ELECTROPHORETIC BLOOD SERUM PATTERNS IN SELECTED SPECIES OF PEROMYSCUS

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ABSTRACT

ELECTROPHORETIC BLOOD SERUM PATTERNS
IN SELECTED SPECIES OF PEROMYSCUS

by Michael K. Petersen

Blood serum proteins of 18 species in the five subgenera (according to Osgood, 1909) of Peromyscus from the United States and México were separated by the disc electrophoretic procedure (following Ornstein and Davis, 1962) in an attempt to classify these animals by differences in these proteins. It was hoped that these findings might be correlated with our present understanding of taxonomic relationships of the various species of Peromyscus based on other morphological characteristics.

Blood was extracted from anesthetized or conscious animals and centrifuged to obtain the clear serum which was frozen until electrophoresis was performed on it. After electrophoresis at a constant current of 5 ma./tube for a distance of 32 mm, the proteins were stained with Amido Blue-Black 10 B and then optically scanned with a Model E Canalco Microdensitometer which produced a graphic curve thought to be characteristic for each species. X and Y coordinates were calculated (and averaged if more than one individual per

species was used) for each protein "peak" and "trough," thus eliminating individual variation so that one curve for each species could be used in analysis of data. Curves were visually compared on the basis of superficial characteristics such as number of bands, symmetry, general appearance, and slope of peaks.

The results indicate that species of Peromyscus can be placed into species groups based on their characteristic densitometric curves. The species groups form a continuum which is in accordance with that of Osgood (1909) who based his on external and cranial characteristics. It can be concluded from this study that the disc electrophoretic technique is probably sensitive to the species group level in the genus Peromyscus. In general, the species curves for Peromyscus also corroborated the findings of other investigators such as Hooper (1957, 1958), Hooper and Musser (1964), Blair (1942), and Rinker (1963) who classified the genus on the basis of phallic characteristics and musculature.

The disc electrophoretic procedure used in this study failed, however, to produce results agreeing with those of a starch gel electrophoretic investigation made by Johnson and Wicks (1959). This indicates that much needs to be accomplished in the standardization of electrophoretic techniques before mammalian relationships can successfully be determined by protein differences.

Geographic and individual variation in blood serum proteins was observed by superimposing the densitometric curves of individuals in each species taken at the same locality and at different localities. Non-geographic variation in curve structure was less than geographic variation. Other causes of variability may include age, sex, diet, physiological stress at time of blood extraction from the animal, disease, time of year when blood was collected, experimental error, and multiple alleles.

Prealbumin protein was found to be prevalent in most Mexican species of Peromyscus, suggesting that this protein may be of a selective advantage as an additional means of transporting thyroxin in the blood stream. This would help an animal to increase its basal metabolic rate in the lower oxygen tensions of higher altitudes.

ELECTROPHORETIC BLOOD SERUM PATTERNS IN SELECTED SPECIES OF PEROMYSCUS

By

Michael K. Petersen

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INTRODUCTION

Mammalian taxonomists are familiar with most of the morphological features of mammals which they are studying. With the advent of modern molecular biology, many workers are now attempting to classify mammals by the differences in their proteins. Electrophoresis is one of several methods employed to separate and distinguish proteins in blood, snake venom, muscle, and other tissues. Electrophoresis (see Bouck and Ball, 1965) literally means carried by electricity. In a proper medium, the net change of a protein molecule determines the direction and relative rate of migration in an electrical field. Proteins of different species can be thus separated, identified and compared.

The objectives of this study were (1) to separate electrophoretically, blood serum samples from several representatives of the five subgenera of Peromyscus (according to Osgood, 1909); (2) to scan densitometrically the resulting protein bands to determine differences between the samples; and (3) to correlate these findings with our present understanding of taxonomic relationships of the various species of Peromyscus based on other morphological characteristics.

Molecular Basis of Evolution

Leone (1964) mentions that genes control protein structure and that proteins remain the same over many generations. Certain of these proteins evolve in parallel, within a given phyletic line, their structures being increasingly similar in more closely related organisms. Anfinson (1959) has proposed the useful concept of protein spectrum: "Since proteins can be modified without loss of function, it seems certain that the permissible degree of modification, in terms of fractions of their total structure, will vary from molecular species to species," some being persistently reproduced. From this comment, Leone (1964) goes on to say that "we may 'express' a species in terms of a hierarchy of protein structures ranging in 'violability' from none to very much. During mutation and natural selection, one end of the spectrum of proteins would remain little changed, and the other end change considerably. This explains the persistence of those proteins which we recognize as accounting for the unity of biochemistry. It also accounts for the heteromorphic evolution as well as for the appearance of new proteins in phylogeny." Thus the disc electrophoretic technique is one appropriate method presently being employed to explain the molecular basis of evolution and to support or refute existing systematic relationships.

Review of Literature

A variety of comparative studies on mammalian blood serum, using several electrophoretic techniques, have been conducted at different taxonomic levels (from between orders to between individuals within a single inbred strain). Qualitatively, mammals have five characteristic blood proteins--one albumin and four globulins (Gleason and Friedberg, 1953), although opossums have less albumin. Reptiles and amphibians have seven to ten characteristic proteins, but their total protein content is lower; relative mobilities are slower and no albumins are present.

Moore (1945) found differences in electrophoretic patterns between such species as man, rhesus monkeys, swine, white rats, cotton rats, cats, guinea pigs, hamsters, and chickens; and, it was noted that each species had a reproducible, characteristic pattern. Young laboratory rats, young cotton rats, and adult cotton rats have an α globulin protein, while in adult laboratory rats it is lacking. β and γ globulins are present in hamsters but not in guinea pigs. In cats the albumin is of a faster mobility than in other mammals and β globulin is not present. The entire protein patterns in humans, rhesus monkeys, and swine were found to be similar to one another, while those of male and female chickens differ. The last two studies serve to indicate that protein differences do occur in many groups of animals,

but these differences have not been used to illustrate any systematic relationships. On the other hand, Shaw and Barto (1965) discovered polymorphic forms of the glucose-6-phosphate enzyme in liver, kidney, and muscle tissue in three inbred strains of Peromyscus maniculatus bairdii. Johnson and Wicks (1959) compared blood of species of several mammalian orders and, in general, their results corroborated the current taxonomic arrangements; however, two exceptions were noted, these being in Peromyscus and in four genera of Microtinae (see discussion).

A comparison of blood serum proteins of several genera of rodents (Perognathus, Sigmodon, Peromyscus and Citellus) by Auernheimer et al. (1959) showed significant differences at the subgeneric and generic levels. Differences were mainly evident in the globulins. The protein bands of two closely related species of Perognathus were indistinguishable. There was no appreciable variation among samples from two to nine individuals per species.

