3	10	2	2	0	0	
4	9	0	6	+6	12	

Table 13.-- continued.

Day	#	*P. leucopus*	*P.m. bairdi*	Difference	Ranks	Rank with less freq. sign
5	10	2	4	+2	6	
6	10	2	2	0	0	
7	10	4	2	-2	6	-6
9	9	3	1	-2	6	-6
11	9	1	4	+3	9	
13	8	1	3	+2	6	
15	10	2	4	+2	6	
18	7	0	4	+4	11	
21	9	1	4	+3	9.5	
24	7	0	1	+1	2	
136		20	39			14

Vertebral Column

The vertebral column presents an interesting aspect of this study because although it is basically one unit, it is divided into five morphologically and functionally distinct units. For this reason, it is possible to glean information from this structure by analyzing its five regions: cervical, thoracic, lumbar, sacral, and caudal, in addition to studying it as a whole.

The aspect of the vertebral column chosen for study was the fusion of the dorsal portion of the two lateral arches. This was a good area of study for several reasons. First, this fusion could be studied in all sections of the column. Second, it provided an easily recognizable quantitative measure of growth; being either fused or not. Third, it was felt that this dorsal fusion was a good index of bone growth in the vertebral column. In all cases, information was gathered only on the status of the fusion of the dorsal aspect of the vertebral column.

The vertebral column in the two species is essentially identical and is similar to that of the laboratory mouse, *Mus musculus* (Wirtschafter, 1960). There are of course seven cervicals, including an atlas and an axis. The remaining vertebrae are divided into thirteen thoracics, six lumbar, three sacral, and approximately 25 caudals. Since there are differences between the sections concerning the times of fusion, each will be discussed separately.

The vertebral column, taken as a whole, appears to be equal in its rate of development in the two species. A chi-square test at the 5% level of significance with 7 degrees of freedom produces a value of 13.9831 (Table 14). To be significant, the value would have to be at least 14.07. The chi-square value for the vertebral column lies between the .05 and the .10 level. This agrees with the individual components, in which only the thoracic vertebrae showed a significant difference between the two species, with P.m. bairdi showing significantly more rapid fusion. The other sections revealed no differences between the species.

Table 14.-- Chi-square analysis of the vertebral column.

	P. leucopus		P.m. bairdi		
Day	Obs.	Exp.	Obs.	Exp.	Total
3	5	4.32	4	4.68	9
4	17	14.39	13	15.61	30
5	46	38.37	34	41.63	80
6	71	65.71	66	71.29	137
7	92	109.84	137	119.16	229
9	188	178.44	184	193.56	372
11	202	216.11	250	235.19	452
13	252	245.11	259	265.89	511
Total	873	872.99	947	946.91	1820

$$\frac{(.68)^2}{4.32} + \frac{(.68)^2}{4.68} + \frac{(2.61)^2}{14.39} + \frac{(2.61)^2}{15.61} + \frac{(7.63)^2}{38.37} + \frac{(7.63)^2}{41.63}$$

$$+ \frac{(5.29)^2}{65.71} + \frac{(5.29)^2}{71.29} + \frac{(17.84)^2}{109.84} + \frac{(17.84)^2}{119.16} + \frac{(9.56)^2}{178.44}$$

$$+ \frac{(9.56)^2}{193.56} + \frac{(14.81)^2}{216.81} + \frac{(14.81)^2}{235.19} + \frac{(6.89)^2}{245.11} + \frac{(6.89)^2}{265.89} = x^2$$

Table 14.-- continued.

$$\frac{.4624}{4.32} + \frac{.4624}{4.68} + \frac{6.8121}{14.39} + \frac{6.8121}{15.61} + \frac{58.2169}{38.37} + \frac{58.2169}{41.63}$$

$$+ \frac{27.9841}{65.71} + \frac{27.9841}{71.29} + \frac{318.2656}{109.84} + \frac{318.2656}{119.16} + \frac{91.3936}{178.44}$$

$$+ \frac{91.3936}{193.56} + \frac{216.3361}{216.81} + \frac{216.3361}{235.19} + \frac{47.4721}{245.11}$$

