EVOLUTIONARY GENETICS OF CERTAIN MICE OF THE PEROMYSCUS BOYLII SPECIES GROUP

DISSERTATION

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By

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The genetic structure of 49 natural populations of four species (P. attwateri, P. boylii, P. pectoralis, and P. polius) of the Peromyscus boylii species group was analyzed through application of chromosomal and electrophoretic techniques.

Chromosomal variation within and among populations of the boylii species group was analyzed from 178 specimens. Electrophoretic techniques were utilized for the demonstration of variation in enzymes and other proteins encoded by structural loci and applied to the study of the evolution of the boylii species group by estimation of levels of genetic heterozygosity within populations, estimation of degree of genetic similarity between conspecific populations and between species, and determination of patterns of geographic variation in allelic frequencies and levels of heterozygosity.

Six distinct chromosomal patterns were observed among the populations of the four species of the boylii species group. All specimens had a diploid number of 48 and the major difference in chromosomal morphology was in the number of pairs of large to medium biarmed autosomes. Little or no

chromosomal variation was observed in three species (*attwateri*, *pectoralis* and *polius*), but considerable chromosomal variation occurred among populations of *P. boylii*. Generally, the chromosomal variation in *P. boylii* was between allopatric populations, with each chromosomal pattern limited to a recognized subspecies. Polymorphism was observed in two populations. The polymorphism observed in *P. polius* was the result of pericentric inversion involving the smallest pair of metacentric autosomes. The polymorphism observed in *P. boylii spicilegus* was interpreted at the result of gene flow between *P. boylii rowleyi* and *P. boylii spicilegus*.

In addition to chromosomal evidence, analysis of electrophoretic data demonstrated and suggested effective gene flow between the chromosomal forms of *P. boylii*. Electrophoretically demonstrable variation was analyzed in 11 proteins encoded by 17 autosomal loci. Of the 17 structural loci, 11 were polymorphic in one or more populations. No more than five loci were observed to be polymorphic within a single population, with a mean number of polymorphic loci of 2.26.

The levels of genetic variability, as measured by the proportion of loci in a heterozygous state in the average individual of a population, for populations of the *boylii* species group were within the range reported for other rodents. Populations of *P. attwateri* exhibited levels of heterozygosity considerably lower than those reported for

other species of Peromyscus. The low level of genetic variability in P. attwateri was probably the result of founder effect of the original population which was isolated on the Edwards Plateau during the late Pleistocene and subsequent genetic drift which allowed fixation of genes within this isolated population.

Geographic variation in levels of heterozygosity observed among populations of P. attwateri and P. boylii indicates a north to south cline of increasing heterozygosity with the most variable populations occurring in the southern portion of the range of each species. A wide range of the levels of heterozygosity and genetic similarity was observed among populations of P. pectoralis and is the result of genetic contribution from three Pleistocene refugia.

Analysis of paired combinations of populations of P. boylii indicated that the various chromosomal forms of P. boylii form a conspecific unit. Populations of P. attwateri were genetically distinct from other members of this group. Examination of specific loci for allelic homology suggested that P. attwateri arose from genetic divergence of a population of P. boylii isolated on the Edwards Plateau during the late Pleistocene.

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CHAPTER I

INTRODUCTION

The rodent genus Peromyscus is a wide-ranging taxon with one or more species occupying almost every terrestrial habitat of North and Central America. Seven subgenera and approximately 57 species are currently recognized (Hooper, 1968). This diversification of forms offers the evolutionary biologist an actively evolving group for study. A continuum of the stages of the evolutionary process is represented in this taxon from isolated populations, indicative of incipient species, to subgenera comprised of several species groups (Dice, 1968). The largest subgenus, Peromyscus, includes seven species groups or assemblages of species with close phylogenetic relationship. Members of the same species groups crossed in the laboratory are typically partially interfertile, while members of different species groups are completely infertile (Dice, 1968).