METHODS

Eighteen species of *Peromyscus* were used in this study. Adults of either sex were selected. The kinds, localities of capture and number of specimens studied are as follows:

Collecting Localities

P. boylii: México, 5 mi. W Atenquique, 6500 ft., Jalisco, 1

" " México, 4 mi. NW Huitzilac, 9200 ft., Morelos, 3

" " México, 12 mi. SW Xochipala, 8200 ft., Guerrero, 4

" " México, 2 mi. W Xochipala, 4400 ft., Guerrero, 1

" " México, 6 mi. NW Chilpancingo, 5500 ft., Guerrero, 1

" " México, 10 mi. N Ixtlán de Juarez, 9300 ft., Oaxaca, 1

**P. californicus*: California, Berkeley, Alameda Co., 5

**P. crinitus*: California, southern part of state, 4

P. difficilis: México, 4 mi. NW Huitzilac, 9200 ft., Morelos, 4

" " México, 3 mi. ENE Zoquiapan, 9200 ft., Edo. México, 1

" " México, 1½ mi. ENE Zoquiapan, 8600 ft., Edo. México, 1

**P. eremicus*: California, no specific locality, 2

P. evides: México, 6 mi. NW Chilpancingo, 5500 ft., Guerrero, 1

*P. floridanus: Florida, no specific locality, 2

P. leucopus novaboracensis: Michigan, East Lansing, Ingham Co., 9

P. l. texanus: México, 5 mi. SW Chapulhuacán, 5100 ft., Hidalgo, 4

*P. maniculatus bairdii: Michigan, East Lansing, Ingham Co., 7

P. m. gracilis: Michigan, Cusino Wildlife Station, Schoolcraft Co., 4.

P. m. labecula: México, 3 mi. SW Yurecuaro, 5200 ft., Michoacán, 5

" " " México, 5 mi. WSW Amealco, 8700 ft., Queretaro, 1

P. megalops: México, 12 mi. SW Xochipala, 8200 ft., Querrero, 3

" " México, 8 mi. SSW Juchatengo, 6300 ft., Oaxaca, 2

P. melanophrys: México, 5 mi. WSW Amealco, 8700 ft., Queretaro, 1

" " México, 1 mi. N Santa Rosa, 3600 ft., Zacatecas, 2

P. melanotis: México, ½ mi. NW Llano Grande, 10,600 ft., Edo. México, 1

" " México, 4 mi. NW Huitzilac, 9200 ft., Morelos, 1

P. mexicanus: México, 4 mi. S Valle Nacional, 2600 ft., Oaxaca, 2

P. nuttalli: Kentucky, 6 mi. E Vanceburg, Lewis Co., 2

*P. ochraventer: México, 8 mi. W El Naranjo, 2400 ft., San Luis Potosí, 1

*P. polionotus: Florida, Oscala National Forest, 5

P. thomasi: México, Puerto Chico, 8400 ft., Guerrero, 2

P. truei: México, 2 mi. NE Boquilla, 6200 ft., Durango, 1

*Denotes laboratory-reared animals. The others were wild-taken in live traps.

Procedure

Blood was either extracted from the beating heart of an anesthetized animal (using ether or chloroform) with a 3 cc syringe and #23 needle or from the suborbital sinus of a conscious animal with 1.5 mm diameter capillary tubing. Next the blood was placed in standard 3-inch test tubes and centrifuged at 4500 r.p.m. in an International Clinical Model Centrifuge for five minutes, after which the clear serum was drawn off and frozen until needed for further use.

The disc electrophoretic procedure followed was essentially the same as that employed by Ornstein and Davis (1962). A 10 per cent sucrose solution was used instead of upper gel to dilute the serum. In order to get the best resolution of protein bands, dilutions were varied from 30 to 60 parts sucrose solution to one part serum. It was found that a 40:1 dilution using 20 λ of serum was optimal. After proper dilution, the serum from one animal was divided into triplicates so that each of the three could be run separately to allow for possible loss of any runs. Nine tubes (serum from three mice) were electrophoresed simultaneously at a constant current of 5 ma./tube for a distance

of 32 mm. Upon completion of this operation the nine runs were stained in Amido Blue-Black 10B for ten minutes and then destained electrically in 7 per cent acetic acid for three hours, after which they were stored in individual test tubes in 7 per cent acetic acid.

Analysis of Data

The electrophoretic runs were optically scanned by a Model E Canalco Microdensitometer. A graphic curve (extending approximately five times the length of the actual run) was produced for each animal. In each densitometric curve, the protein bands appeared as peaks (see Figure 1) and were designated by cardinal numbers. Then X and Y coordinates were calculated for every "peak" and "trough," using as an origin the "trough" nearest the point of separation between the upper and lower gels. A species curve thought to be characteristic of each taxon was plotted from the X and Y coordinates (or from average values of these if more than one individual per species was used) on graph paper. Analysis of the species curves consisted of visually comparing them on the basis of superficial characteristics such as number of bands, symmetry, general appearance, and slope of peaks. In the present study, owing to lack of a sufficient number of samples, a statistical analysis was omitted.

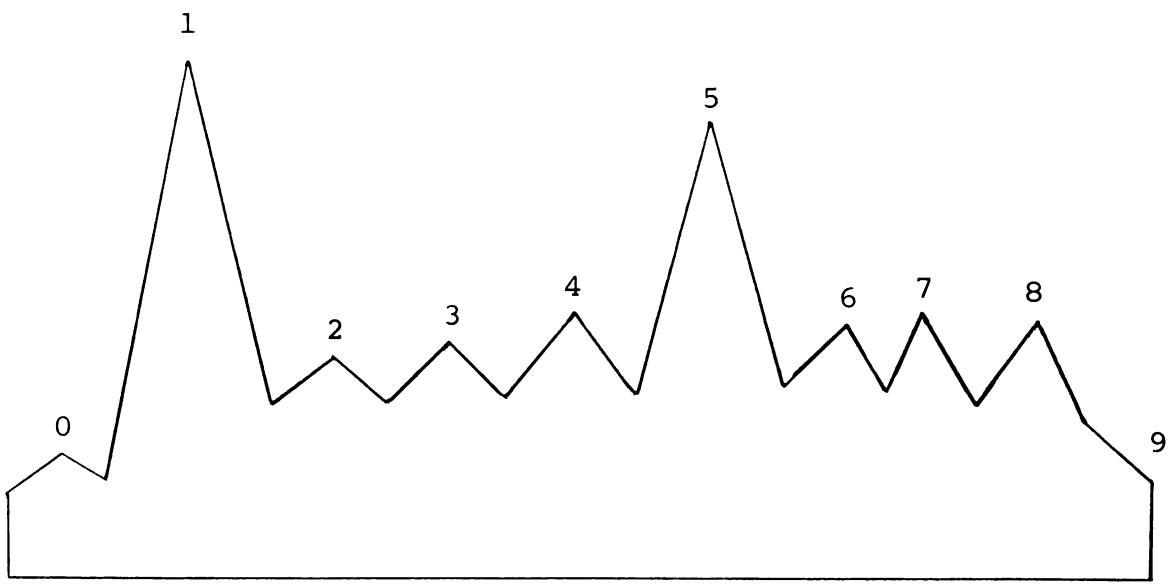


Figure 1. Schematic diagram of a densitometric curve and its nomenclature: 0-prealbumin (pr.A); 1-albumin (A); 2-4-post albumins (po.A); 5-transferrin (T); 6-8-globulins (G); 9-origin of run (X).

Instead, the average densitometric curves for each species have been arbitrarily grouped on the basis of superficial similarities (or dissimilarities). By superimposing all curves within a single species, population (individual) variation and geographic variation were analyzed and compared in P. leucopus and P. boylii.