$$+ \frac{47.4721}{265.89} = x^2$$

$$.1071 + .0988 + .4733 + .4363 + 1.5172 + 1.3984 + .4258$$

$$+ .3925 + 2.8975 + 2.6709 + .5121 + 4721 + 1.0116 + .9325$$

$$+ .1936 + .1785 = 13.7181 = x^2$$

Cervical Vertebrae

The cervicals were the last vertebrae to initiate fusion. The first fusions occurred on the seventh day in *P.m. bairdi*. On that day, two of ten individuals had at least one vertebrae fused, the 7th in one and the 1st plus 3-7th in the other. Unfortunately, as has been mentioned previously, no eight day individuals were collected. By the ninth day, the cervicals were well fused in both species. Seven of eleven specimens in *P.m. bairdi* and four of ten in *P. leucopus* exhibited fusion. Of these eleven individuals, all had the first fused; no other single vertebrae was as well represented in the fused state.

On the eleventh day, all ten of the *P.m. bairdi* showed at least some cervical fusion with two being completely fused. Six of eight individuals in P. leucopus exhibited cervical fusions with three being completely fused. Thirteen-day individuals tended to show complete fusion with one of eight lacking such in P. leucopus, a small individual with #2-5 not fused. Nine (of ten) P.m. bairdi were completely fused with only one individual lacking fusion in the axis. Fifteen day specimens were all completely fused.

The sequence of fusion is difficult to present in detail; however, it appears that the axis fuses first with the others fusing later. The order of the others cannot be precisely determined, but the 7th appears to be the last to fuse in general.

As the cervicals were the last vertebrae to initiate fusion, the number of possible comparisons was reduced to four days: 7, 9, 11, and 13. It should be noted that the only major vertebral difference between the two species occurred on the seventh day when P.m. bairdi had a total of eight fused cervicals, while P. leucopus had none.

A chi-square test at the .05 level of significance and three degrees of freedom produced a value of 7.261 (Table 15). This did not indicate any significant difference between the two species since the value lay between the

.05 and .10 level. Therefore, the cervicals were concluded to have fused at the same rates in both species.

Table 15.-- Chi-square analysis of the cervical vertebrae.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
7	0	3.68	8	4.32	8
9	19	19.31	23	22.69	42
11	31	30.35	35	35.65	66
13	53	49.66	55	58.34	108
	103	103.0	121	121.0	224

$$\frac{(3.68)^2}{3.68} + \frac{(3.68)^2}{4.32} + \frac{(.31)^2}{19.31} + \frac{(.31)^2}{22.69} + \frac{(.35)^2}{30.35} + \frac{(.35)^2}{35.65} + \frac{(3.34)^2}{49.66} + \frac{(3.34)^2}{58.34} = x^2$$

$$\frac{13.5424}{3.68} + \frac{13.5424}{4.32} + \frac{.0931}{19.31} + \frac{.0931}{22.69} + \frac{.4225}{30.35} + \frac{.4225}{35.65} + \frac{11.1556}{49.66} + \frac{11.1556}{58.34} = x^2$$

$$3.68 + 3.135 + .004 + .004 + .013 + .011 + .224 + .191 = 7.261 = x^2$$

Thoracic Vertebrae

Dorsal fusion in the thoracic vertebrae made its first appearance in both species on the sixth day. One individual in nine in *P. leucopus* had the 7-8-9th fused. One of eleven in *P.m. bairdi* had the 6th-11th fused. The number of individuals with fused thoracics was increased on the seventh day when *P. leucopus* had two of ten individuals with thoracic fusions, while *P.m. bairdi* had seven of ten individuals with at least one vertebrae fused.

By the ninth day, almost all individuals had at least some dorsal fusion in the thoracic areas with nine of ten individuals in *P. leucopus* and nine of eleven in *P.m. bairdi*. Three specimens in the *P. maniculatus* group had all the thoracic vertebrae fused, while none of the *P. leucopus* specimens had proceeded that far. Individuals from the eleventh day all had some fusion in the thoracic area. Four of ten individuals in *P.m. bairdi* had complete dorsal fusion; two *P. leucopus* had reached full fusion.

It is difficult to analyze the direction of fusion in the thoracic area but it appears that the middle vertebrae (#3-11) are the first to become fused. In general, the 13th is the last to fuse. Evidence for this is that none of the six-day individuals had #1-2 or #12-13 fused. In fact, the first day that the 13th becomes fused is probably the eighth or ninth day since the first record of the 13th being fused occurred on the ninth day. That is only one out of a total of 21 specimens for both species. *P. leucopus* did not have the 13th fused until the 11th day when three individuals showed that condition.