The boylii species group is comprised of nine species ranging from Honduras northward to Oregon and Utah and eastward to the Ozark Mountains in Missouri and Arkansas. Although the species of the boylii group are morphologically

distinct, they constitute a reasonably compact group which probably stemmed from a single ancestral stock (Hooper, 1968). This species group is comprised of populations which are in various stages of speciation; thus, it presents the evolutionary biologist with an excellant opportunity to study the genetics of speciation.

Recent studies of chromosomal variation in the boylii species group have revealed markedly different karyotypes in allopatric populations of P. boylii (Lee et al., 1972; Schmidly, 1971), suggesting it is not a conspecific unit. The evolutionary significance of these chromosomal patterns in boylii is not presently known, but in one case (attwateri) there is evidence for elevation to specific status (Lee et al. 1972). In addition, Hooper (1968) has indicated that the conspecificity of certain forms such as P. boylii levipes and P. boylii spicilegus is in doubt.

In the past, studies of population genetics of Peromyscus have been restricted primarily to investigations of gross phenotypic characters such as coat color (Blair, 1944, 1947a, 1947b, 1947c). With the development of electrophoretic techniques for demonstrating allelic variation at genetic loci specifying or controlling the structure of enzymes and other proteins, it is now possible to calculate allelic frequencies for populations (Jensen, 1970; Jensen and Rasmussen, 1971; Lewontin and Hubby, 1966; Selander, Hunt, and

Yang, 1969; Selander, Yang, and Hunt, 1969; Selander et al. 1971; and Avise and Selander, 1972). Working on the assumption that the sample of loci controlling the proteins examined is representative of the genome (Hubby and Throckmorton, 1968) it is now possible to compare populations, subspecies, and species with respect to total genetic character or degree of genetic similarity (Selander, Hunt and Yang, 1969; Selander, 1970a, 1970b; Johnson and Selander, 1971; Johnson et al., 1972; Smith et al., 1973; Patton et al., 1972; Webster et al., 1973).

This study was concerned with populations of four species of the boylii species group which occur in northern Mexico and parts of the southern United States (New Mexico, Texas, Oklahoma, and Kansas). Populations of P. attwateri, P. polius, all the recognized subspecies of P. pectoralis, and four subspecies of P. boylii (rowleyi, levipes, spicilegus, and ambiguus) were examined utilizing karyological and electrophoretic techniques.

The major objectives of this study were to assess the levels of genetic similarity between populations, subspecies, and species of the boylii species group; to assess the systematic relationship of these populations and the evolutionary significance of the chromosomal variation within these populations; to analyze geographic patterns of variation in allelic frequencies within a species; to analyze levels and

patterns of heterozygosity within a species; and to analyze small isolated populations for evidence of evolutionary forces which structured these populations.