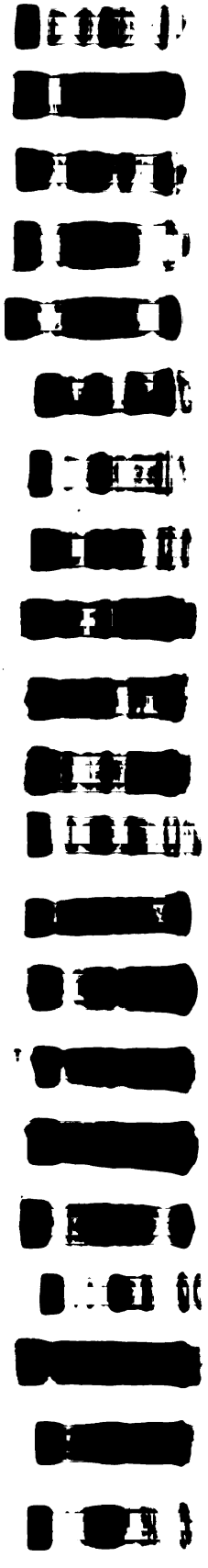
RESULTS

In general, Peromyscus blood serum probably contains proteins similar to those found in other mammals. For this study, however, no attempt was made to name every band with respect to a specific protein but, instead, to use as a guide a schematic densitometric curve (following Ornstein and Davis, 1962), illustrating the peaks and their probable protein identity (see Fig. 1). Plate 1 is a photograph of representative electrophoretic patterns for each species studied. In Figure 2 the species curves (some subspecific) have been plotted. Based on the observable similarities and dissimilarities, the following continuum of peromyscine blood serum relationships is proposed (Fig. 3).

Comparison of the densitometric curves shows that the species of Peromyscus studied can be arranged by means of serum protein bands in the five Osgoodian subgenera, Peromyscus, Podomys, Ochrotomys, Megadontomys, and Haplomylomys. An artificial key to the subgenera based on the configuration of these curves is as follows:

1 . Two T bands present . . . . . Subgenus Haplomylomys
1'. One T band present 2

2 . Four po.A bands present . . . . . Subgenus Megadontomys
2'. Three or less po.A bands present . 3



Peromyscus eremicus
Peromyscus mexicanus
Peromyscus polionotus
Peromyscus californicus
Peromyscus crinitus
Peromyscus thomasi
Peromyscus nuttalli
Peromyscus floridanus
Peromyscus ochraventer
Peromyscus evides
Peromyscus melanophrys
Peromyscus truei
Peromyscus difficilis
Peromyscus boylii
Peromyscus megalops
Peromyscus leucopus texanus
Peromyscus leucopus novaboracensis
Peromyscus melanotis
Peromyscus maniculatus labecula
Peromyscus maniculatus gracilis
Peromyscus maniculatus bairdii

Plate 1. Typical electrophoretic patterns for each species of Peromyscus studied.

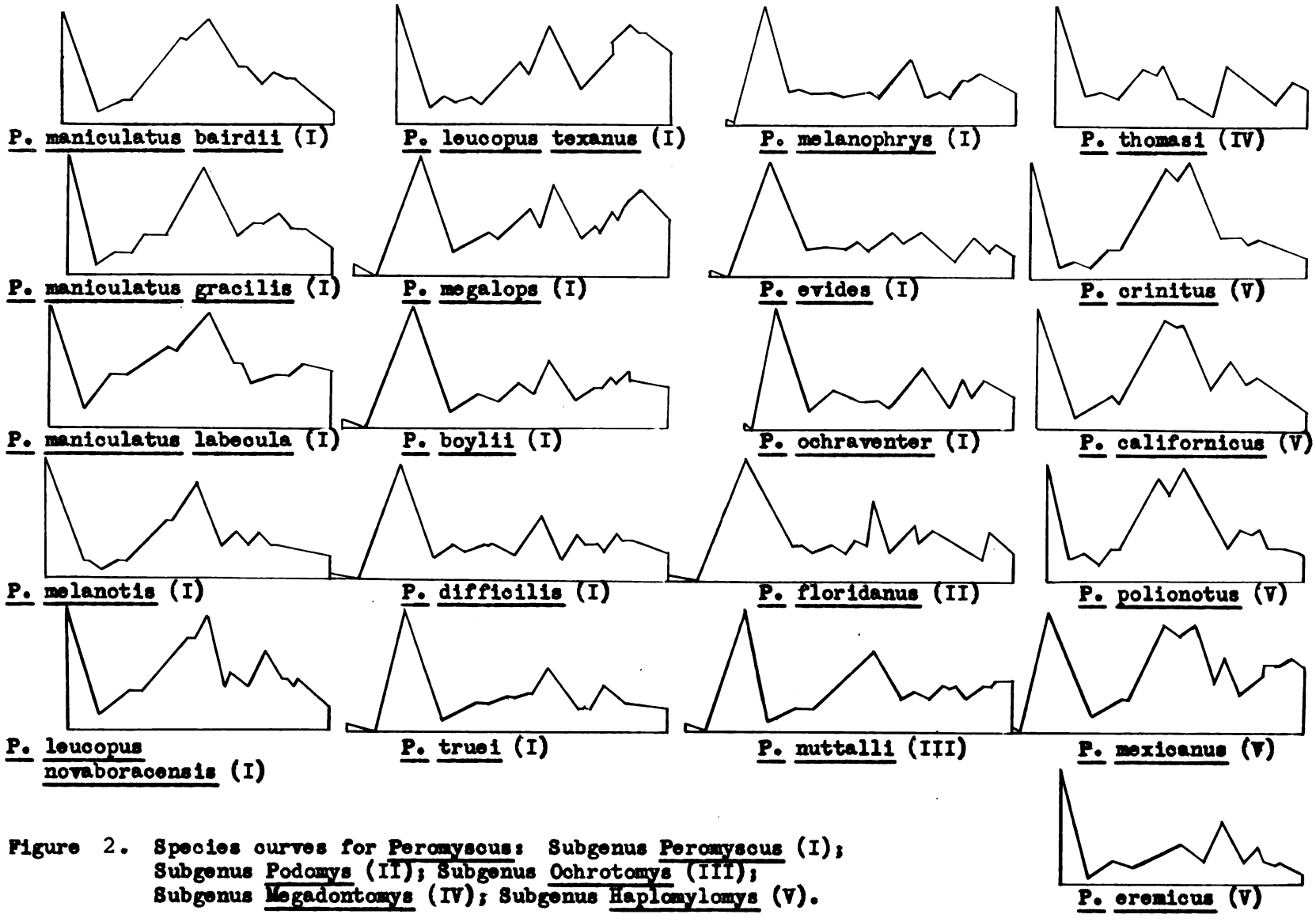


Figure 2. Species curves for Peromyscus: Subgenus Peromyscus (I); Subgenus Podomys (II); Subgenus Ochrotomys (III); Subgenus Megadontomys (IV); Subgenus Haplomylomys (V).