The thoracic vertebrae were the only ones which exhibited a significant difference between the two species. The data suggested that this might be the case since *P.m. bairdi* had more fused thoracics in each age class from 6 - 13 days. A chi-square test on the total number of vertebrae fused each day proved to be significant at the .05 level of significance with four degrees of freedom (Table 16). The chi-square value of 16.117 fell between the .01 and .001 levels of significance.

Table 16.--Chi-square analysis of the thoracic vertebrae.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
6	3	6.32	11	7.68	14
7	15	27.52	46	33.48	61
9	71	64.96	73	79.04	144
11	88	87.07	105	105.93	193
13	100	91.13	102	110.87	202
	277	277.0	337	337.0	614

$$\frac{(3.32)^2}{6.32} + \frac{(3.32)^2}{7.68} + \frac{(12.52)^2}{27.52} + \frac{(12.52)^2}{33.48} + \frac{(6.04)^2}{64.96}$$

$$+ \frac{(6.04)^2}{79.04} + \frac{(.93)^2}{87.07} + \frac{(.93)^2}{105.93} + \frac{(8.87)^2}{91.13} + \frac{(8.87)^2}{110.87} = x^2$$

Table 16.-- continued.

$$\frac{11.0224}{6.32} + \frac{11.0224}{7.68} + \frac{156.7504}{27.52} + \frac{156.7504}{33.48} + \frac{36.4816}{64.96}$$

$$+ \frac{36.4816}{79.04} + \frac{.8649}{87.07} + \frac{.8649}{105.93} + \frac{78.6769}{91.13} + \frac{78.6769}{110.87} = x^2$$

$$1.744 + 1.435 + 5.695 + 4.632 + .561 + .451 + .009 + .008 + .863 + .709 = 16.117 = x^2$$

To determine whether the difference lay in the number of individuals with fused vertebrae or the number of vertebrae per individual, a chi-square test was run on the number of individuals with fused thoracics on each day. Using the .05 level of significance and four degrees of freedom, the chi-square value was 2.585 (Table 17), which fell between the .50 and .70 level of significance.

Table 17.--Chi-square analysis of the thoracic vertebrae.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
6	1	.94	1	1.06	2
7	2	4.21	7	4.79	9
9	9	7.95	8	9.05	17
11	9	8.42	9	9.58	18
13	8	7.48	8	8.52	16
	29	29.0	33	33.0	62

Table 17.-- continued.

$$\frac{(.06)^2}{.94} + \frac{(.06)^2}{1.06} + \frac{(2.21)^2}{4.21} + \frac{(2.21)^2}{4.79} + \frac{(1.05)^2}{7.95} + \frac{(1.05)^2}{9.05} + \frac{(.58)^2}{8.42} + \frac{(.58)^2}{9.52} + \frac{(.52)^2}{7.48} + \frac{(.52)^2}{8.52} = x^2$$

$$\frac{.0036}{.94} + \frac{.0036}{1.06} + \frac{4.8841}{4.21} + \frac{4.8841}{4.79} + \frac{1.1025}{7.95} + \frac{1.1025}{9.05} + \frac{.3364}{8.42} + \frac{.3364}{9.52} + \frac{.2704}{7.48} + \frac{.2704}{8.52} = x^2$$

$$.003 + .003 + 1.16 + 1.1019 + .138 + .121 + .039 + .035 + .036 + .031 = 2.585 = x^2$$

The results indicate that the differences between species is due to the number of fused vertebrae per individual and not due ot *P.m. bairdi* having significantly more individuals with fused vertebrae.

Lumbar Vertebrae

First signs of lumbar fusion occurred on the fifth day in both species. *P.m. bairdi* had four of ten individuals and *P. leucopus* had two of eleven. In both species only #5-6 were fused. Six-day individuals exhibited much the same pattern, with eight of nine in *P. leucopus* and eight of eleven in *P.m. bairdi* showing fusion. However, of these sixteen total individuals, only one of each species had the 4th lumbar vertebrae fused. None had #1-3 fused.