CHAPTER II

MATERIALS AND METHODS

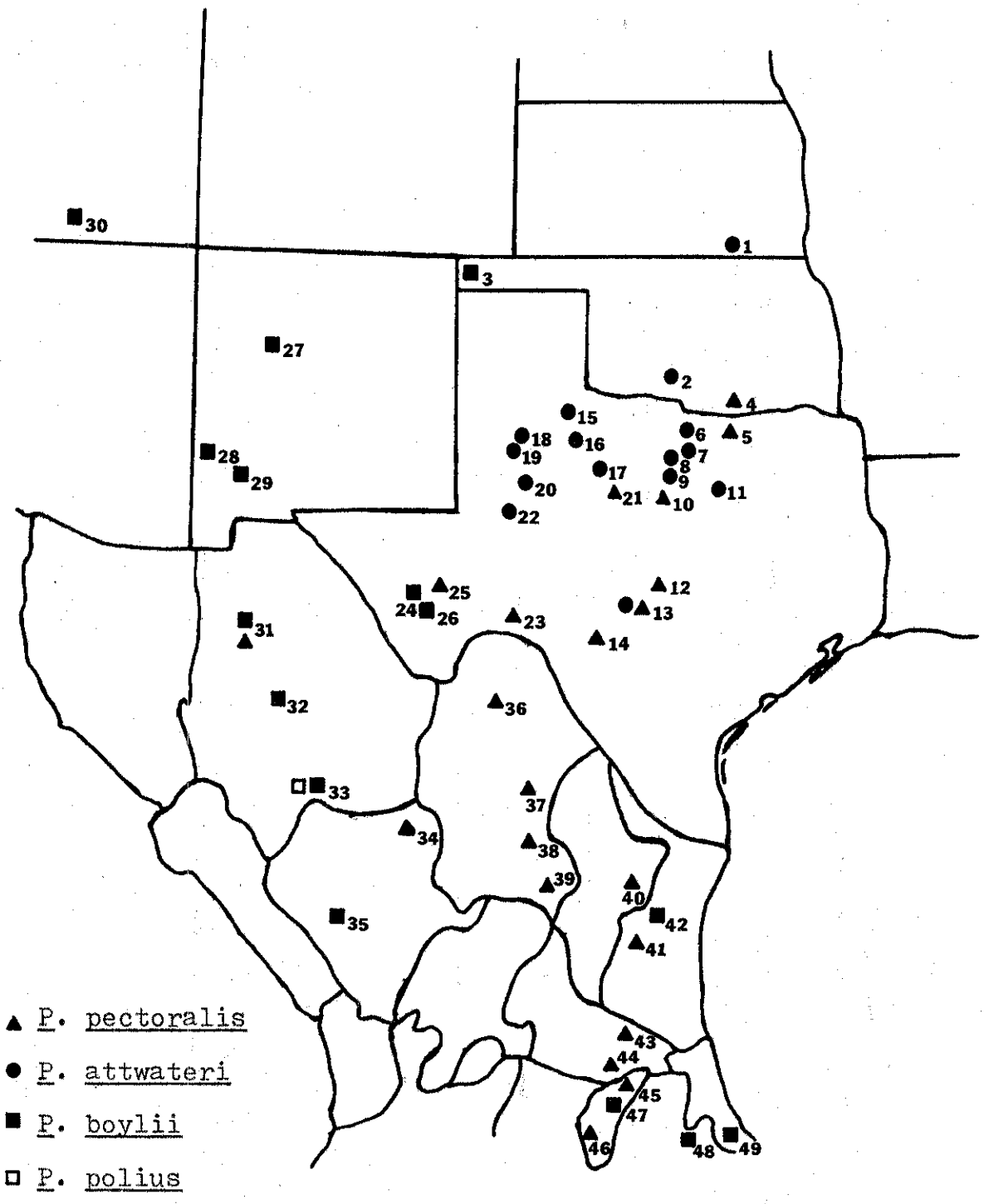
Samples

Mice (n=389) were obtained from 49 naturally occurring populations (Fig. 1) using Sherman live traps, and were housed in plastic cages in the laboratory and provided an ad libitum diet of Purina Laboratory Chow and water. Females were maintained in the laboratory for a period of at least 1 month in order to collect offspring of any pregnant mice captured. No differences were observed in electrophoretic patterns of mice bled on the first day of captivity and the same mice bled several months or even a year later. Representative specimens were deposited in the mammal collection of North Texas State University.

Chromosomal Techniques

Representative specimens from all populations were processed for somatic (bone marrow) metaphase chromosomes using the procedures outlined by Patton (1967) and modified by Lee (1969). In this procedure velban (Vinblastine sulfate), 0.004% solution, a mitotic inhibitor, was injected intraperitoneally with an optimum period of exposure of 50 to 60 min. After sacrificing the animal the bones of both

Fig. 1--Sample localities for mice of the boylii species group. Localities numbered according to appendix.