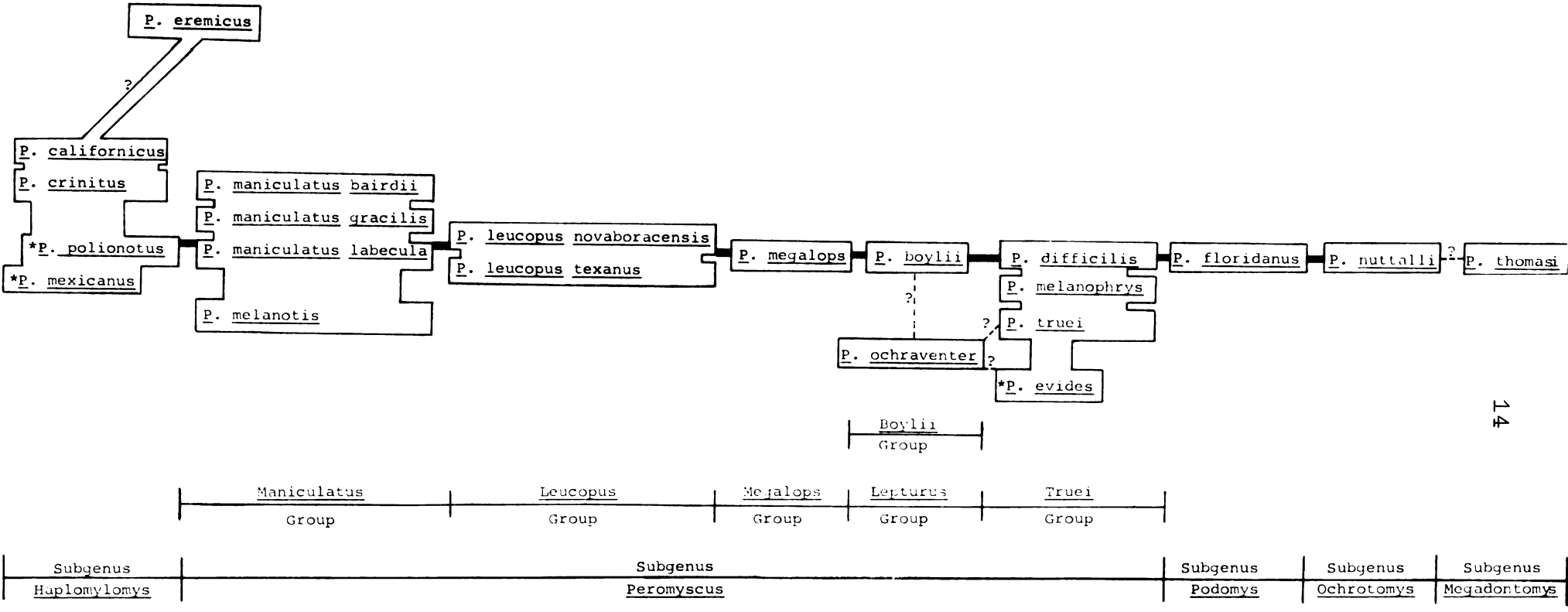


Figure 3. Relationships in the genus Peromyscus based on electrophoretic patterns of blood serum.

*Species whose electrophoretic relationships do not agree with Osgood's classification.

3 . One po.A band present . . . . . Subgenus Ochrotomys
3'. Two or more po.A bands present . 4

4 . T band narrow; G bands not separated from each other by an equal distance . . . . . Subgenus Podomys
4'. T band wider; G bands separated from each other by approximately an equal distance . . . . . Subgenus Peromyscus

Descriptions of Species Curves

In the following accounts the species of Peromyscus studied are arranged in groups based on the general characteristics of their densitometric curves. All species curves discussed in the following accounts were compared with the curve of Peromyscus maniculatus as a base for three reasons. First, this species is widely distributed; second, it is a member of the subgenus Peromyscus, which forms the main aggregate of species within this genus; and third, its species curve is fairly symmetrical (seen by placing a straight edge along imaginary lines from the origin and lowest po.A to the T band peak), allowing a good reference with which to compare the curves of other species.

Subgenus Peromyscus

An artificial key to species groups of the subgenus Peromyscus based on the configuration of the densitometric curves is as follows:

1 . T band tall and quite wide: po.A and G bands short, sloping away from T band in a symmetrical manner . . . . Maniculatus Group
1'. T band and not as wide: G bands taller and more varied in height . . . 2

2 . Po.A bands nearest albumin band less than 50 per cent as tall as T band; pr.A band absent . . . . . Leucopus Group
2'. Po.A bands nearest albumin band at least 50 per cent as tall as the T band; pr.A band present 3

3 . Densitometric curve low on left side of the tall T band and higher on right side of T band . . . . . Megalops Group
3'. Densitometric curve about the same height on either side of the shorter T band 4

4 . Po.A bands well-defined . . . . . Boylii Group
4'. Po.A bands less well-defined 5

5 . T band about half-way between origin of run and albumin band . . . . Truei Group
5'. T band nearer to origin of run than to albumin band . . . . . Lepturus Group

Maniculatus Group

Peromyscus maniculatus bairdii - The curve, which lacks a pr.A band, (see Fig. 2), in general, slopes down equally from either side of the almost equilateral T band which is fairly wide. The po.A bands start low from A and rise sharply in the vicinity of T, while the G bands are medial in distance from T to X and descend at about the same angle.

P. m. gracilis - In most respects, this curve, which lacks a pr.A band, is similar to that of P. m. bairdii; however, the po.A immediately to the left of T is lower, and the middle G bands are more elevated.

P. m. labecula - This curve, which lacks a pr.A band, also resembles those of the above subspecies but has taller po.A bands and the middle G band sags in contrast to that of P. m. gracilis.

P. melanotis - This species curve, which lacks a pr.A band, is also similar to those of the previously mentioned species. The G bands do not slope down as much making the total curve seem to be somewhat more extended than in the other members of this group.

Leucopus Group

P. leucopus novaboracensis - The curve, which lacks a pr.A band, is grossly similar to those of the Maniculatus Group; however, the po.A bands are lower, the G bands higher, and the T band thinner.

P. l. texanus - This curve resembles that of P. l. novaboracensis but has three instead of two po.A bands and the G bands are higher due to hemolysis at time of sample collecting. The T band is wider and less symmetrical while no pr.A is evident.

Megalops Group

P. megalops - The curve, which has one pr.A band, is more like those in the Leucopus Group than in the Maniculatus

Group. The po.A bands are higher and slope toward the T band, while the G bands slope away from this tall medial band.

Boylii Group

P. boylii - There is a slight resemblance of this curve to that of P. megalops; however, the protein bands are lower, including the T band. The po.A bands slope toward the T band, while the right-hand G bands are taller than their counterpart to the left. In total, the curve seems to be more elongated (as in most of the following species) than those of the previously mentioned species. One specimen had two pr.A bands, whereas all the rest had only one.

Truei Group

P. difficilis - This curve, which has one pr.A band, is somewhat similar to that of P. boylii, but the po.A bands are low and more level, whereas the G bands sag in the middle.

P. truei - This species curve, which has one pr.A band, resembles that of P. difficilis but has one less G band. The middle G band is much higher than its counterpart while the po.A bands slope toward the T band.

P. melanophrys - The G bands are taller toward the origin than their left-hand counterpart and the po.A bands are mostly level. This species curve more closely resembles that of P. difficilis than that of P. truei, but two of the three individuals had two pr.A bands.

P. evides - From the above three species, P. evides can be recognized by its having a low set of protein bands and one pr.A band. With respect to the T band, it is most like P. truei (has a low T); however, the po.A bands are more level, resembling those of difficilis and melanophrys. In order of greatest resemblance the bands of P. evides are closest to those of P. truei, P. difficilis, P. melanophrys, and somewhat like P. boylii. Based on other morphological features evides has been placed either as a subspecies of P. boylii or in the Boylii group.

Lepturus Group

P. ochraventer - It is evident in Figure 2 that this species curve, which has one pr.A band, is different from those of any other group, but seems to fall somewhere near the Boylii Group and the Truei Group. There are two G bands present (P. truei has the fewest number of G bands in the latter group) and they most resemble the G bands of P. evides while the T band is wide and symmetrical as in difficilis.

In total, its pattern is equally similar to those of P. boylii and P. evides. I follow Hall and Kelson (1959:649) in placing P. ochraventer in the Lepturus Group.

Subgenus Podomys

P. floridanus - This curve, which has one pr.A band, is also elongated and has many bands. Characteristically, the T band is narrow and symmetrical, while the first two G bands slant away and down from the T band and are close together in relation to the one nearest the origin (X).