Little change was evident between the sixth and seventh day individuals. The percentages were 70% (7/10) for P. leucopus and 80% (8/10) for P.m. bairdi. Of these, five of the P. maniculatus and only one of the P. leucopus had other than the 5th and 6th fused. The third was present in one of the P. leucopus and three of the P. maniculatus.

One nine-day individual in P. maniculatus had all the lumbar fused. At that age, all ten of the P. leucopus and nine of eleven P. maniculatus had some fusion. Even in some of the thirteen-day individuals, there were many individuals which lacked complete fusion of the dorsal aspect of the lumbar vertebrae.

There is a definite trend in both species to have the 5th and 6th lumbars fused long before the rest. It is possible that this may be a functional adaptation due to the proximity of the sacral vertebrae.

The number of lumbar vertebrae was almost identical in each species over the days sampled (4 - 13). A chi-square test was run on the data at the .05 level of significance with six degrees of freedom, to test for the possibility of significant differences. The value of the chi-square of 3.205 was significant between the .70 and .80 levels (Table 18). From this result, it was concluded that there was no significant difference between the two species in the number of fused lumbar vertebrae.

Table 18.-- Chi-square analysis of the lumbar vertebrae.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
4	1	.92	1	1.08	2
5	4	5.07	7	5.93	11
6	14	11.52	11	13.48	25
7	15	17.50	23	20.50	38
9	28	26.71	30	31.29	58
11	33	36.38	46	42.62	79
13	45	41.91	46	49.09	91
	140	140.0	164	163.99	304

$$\frac{(.08)^2}{.92} + \frac{(.08)}{1.08} + \frac{(1.07)^2}{5.07} + \frac{(1.07)^2}{5.93} + \frac{(2.48)^2}{11.52}$$

$$+ \frac{(2.48)^2}{13.48} + \frac{(2.50)^2}{17.50} + \frac{(2.50)^2}{20.50} + \frac{(1.29)^2}{26.71} + \frac{(1.29)^2}{31.29}$$

$$+ \frac{(3.38)^2}{36.38} + \frac{(3.38)^2}{42.62} + \frac{(3.09)^2}{41.91} + \frac{(3.09)^2}{49.09} = x^2$$

$$\frac{.0064}{.92} + \frac{.0064}{1.08} + \frac{1.1449}{5.07} + \frac{1.1449}{5.93} + \frac{6.1504}{11.52} + \frac{6.1504}{13.48}$$

$$+ \frac{6.250}{17.50} + \frac{6.250}{20.50} + \frac{1.6641}{26.71} + \frac{1.6641}{31.29} + \frac{11.4244}{36.38} + \frac{11.4244}{42.62}$$

$$+ \frac{9.5481}{41.91} + \frac{9.5481}{49.09} = x^2$$

$$.006 + .005 + .225 + .193 + .533 + .456 + .356 + .304$$

$$+ .062 + .053 + .314 + .268 + .227 + .194 = 3.205 = x^2$$

Sacral Vertebrae

The sacral vertebrae consist of three elements which fuse to form a solid base of attachment for the ilium. The sacrals, along with the caudals, begin to exhibit dorsal fusions on the third day. In each species, one individual (of nine in P. leucopus and eleven in P. maniculatus) had the 2nd and 3rd fused. Four-day individuals in P. leucopus exhibited fusions in the 2nd or 3rd or both. P. maniculatus had two of eleven with the 2nd and 3rd fused.

The first sacral did not beome fused until the fifth day when one individual from each species had all the sacrals fused. In addition, seven of eleven P. leucopus had at least one fusion while four of ten P. maniculatus had a similar condition. By the sixth day, only two of twenty individuals lacked any fusion in the sacrum.

On the seventh day and ninth day, all P. leucopus had fusion of at least one sacral vertebrae, while P.m. bairdi lacked one individual on each day. However, on the eleventh day, P. leucopus lacked ossification in two out of nine individuals, while P.m. bairdi had all with fusions.

The 2nd and the 3rd sacral vertebrae appear to ossify first followed by the first. The 2nd and 3rd first appear on the third day. The first does not appear until the fifth day in both species. The first never appears alone.