hind legs were removed, all muscle was detached, and the bones were splintered into a centrifuge tube containing 10 cc of 1% sodium citrate solution at 37°C. After vigorous shaking, the tube, containing bone marrow cells and bone chips, was incubated for 12 min. at 37°C. The tube was then gently inverted several times to resuspend the cells. Bone chips were allowed to sediment and the supernatant was decanted into another tube. The suspension of cells was centrifuged for 2 min. at 500 rpm resulting in a button of cells in the bottom of the tube. The supernatant was decanted, and 9 cc of Carnoy's fixative, one part glacial acetic acid to three parts anhydrous methanol, were added carefully. The tube was stoppered and refrigerated for at least 12 h., after which the button of cells was resuspended to insure total fixation of cells. The tube was stoppered and refrigerated for at least 2 additional h., after which the extraneous material was removed, and the suspension was centrifuged again at 500 rpm for 2 min. until the button of cells formed. The supernatant was decanted, 10 cc of new fixative was added, and the button was resuspended. This washing procedure was repeated three times. On the third wash 1 cc of fixative was added, the button was resuspended, and the tube was stoppered and returned to the refrigerator for at least 30 min. The suspension was then dropped onto chilled, wet slides and flame-dried. Slides were stained

for 20 min. in a 4% solution of buffered Giemsa stain and washed for 30 sec. in acetone. Meiotic chromosomes were prepared from testicular tubules by the same procedure.

Karyograms of the mitotic chromosomes were constructed from photomicrographs and major classes of chromosomes were established as follows: metacentric chromosomes (arm ratio approximately 1:1), submetacentric chromosomes (arm ratio from 1:1.1 to 1:1.9), subtelocentric chromosomes (arm ratio 1:2 or greater), and acrocentric chromosomes (second arms minute or absent).

Horizontal Starch Gel Electrophoresis

Preparation of Tissue Extracts

Blood was obtained by inserting a 1.0 x 100 mm capillary tube into the suborbital canthal sinus. For serum samples, approximately two capillary tubes of blood were collected in a dry 6 x 50 mm culture tube and allowed to clot for approximately 20 min. The samples were then centrifuged at 1000 x g for 10 min. Serum samples were placed in a clean culture tube and were either used immediately or stored at -20°C for several weeks. No differences were observed in protein patterns of fresh sera and that stored for several weeks.

Transferrin samples were prepared by removing one drop of the serum and placing it in a clean 10 x 75 mm culture

tube containing one drop of a 0.15% ferric chloride solution. Four drops of a 0.6% rivanol solution (2-ethoxy-6,9-diamino-acridine lactate) were added, the solution was thoroughly mixed by shaking, and the precipitate was centrifuged at 1000 x g for 3 min. (Chen and Sutton, 1967). This process removed most of the proteins other than transferrin (Sutton and Karp, 1965). Electrophoresis of the clear yellow supernatant was completed within 6 h. of preparation of samples.

The remaining serum was diluted with an equal volume of buffered saline solution (1.21 g dibasic sodium phosphate, 0.62 g monobasic potassium phosphate, 14.45 g sodium chloride per 1700 ml of deionized water (Jensen, 1970). Albumins and plasma esterases were run from dilute serum samples.

For hemoglobin and erythrocyte protein samples, approximately one capillary tube of blood was collected in a 10 x 75 mm test tube containing 0.5 ml of 4% sodium citrate solution. Samples were centrifuged at 1000 x g for three min. Erythrocytes were washed three times in buffered saline solution and hemolysed following the third washing by addition of three to four drops of deionized water. Hemolysed erythrocyte preparations were centrifuged at 1000 x g for 10 min. to precipitate unlysed cells. Hemoglobin samples were run within 5 h. of bleeding. Storage, even at low temperature, was reported to result in denaturation of hemoglobin (Jensen, 1970; Selander, Yang, and Hunt, 1969).

Tissue extracts were prepared by homogenizing samples of liver, kidney, and testes in two volumes of buffer (0.1 M tris, 0.001 M EDTA, pH adjusted to 7.0 with dilute hydrochloric acid) for approximately 1 min. in a cooled 7 ml glass tissue grinder (Selander et al., 1971). Extracts were centrifuged at 10,000 rpm at 0°C for 30 min. The supernatant solution was removed and was used immediately or stored at -20°C for as long as a month.