Subgenus Ochrotomys

P. nuttalli - It can be seen that the curve for this species, which has one pr.A band, (regarded as a distinct genus by Hooper, 1958) is different from the preceding ones; however, it is still elongated. There is only one po.A, but there are five G bands, whereas the T band is tall, wide, and not as symmetrical.

Subgenus Megadontomys

P. thomasi - It is evident that this species curve, which lacks a pr.A band, is different from those of all other Peromyscus. There is no prominent T, but if the tall, wide, second band before X is designated as T, the run will have four po.A bands (two of them major and of about equal height) and only one G band. Between the large,

"double-humped" po.A band and the T band, there is a "sag" in this run while the T band is quite symmetrical but is inclined to the left.

Subgenus Haplomylomys

P. crinitus - This species, which lacks a pr.A band in its curve, and the four following ones have the shortest curves of any of the species studied except for P. thomasi. The curves also have a fairly symmetrical appearance, however, a salient characteristic of this subgenus is the "double-transferrin" protein. P. crinitus has two po.A bands and two G bands in its curve.

P. californicus - The curve, which lacks a pr.A band, is similar to that of P. crinitus, but it has only one po.A band while the G bands are more prominent and taper off more than in the last species.

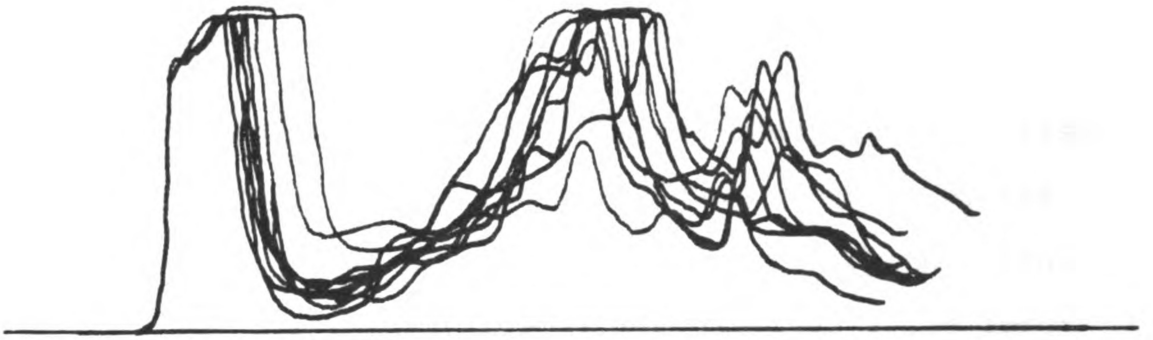
P. polionotus - This curve, which lacks a pr.A band, is quite similar to those of the two above species, but the right-hand "hump" of the double T is higher than the other while there are two po.A bands and three G bands. The serum protein pattern also is superficially similar to those of the Maniculatus Group because of the disparities noted on the double T and because of the presnece of three G bands.

P. mexicanus - This species is also placed in the Subgenus Haplomylomys because of the prominent double T in the curve; however, there is only one po.A band, one pr.A band, and three G bands which set it somewhat apart from the other members of this group. Hemolysis occurred in both samples, possibly accounting for the height of the two right-hand G bands. There is, however, one large G band to the immediate right of the double T.

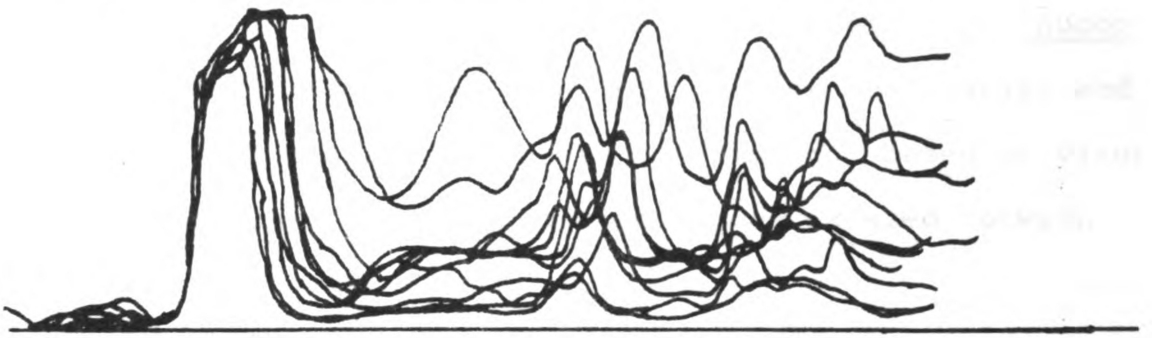
P. eremicus - The curve of this species, which lacks a pr.A band, is quite different from the four above. There is no definite double T band; instead, one fairly tall, equilateral, so-designated T band and one lower large band to its immediate left are present. This latter band is also equilateral but more "spread out," making it possible that this band is also of "double hump" origin. One po.A band and two G bands comprise the remainder of the curve that is not as elongated as those of the Truei Group, with which it also has a superficial similarity.

Variability

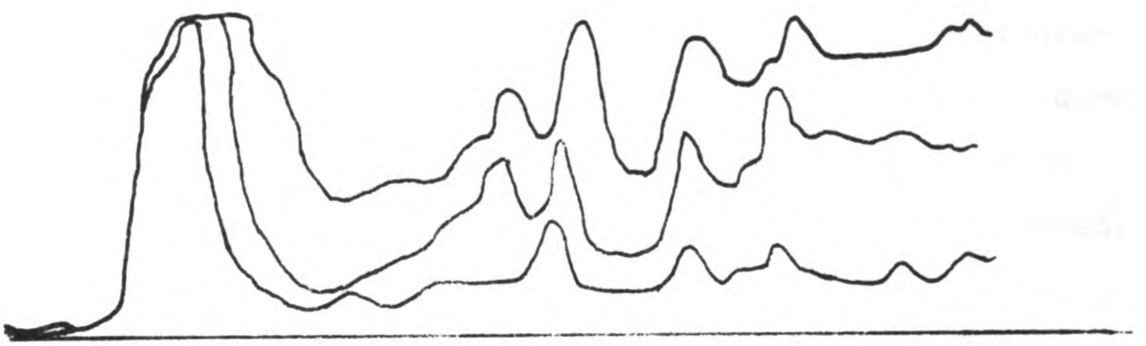
Figure 2 illustrated species curves in which all samples of a given species were averaged together, essentially eliminating any intraspecific or individual variation. In Figure 4b the individual densitometric curves for a typical species, P. boylii, have been superimposed, indicating that considerable geographic variation does exist in blood



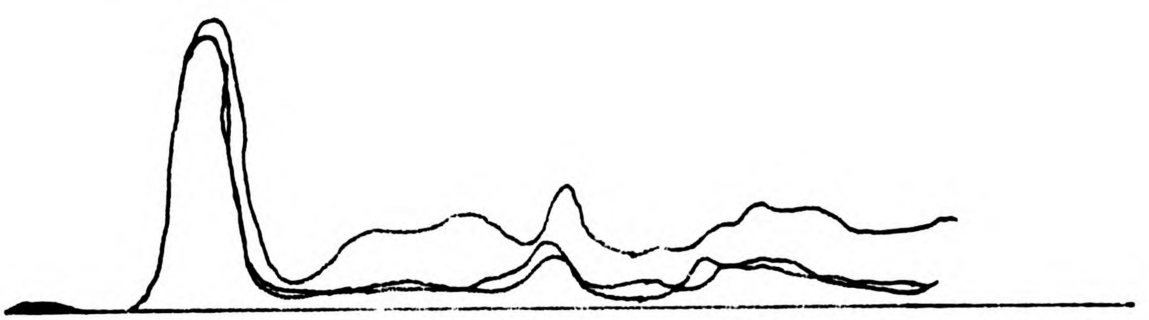
(a) P. leucopus--East Lansing, Ingham Co., Michigan