As with the other divisions of the vertebral column, the data from the sacral vertebrae were analyzed with a chi-square test with the hope of establishing the presence or absence of significant differences between the species. The data did not suggest that there was any significant difference since each age class had not more than a total difference of six fused vertebrae. The results of the test at the .05 level of significance with six degrees of freedom was a chi-square value of 3.294 (Table19). This figure was significant at between the .70 and .80 levels.

Table 19.--Chi-square analysis of the sacral vertebrae.

	P. leucopus		*P.m. bairdi*		
Day	Obs.	Exp.	Obs.	Exp.	Total
3	2	2.09	2	1.91	4
4	8	6.28	4	5.72	12
5	15	13.61	11	12.39	26
6	22	20.41	17	18.59	39
7	25	26.69	26	24.31	51
9	30	28.79	25	26.21	55
11	21	25.12	27	22.88	48
	123	122.89	112	112.01	235

Table 19.-- continued.

$$\frac{(.09)^2}{2.09} + \frac{(.09)^2}{1.91} + \frac{(1.72)^2}{6.28} + \frac{(1.72)^2}{5.72} + \frac{(1.39)^2}{13.61}$$
$$+ \frac{(1.39)^2}{12.39} + \frac{(1.59)^2}{20.41} + \frac{(1.59)^2}{18.59} + \frac{(1.69)^2}{26.69} + \frac{(1.69)^2}{24.31}$$
$$+ \frac{(1.21)^2}{28.79} + \frac{(1.21)^2}{26.21} + \frac{(4.12)^2}{25.12} + \frac{(4.12)^2}{22.88} = x^2$$

$$\frac{.0081}{2.09} + \frac{.0081}{1.91} + \frac{2.9584}{6.28} + \frac{2.9584}{5.72} + \frac{1.9321}{13.61} + \frac{1.9321}{12.39}$$
$$+ \frac{2.5281}{20.41} + \frac{2.5281}{18.59} + \frac{2.8561}{26.69} + \frac{2.8561}{24.31} + \frac{1.4641}{28.79}$$
$$+ \frac{1.4641}{26.21} + \frac{16.9744}{25.12} + \frac{16.9744}{22.88} = x^2$$

$$.003 + .004 + .471 + .517 + .141 + .155 + .123 + .135$$
$$+ .107 + .117 + .050 + .055 + .675 + .741 = 3.294 = x^2$$

Apparently there is no difference between the species in the number of sacral vertebrae fused on each day.

Caudal Vertebrae

The caudal vertebrae present an unusual situation in that not all of them posses neural arches. Only the first five or six have neural arches while the remainder of the 25 or 26 vertebrae consist only of the centrum.

In examining the tail, it was found that caudals showed first fusion in the three-day individuals, with one of nine *P. leucopus* and two of eleven *P.m. bairdi* fused.

Each species had two four-day individuals with fused caudals out of the same number of specimens as at three days.

Eight of eleven P. leucopus showed caudal fusion in five-day individuals. These ranged in number from one to four in number in both species. By the sixth day, all eleven P.m. bairdi were fused and eight of nine P. leucopus were fused. All individuals were fused on the seventh day.

The analysis of the caudal vertebrae was done on only the first four arched vertebrae. A chi-square test was run with a .05 level of significance and six degrees of freedom and indicated no significant developmental differences between the two species. The chi-square value was 3.011 which fell between the .80 and .90 levels (Table 20).

Table 20.-- Chi-square analysis of the caudal vertebrae.

	P. leucopus		P.m. bairdi		
Day	Obs.	Exp.	Obs.	Exp.	Total
3	3	2.63	2	2.37	5
4	8	8.43	8	7.57	16
5	27	23.71	18	21.29	45
6	32	31.09	27	27.91	59
7	37	37.41	34	33.59	71
9	40	38.47	33	34.53	73
11	29	34.25	36	30.75	65
	176	175.99	158	158.00	334

Table 20.-- continued.