(b) P. boylii--From six Mexican localities



(c) P. boylii--4 mi. NW Huitzilac, 9200 ft., Morelos



(d) P. boylii--12 mi. SW Xochipala, 8200 ft., Guerrero

Figure 4. Individual and geographic variation in blood serum patterns of Peromyscus.

serum proteins both qualitatively and quantatively. Also, some nongeographic variation is evident as shown by the superimposed curves for nine samples of P. leucopus from one locality (Fig. 4a). Yet if one examines blood serum proteins of individual boylii from each geographic locality (Fig. 4c and d), they tend, unlike the samples of P. leucopus serum proteins, to exhibit a highly similar qualitative and quantitative pattern. There was no evidence, based on visual examination, that appreciable differences existed between blood serum patterns of the sexes.

Presence or Absence of Prealbumin Protein

In serum patterns, the presence or absence of prealbumin was noted with respect to certain geographic regions. For example (see Fig. 2 and Table 1), in eight of twelve Mexican species of Peromyscus, prealbumin protein occurred. P. maniculatus labecula and P. melanotis of the Maniculatus Group, and P. leucopus texanus of the Leucopus Group did not exhibit this protein, nor did other members of their respective groups. P. thomasi, which already is recognized as belonging to a different subgenus, also lacked this protein. Of the nine species of Peromyscus from the United States used in this study, only two had prealbumin. Both P. nuttalli and P. floridanus, which belong in different subgenera, did carry prealbumin.

Table 1. Presence or absence of prealbumin protein in blood serum of *Peromyscus*

Peromyscus from México	*Peromyscus* from United States
P. leucopus texanus	*P. leucopus novaboracensis*
P. maniculatus labecula	*P. maniculatus bairdii*
P. melanotis	*P. maniculatus gracilis*
**P. megalops*	*P. polionotus*
**P. mexicanus*	*P. crinitus*
**P. ochraventer*	*P. californicus*
**P. boylii*	*P. eremicus*
**P. difficilis*	**P. floridanus*
**P. truei*	**P. nuttalli*
**P. melanophrys*	
**P. evides*	
P. thomasi	

*Prealbumin protein present in blood serum.

DISCUSSION

Interpretation of Results

In order to illustrate relationships, the species or species groups of Peromyscus have been arranged in a diagrammatic series (see Figure 3). Species whose relationships are obviously close have been connected with solid lines. Those whose relationships seem questionable are joined with dotted lines. Within a species group, some species curves are more similar to one another than to others. The probable degree of similarity is shown by varying widths of "bottlenecks"--a narrow "bottleneck" indicating greater divergence.

On examining the diagram of proposed blood serum relationships, it can be seen that most of the species of Peromyscus fall in an almost continuous series with respect to their electrophoretic bands. This continuum is in accordance with that of Osgood (1909), who based his on external and cranial characteristics. He states that the subgenera Haplomylomys and Megadontomys are fairly circumscribed and definable but seem to be at opposite ends of a continuous series in which the subgenus Peromyscus is in the middle and combines most of the characters of the former two subgenera.

Hooper and Musser (1964) suggest that "Peromyscus consists of 13 groups of species (based on morphology of glans, bacula, and skulls and on ecologic distribution). Seven of these groups comprise the main cluster, and six are peripheral in regard to amount of differentiation." They give full generic recognition to Ochrotomys. The main cluster is composed of the subgenus Peromyscus, while the remaining groups are each treated as six subgenera. Likewise, several groups in my study are peripheral (Haplomylomys, Podomys, Ochrotomys, and Megadontomys) with respect to blood serum patterns.

Within the subgenus Peromyscus, the symmetrical electrophoretic curves of the Maniculatus Group place these species in a group by themselves. To its right with decreasing similarity follow the Leucopus and Megalops Groups, the latter being somewhat intermediate between the Maniculatus and Boylii Groups. Those from the Boylii Group on toward the right-hand part of the diagram generally exhibit more-elongated runs. Boylii, difficilis, melanophrys, truei, evides, and ochraventer seem to be clumped fairly close together; however, it is not known exactly where ochraventer and evides fit in this cluster. For the subgenus Megadontomys, thomasi has a curve radically unlike any of the other Peromyscus. Members of the subgenus Haplomylomys are placed at the opposite end of the series from Megadontomys because

of their characteristic "double transferrin" bands. There is no apparent reason why polionotus and mexicanus fall in this group, or why eremicus has a somewhat different pattern from other Haplomylomys.

From these data and results the author feels that the use of disc electrophoresis to analyze blood proteins in Peromyscus is most useful as a taxonomic index at the species group level. This is especially true in the Maniculatus and Leucopus Groups where in each several subspecies were found to have similar patterns. Distinct pattern differences occur at the level of the subgenus distinguishing each one from all others. These differences are greater than those found between species of one subgenus.

Hooper and Musser (1964) note contrasting general appearances between tropical and temperate species of Peromyscus and have postulated functional significance for these differences. The temperate forms typically have a bicolor tail, rounded brain case, and large auditory bullae; while in tropical forms the tail is mottled below or is unicolor, the brain case is more elongate, and the auditory bullae are smaller. In serum patterns, the presence or absence of prealbumin was noted (see Results, p. 24) with respect to certain geographic regions. This observation suggests, but does not demonstrate that prealbumin may be of importance to small mammals at higher altitudes.

Putnam (1961) states that prealbumin and albumin (in part) function to transport thyroxin in the blood. All of the mammals in my study had an albumin band; in general, mountain-dwelling mammals of México also carried the prealbumin band. Prealbumin might be an added means of supplying a mouse with thyroxin (to increase basal metabolic rate) in order to cope with lower oxygen tensions at higher altitudes. P. melanotis and P. thomasi are both animals of high elevations which did not have this protein. It is possible that these two species are recent arrivals in high altitudes and as yet have not diverged enough to have acquired prealbumin by natural selection, or they have some other physiological adaptation to increase BMR in these habitats.

Comparison with Other Morphological Studies of Peromyscus

Table 2 presents a summary of the species relationships in Peromyscus as shown by several morphological studies. In Osgood's revision of the genus Peromyscus in 1909, special emphasis was placed on pelage and cranial characteristics. The relationships of the blood serum patterns seem to follow very closely the arrangement of species made by Osgood, with only four or five disagreements noted. P. polionotus and mexicanus were placed by Osgood in the Subgenus Peromyscus; however, their blood serum patterns are like those of Haplomylomys. Osgood regarded evides as a

Table 2. Summary of relationships of species of Peromyscus as described by several workers

Osgood (1909)	Hooper (1957)	Hooper (1958)
Genus Peromyscus	Genus Peromyscus	Genus Peromyscus
Subgenus Peromyscus	Subgenus Peromyscus	Maniculatus Division
maniculatus group	P. maniculatus	Maniculatus Group
P. maniculatus	P. melanotis	P. maniculatus
P. polionotus	P. leucopus	P. leucopus
P. melanotis	P. melanophrys	P. polionotus
leucopus group	P. truei	Boylii Group
P. leucopus	P. boylii	P. boylii
boylii group	P. difficilis	P. truei
P. boylii	P. mexicanus	P. mexicanus
truei group	Subgenus Haplomylomys	P. difficilis
P. truei	P. crinitus	P. melanophrys
P. difficilis	P. eremicus	Undiscerned Forms
melanophrys group	P. californicus	P. megalops
P. melanophrys	Subgenus Ochrotomys	P. crinitus
mexicanus group	P. nuttalli	Eremicus Division
P. mexicanus		P. eremicus
megalops group	Rinker (1963)	P. californicus
P. megalops		Floridanus Division
Subgenus Haplomylomys	Genus Peromyscus	P. floridanus
P. eremicus	Subgenus Peromyscus	Thomasi Division
P. crinitus	P. leucopus	P. thomasi
P. californicus	Subgenus Haplomylomys	Nuttalli Division
Subgenus Megadontomys	P. eremicus	P. (Ochrotomys) nuttalli
P. thomasi	Subgenus Ochrotomys	
Subgenus Podomys	P. nuttalli	
P. floridanus		
Subgenus Ochrotomys		
P. nuttalli		

Table 2--Continued

Hooper and Musser (1964)	Blair (1942)	Groupings in the present study
Genus Peromyscus	Genus Peromyscus	Genus Peromyscus
Subgenus Peromyscus	Subgenus Peromyscus	Subgenus Peromyscus
maniculatus group	maniculatus and	Maniculatus Group
P. maniculatus	leucopus groups	P. maniculatus
P. polionotus	P. maniculatus	P. melanotis
P. melanotis	P. leucopus	Leucopus Group
leucopus group	P. polionotus	P. leucopus
P. leucopus	boylii and truei groups	Megalops Group
crinitus group	P. boylii	P. megalops
P. crinitus	P. truei	Boylii Group
boylii group	Subgenus Haplomylomys	P. boylii
P. boylii	P. californicus	Truei Group
P. evides	P. eremicus	P. truei
truei group	P. crinitus	P. difficilis
P. truei	Subgenus Podomys	P. melanophrys
P. difficilis	P. floridanus	P. evides
melanophrys group	Subgenus Ochrotomys	Lepturus Group
P. melanophrys	P. nuttalli	P. ochraventer
mexicanus group		Subgenus Podomys
P. mexicanus	Johnson and Wicks (1959)	P. floridanus
P. ochraventer		Subgenus Ochrotomys
P. megalops	Genus Peromyscus	P. nuttalli
Subgenus Haplomylomys	Subgenus Peromyscus	Subgenus Megadontomys
P. eremicus	P. maniculatus	P. thomasi
P. californicus	P. polionotus	Subgenus Haplomylomys
Subgenus Podomys	P. crinitus	P. crinitus
P. floridanus	Subgenus Haplomylomys	P. californicus
Subgenus Megadontomys	P. californicus	P. eremicus
P. thomasi	Subgenus Podomys	P. polionotus
Genus Ochrotomys	P. floridanus	P. mexicanus
Ochrotomys nuttalli	P. gossypinus	

subspecies of boylii, while Hooper and Musser (1964) give it full specific rank--the electrophoretic pattern supports the latter workers' conclusions. The electrophoretic curve of melanophrys is similar to those of members of the Truei Group rather than being separated as the distinct Melanophrys Group of Osgood; however, melanophrys is listed by him as being closely related to difficilis and truei.

Hooper (1957) feels that too much emphasis was made on dental topography in Osgood's revision. In a study of lophs and styles of molars, the former worker questioned whether three subgenera, Haplomylomys, Peromyscus, and Ochrotomys, could be naturally grouped by these characteristics. Geographic variation in the lophs and styles of leucopus and boylii, according to Hooper (op. cit.), sometimes exceeds those that contrast full species. He felt that these structures could not be relied upon in a systematic analysis of Peromyscus. Thus at the level of the species group, electrophoresis may be a more sensitive taxonomic indicator than certain dental features.

Phallic characters have been used to show systematic relationships in cricetine rodents. Blair (1942), who studied four of the five subgenera of Peromyscus of Osgood, could easily discern them by their characteristic types of bacula. He found the baculum of polionotus to resemble that of maniculatus, whereas the serum pattern of the former

shows greater resemblance to those of Haplomylomys. Blair also found that the bacula of boylii and truei could not be distinguished; whereas, their serum patterns are somewhat different. He distinguished two types of bacula in the subgenus Haplomylomys. Californicus had one type and eremicus and crinitus another. The differences between these two types, according to Blair, were as great as those that distinguish the maniculatus group from the boylii group. The serum patterns of Haplomylomys show that eremicus is set apart from crinitus and californicus. The subgenera Peromyscus, Haplomylomys, and Podomys form a fairly compact group on the basis of general bacula characteristics, while Ochrotomys is peripheral. In serum patterns, Haplomylomys and Ochrotomys are both peripheral, the former being most remote.

The species arrangement based on electrophoretic blood serum patterns is also in general agreement with that based on the characteristics of the Peromyscine male phalli (Hooper, 1958). On the basis of male phalli, Peromyscus can be divided into several major species divisions--Floridanus, Nuttalli, Thomasi, Eremicus, Banderanus, Maniculatus and Lepturus, the first four corresponding to Osgood's Podomys, Ochrotomys, Megadontomys and Haplomylomys, respectively; the rest to Peromyscus. Hooper places crinitus in his Maniculatus Division, whereas its blood serum pattern clearly

places it in Haplomylomys. His Maniculatus Division includes a Maniculatus Group consisting of maniculatus, polionotus, leucopus, and gossypinus; it is generally comparable to my Maniculatus and Leucopus Groups, with the exception of polionotus, which in serum pattern resembles Haplomylomys. His Boylii Group corresponds roughly to my Boylii Group and Truei Group combined. He has not resolved the status of megalops; I have placed this species in the continuum between the Leucopus and Boylii Groups. I do not feel that the serum pattern of nuttalli warrants Hooper's generic ranking of Ochrotomys, but believe, rather, that it should remain as a peripheral subgenus (Ochrotomys) within Peromyscus. Hooper and Musser (1964) place ochraventer in their Boylii Group (based on glans and baculum) and in their Mexicanus Group (based on total evidence). There is doubt as to exactly where this species fits from the standpoint of its serum pattern, but I have placed it near the Boylii Group and Truei Group.

Myologically, three subgenera of Peromyscus (Peromyscus, Haplomylomys, and Ochrotomys) were found by Rinker (1963) to be a closely related unit, with few features separating them. Nuttalli and eremicus both differed from leucopus to approximately the same degree but each in a different manner. The same is approximately true in their blood serum patterns. Rinker felt that "the muscular system

provides minimal evidence which could be of taxonomic value" below the subgeneric level.

In summary, the disc electrophoretic blood serum patterns generally support existing taxonomic categories based on other morphological classifications of Peromyscus.

Comparison with Other Electrophoretic Studies

To my knowledge, the only published electrophoretic systematic study of Peromyscus was performed by Johnson and Wicks (1959). For the most part their starch gel technique failed to reflect existing taxonomic relationships in either Peromyscus or in voles of the subfamily Microtinae. P. crinitus and californicus (both of Osgood's Haplomylomys) had dissimilar patterns, with the former having a pattern similar to that of maniculatus. This supports Hooper's (1958) contention that crinitus should be placed in the subgenus Peromyscus and disagrees with the disc electrophoretic relationships shown in my study. Also differing from present taxonomic status in Peromyscus were their findings that gossypinus' pattern (a species not studied by me) was unlike that of maniculatus and similar to that of floridanus. However, these workers did show a close relationship between the serum patterns of maniculatus and polionotus, two species which have common morphological characteristics.