$$\frac{(.37)^2}{2.63} + \frac{(.37)^2}{2.37} + \frac{(.43)^2}{8.43} + \frac{(.43)^2}{7.57} + \frac{(3.29)^2}{23.71}$$

$$+ \frac{(3.29)^2}{21.29} + \frac{(.91)^2}{30.09} + \frac{(.91)^2}{27.91} + \frac{(.41)^2}{37.41} + \frac{(.41)^2}{33.59}$$

$$+ \frac{(1.53)^2}{38.47} + \frac{(1.53)^2}{34.53} + \frac{(5.25)^2}{34.25} + \frac{(5.25)^2}{30.75} = x^2$$

$$\frac{.1369}{2.63} + \frac{.1369}{2.37} + \frac{.1849}{8.43} + \frac{.1849}{7.57} + \frac{10.8241}{23.71} + \frac{10.8241}{21.29}$$

$$+ \frac{.8281}{30.09} + \frac{.8281}{27.91} + \frac{.1681}{37.41} + \frac{.1681}{33.59} + \frac{2.3409}{38.47}$$

$$+ \frac{2.3409}{34.53} + \frac{27.5625}{34.25} + \frac{27.5625}{30.75} = x^2$$

$$.052 + .057 + .021 + .024 + .456 + .508 + .027 + .027$$

$$+ .029 + .004 + .005 + .060 + .068 + .804 + .896 = 3.011 = x^2$$

DISCUSSION AND CONCLUSION

As might be expected from a comparative study of two closely related forms, the results of this study indicate that there are few significant differences in bone development between P.m. bairdi and P. leucopus. The fact that some were found substantiates the belief that the Alizarin-KOH clearing and staining technique is an adequate tool in this type of research for comparisons at the sub-generic level. In addition, the results of this research suggest that the differences between the two species in the rates of bone development might at least partially mirror the dissimilar environments of the two species and the differential selective pressures inherent therein.

The similarities between the two species were more numerous than the differences. The rates of ossification of the olecranon, patella, vertebral column (entire), cervical vertebrae, sacral vertebrae, lumbar vertebrae, caudal vertebrae, and the fusion of the tibia-fibula exhibited no significant differences between the two species. The degree of similarity between the species in the various characters studied were not consistent and as can be seen in the results of the statistical analyses, the probabilities of the characters not being significantly different ranged from 5-10% to 80-90%.

Those areas which exhibited significant differences in bone development were the ankle, wrist, femural condyles, and the thoracic vertebrae. The probabilities that these structures were in actuality not significantly different ranged between .02 and .001 indicating that these structures were highly different. There is a possibility that the two species differed in other areas of bone development, but of the areas examined, only these four showed differences between the species.

It was inferred earlier that these disparities in ossification between P.m. bairdi and P. leucopus may be the result of differences in their habitats, as reflected in differential selective pressure. This cannot be proven with the results of this research; it can only be presented as a logical possibility. Further evidence for this contention could be obtained from an analysis of a series of individuals of the woodland deer mouse, P. maniculatus gracilis. This species is very closely related to P.m. bairdi; however, it lives in habitats very similar to those of P. leucopus. Were this sub-species to mimic the bone development of P. leucopus more closely than that of P.m. bairdi, credence could be attributed to the suggestion that the more rapid development of the grassland forms was due to the selective pressure favoring early mobility.

At this point, it might be noted that three of the four structures with significant differences are directly associated with locomotion (wrist, ankle, and femural condyles). This compares with three structures directly associated with locomotion which do no exhibit differences (patella, olecranon, and tibia-fibula). In those areas not associated with locomotion only one area, the thoracic vertebrae, shows significant differences, compared with five which do not. This indicates that skeletal elements associated with locomotion show more variability than those which are not, and thus may be influenced by environmental pressures.

In considering certain structures, a number of elements were lumped to produce an entity, such as the wrist. However, if all the elements are considered separately, there are a total of twenty-six. An interesting trend may be seen in the days which each of these elements first appears. Of the twenty-six characters studied, P.m. bairdi had eleven which ossified before the corresponding structures in P. leucopus. Fifteen elements appeared on the same day, while, in no case did a bone from P. leucopus ever appear prior to a corresponding one in P.m. bairdi. In a sign test run on these data, the probability that there is no significant difference between species when P.m. bairdi preceeds P. leucopus eleven times to none is .000 (Siegal, 1956), as seen in Table 21. This definitely suggests that ossification is tending to take place in P.m. bairdi faster than in P. leucopus.

Table 21.-- Days when ossification first takes place in each species.