Johnson and Wicks concluded that their starch gel electrophoretic evidence did not correlate well with the present morphological relationships in Peromyscus and several Microtine rodents, but did correlate well in several other mammals examined. The results from Johnson and Wicks' study show that in Peromyscus interspecific variation within a single subgenus can be greater than that between representatives of different subgenera.

There are two main reasons why my results differ from other electrophoretic studies (especially Johnson and Wicks, 1959). The first is that many varied electrophoretic techniques have been used (starch gel, paper strip, etc.), each employing slightly different chemicals. The method used in my study is recognized by Ornstein and Davis (1926) as being the most sensitive (separating greatest number of proteins). Thus, each technique will yield results sufficiently different as not to be comparable. Secondly, in most mammalian electrophoretic studies, suprageneric taxons have been compared rather than species or species groups.

It should be kept in mind that differences in electrophoretic patterns can be due to factors other than genetic control of protein configurations. Some of these factors may include age, sex, diet, physiological stress, disease, season of year when blood was collected, and experimental error in electrophoretic procedure. Peacock et al.

(1965) while studying human serum electrophoretic patterns found that neither age, sex, nor the four blood groups (A, B, AB, O) could be correlated with the twelve different patterns found; however, disease caused changes in relative mobilities of the proteins. Atchley et al. (1961) inoculated guinea pigs with Entamoeba histolytica and found decreased albumin and increased globulin fractions. In white-tailed deer in Florida several polymorphic hemoglobin patterns have been found which are under genetic control. Certain of these hemoglobins can determine whether or not sickling cell disease will occur (Kitchen et al., 1964). Bouck and Ball (1965) demonstrated that stress under low oxygen tensions can alter serum electrophoretic patterns in fish.

Thus, some of the individual (non-geographic) variation which was evident in P. leucopus novaboracensis may be explained from the above factors; however, it can be assumed that these individuals were all healthy adults, that they had the same diet in nature, that they were under the same degree of physiological stress when their blood was collected, and that their blood was taken on the same day of the year. Since these variables have been essentially eliminated, it is highly possible that the serum proteins of Peromyscus are under the control of multiple alleles, which would account for the individual variation present.

Extreme variation was found by Goodman *et al.* (1965) to exist in the transferrin patterns of macaque monkeys. Six [alleged] species of this monkey had eleven molecular forms and 34 phenotypes of the transferrin protein. Stump-tailed macaques exhibited monomorphic forms of transferrin in one part of their range and polymorphic forms in the remaining part. Island populations tended to be homozygous for alleles controlling this protein. From these results, they concluded that these monkeys are a monophyletic assemblage of "semispecies." Although variation did occur (Fig. 4) within species of *Peromyscus* (some more than others), a general pattern was evident for each. The transferrin polymorphism of macaques illustrates greater variation than is observable for any of the proteins in *Peromyscus*.

SUMMARY AND CONCLUSIONS

Blood serum proteins of eighteen species of Peromyscus from the United States and México (some wild-caught and some laboratory-reared) were separated by the disc electrophoretic technique of Ornstein and Davis (1962). The separated proteins, stained in Amido Blue-Black 10 B, were optically scanned by a Model E Canalco Microdensitometer which produced a graphic curve for each species. These curves were then compared in an effort to determine systematic relationships between the species and to compare these with findings of other workers using morphological structures.

The writer suggests that the disc electrophoretic technique used in this study is sensitive to the species group level in Peromyscus. From representative densitometric curves of each species, a series of groups has been formed which corresponds to morphological relationships in Peromyscus as stated by Osgood in 1909. In general, the results of this study verify our present understanding of the taxonomic status of species in the genus. It is presently difficult to compare results of electrophoretic studies for taxonomic purposes because of the lack of

uniformity in the use of various techniques. Once standard procedures are obtained, this method of distinguishing proteins should be most helpful in understanding mammalian relationships.

LITERATURE CITED

Anfinson, C. B.
1959. The Molecular Basis of Evolution, Wiley: New York. 208 pp. illus.

Atchley, F. O.
1961. Electrophoretic studies of blood serum proteins in guinea pigs inoculated with Entamoeba histolytica. Jour. Parasit. 47(2): 297-301.

Auernheimer, A. H., W. Cutter, and F. O. Atchley.
1959. Electrophoretic studies on blood serum proteins of rodents. Jour. Mamm. 41(3): 405-407.

Blair, W. F.
1942. Systematic relationships of Peromyscus and several related genera as shown by the baculum. Jour. Mamm. 23(2): 196-204. 2 figs.

Bouck, G. R. and R. C. Ball.
1965. Influence of a diurnal oxygen pulse on fish serum proteins. Trans. Amer. Fish. Soc. 94(4): 363-370.

Gleason, T. L. and F. Friedberg.
1953. Filter paper electrophoresis of serum proteins from small animals. Physiol. Zool. 26: 95-100.

Goodman, M., A. Kulkarni, E. Poulik, and E. Reklys.
1965. Species and geographic differences in the transferrin polymorphism of macaques. Sci. 147 (3660): 884-886. 2 figs.

Hall, E. Raymond and K. R. Kelson.
1959. The Mammals of North America. Vol. II. Ronald Press: New York. Pp. viii + 1083 + 79, 553 figs.

Hooper, E. T.
1957. Dental patterns in mice of the genus Peromyscus. Misc. Publ. Univ. Mich. Mus. Zool. No. 99: 1-59. 24 figs.

Hooper, E. T.
1958. The male phallus in mice of the genus Peromyscus. Misc. Publ. Univ. Mich. Mus. Zool. No. 105: 1-40.

_____, and G. G. Musser.
1964. Notes on classification of the rodent genus Peromyscus. Occ. Papers Univ. Mich. Mus. Zool. No. 635: 1-13. 2 figs.

Johnson, M. L. and M. J. Wicks.
1959. Serum protein electrophoresis in mammals--Taxonomic implications. Syst. Zool. 8: 88-95.

Kitchen, H., F. W. Putnam, and W. J. Taylor.
1964. Hemoglobin polymorphism: Its relation to sickling of erythrocytes in white-tailed deer. Sci. 144: 1237-1239.

Leone, C. A.
1964. Taxonomic Biochemistry and Serology. Ronald Press: New York. 728 pp. illus.

Moore, D. H.
1945. Species differences in protein patterns. Jour. Biol. Chem. 161: 21-32.

Ornstein, L. and B. J. Davis.
1962. Disc Electrophoresis. Parts I and II. Distillation Products Industries. Rochester, New York. 22 pp. illus.

Osgood, W. H.
1909. Revision of the mice of the American genus Peromyscus. North Amer. Fauna. 28: 1-285. 12 figs. illus.

Peacock, A. C., S. L. Bunting, and K. G. Queen.
1965. Serum protein electrophoresis in acrylamide gel: Patterns from normal human subjects. Sci. 147(3664): 1451-1453.

Putnam, F. W.
1960. The Plasma Proteins. Academic Press: New York. 518 pp.

Rinker, G. C.
1963. A comparative myological study of three subgenera of Peromyscus. Occ. Papers Univ. Mich. Mus. Zool. No. 632: 1-18. 1 fig.

Shaw, C. R. and E. Barto.
1965. Autosomally determined polymorphism of glucose-6-phosphate dehydrogenase in Peromyscus. Sci. 148(3673): 1099-1100.