	P.m. bairdi	P. leucopus
Olecranon *	4	5
Distal ossification of femur *	4	6
Patella *	7	9
Tibia-Fibula *	4	5
Astragalus	0	0
Calcaneus	0	0
Tarsal 1 *	2	4
Tarsal 2 *	5	6
Tarsal 3	4	4
Tarsal 4-5	3	3
Centrale *	5	6
Tibiale *	7	9
Cervical vertebrae	5	5
Thoracic vertebrae	5	5
Lumbar vertebrae	4	4

Table 21.-- continued.

	P.m. bairdi	*P. leucopus*
Sacral vertebrae	3	3
Caudal vertebrae	3	3
Ulnare	2	2
Pisiform *	6	7
Sesamoid	4	4
Centrale	2	2
Fused R-I *	3	5
Carpal 1 *	4	5
Carpal 2	4	4
Carpal 3	4	4
Carpal 4-5	2	2

* Indicates structure first appearing on different days.

The manifestation of the seventh sternal element in both species represents an anomolous condition. Skeletal variations are very common in mammals, particularly in island forms. There is no significant difference between the species, even though *P.m. bairdi* possessed this structure almost twice as often as *P. leucopus* (28.7% vs. 14.7%).

The results of this research are of importance in laying the groundwork for the study of some of the other species in the genus *Peromyscus*, particularly *P.m. gracilis*.

Members of this genus have been studied intensively for many years with the hope of determining ecological and phylogenetic relationships. The KOH-Alizarin clearing and staining technique may add a new avenue of research in this area of Mammalogy.

The determination of an aging schedule is somewhat tenuous. The great amount of variability within the species precludes a high degree of precision. In all cases an underestimation of age would result, as there are a few individuals which exhibit bone development at least one day prior to the majority. Since an aging table would have to be based on the minimum ages of individuals exhibiting ossified structures, an underestimation of age would be likely.

Thus, this thesis presents the results of what might be termed a pilot study for a more detailed research project in the comparative osteology of the genus *Peromyscus*. It establishes the methods and procedures which may be used in analyzing other species, as well as pointing to certain pitfalls which are inherent in this type of study.

BIBLIOGRAPHY

Burt, William H., 1957, Mammals of the Great Lakes Region, University of Michigan Press, Ann Arbor, 246 pp.

Crary, D.D. and P.B. Sawin, 1949, "Morphogenic Studies in the Rabbit VI. Genetic Factors Influencing the Ossification Patterns of the Limbs", Genetics 34: 508-23.

_______. 1957, "Morphogenic Studies on the Rabbit XVIII. Growth of Ossification Centers of the Vertebral Centra During the 21st Day", The Anatomical Record 127: 131-50.

_______, and Nancy Atkinson, 1958, "Morphogenic Studies of the Rabbit XXI. The Nature of Disproportionate Dwarfism Induced by the Da Gene Revealed by Early Fetal Ossification Pattern", American Journal of Anatomy 103(1): 69-98.

Hamilton, William J., Jr., 1939, American Mammals, McGraw-Hill Book Co., New York, 434 pp.

_______. 1943, The Mammals of the Eastern United States, Cornell University Press, Ithaca, New York, 432 pp.

Hyman, Libbie H., 1922, Comparative Vertebrate Anatomy, University of Chicago Press, Chicago, 544 pp.

Little, Malcomb E., 1937, Structures of the Vertebrates, Farrar and Rinehart Co., New York, 488 pp.

Nesslinger, Carlita L., 1956, "Ossification Centers and Skeletal Development in Postnatal Virginia Opossum", Journal of Mammalogy 37: 382-94.

Rowett, H.G.Q., 1957, Dissection Guides: III, The Rat, Rinehart and Co., New York, 64pp.

Sawin, P.B. and Crary, D.D., 1956, "Morphogenic Studies of the Rabbit XVI. Quantitative Racial Differences in Ossification Pattern of the Vertebrae of Embryos as an Approach to Basic Principles of Mammalian Growth", American Journal of Physical Anthropology 14: 624-48.

Sawin, P.B. and Muriel Trask, 1965, "Morphogenic Studies of the Rabbit XXXV. Pleiotropic Effects of the Dachs Gene and the Radient Growth Pattern", Journal of Morphology 117: 87-114.

Siegal, Sidney, 1956, Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill Book Co., New York, 312 pp.

Wirtschafter, Zolton Tillson, 1960, The Genesis of the Mouse Skeleton: A Laboratory Atlas, Thomas Co., Springfield, Illinois, 169 pp.