Organs not homogenized immediately after the death of the animal were dissected and frozen in two volumes of homogenizing buffer. Selander et al. (1971) reported that most proteins remain undenatured in intact organs frozen for weeks or months at -20°C, but in solution the activity of many enzymes was soon lost at this temperature.

Electrophoretic Apparatus and Techniques

Horizontal starch gel electrophoresis (Smithies, 1955) was utilized to fractionate all samples. Gel molds were modified from those described by Kristjansson (1963). The mold consisted of a glass plate (152 mm x 220 mm x 6 mm) and four plexiglass strips, two 6 mm x 19 mm x 196 mm and two 6 mm x 19 mm x 114 mm. The plexiglass strips were held in place with petroleum jelly. When the liquid gel was poured into the mold, a plexiglass plate (152 mm x 220 mm x 6 mm) was used to cover the mold.

All gels were prepared from a 12% suspension of hydrolysed starch (Connaught National Laboratories, University of Toronto, Toronto, Canada). Buffer was heated to boiling in a 1000 ml round-bottom flask, suspended starch was poured into the heated buffer, shaken vigorously for 1 min., and degassed with an aspirator (approximately 1 min.). After degassing, the clear liquid gel was poured into the mold, covered with a plexiglass plate, and allowed to cool at room temperature for a minimum of 90 min.

After the gels had cooled the plexiglass plate and long plexiglass strips were removed. Gels were cut parallel to and 2.0 cm from one of the short sides of the gel to form an insertion line and the smaller portion of the gel was gently pushed back. All samples were absorbed into no. 3 filter paper (4 mm x 5 mm), except albumin and plasma esterase samples which were absorbed into no. 1 filter paper. Filter paper inserts were blotted and placed approximately 3 mm apart against the exposed cut surface of the smaller portion of the cut gel. After the samples were placed on the gel, the smaller portion was carefully pushed back in contact with the larger portion of the gel.

Saran Wrap (Dow Chemical Company) was used to cover the surface of the gel during electrophoresis (Kristjansson, 1963). The edges of the Saran Wrap were folded back to expose approximately 1.7 cm of the gel, to allow contact with

the bridge from the electrode chambers of the electrophoresis apparatus.

The electrophoresis chamber consisted of a plexiglass tray (405 mm x 360 mm x 88 mm) which was divided into three compartments. The central compartment (157 mm x 405 mm x 57 mm), above which the gel was placed, was filled with ice to cool the gel during electrophoresis. The two outer electrode chambers (405 mm x 101 mm) each contained a 304 mm no. 22 platinum wire. The gel was supported on a glass plate placed across the central compartment and sponge cloths (203 mm x 139 mm x 6 mm) were used as bridges between the gel and the electrode buffer. A glass plate was placed on top of sponge cloth bridges to hold them flat in contact with the gel. Power was supplied by either a Gelman Electrophoresis Power Supply Model 38206 or a Heathkit 1P17 H.V. Power Supply. All electrophoresis was completed in a controlled temperature chamber between 0° and $4^{\circ}C$.

Buffer Systems

Seven buffer systems were used to separate the various proteins examined in this study. Hemoglobins and esterase-1 (erythrocyte esterase) were separated in a discontinuous buffer system consisting of a 0.01 M tris-hydrochloric acid gel buffer pH 8.5 (1.21 g tris in 1000 ml of deionized water, pH adjusted to 8.5 with 1 N hydrochloric acid) and an electrode buffer of 0.3 M sodium borate pH 8.2 (18.55 g boric

acid in 800 ml deionized water, pH adjusted to 8.2 with 1.0 M sodium hydroxide, and diluted to 1 l) (Selander et al., 1971). Most efficient separation was obtained at 25 ma with voltage not exceeding 250 v. Two other erythrocyte esterases and 6-phosphogluconate dehydrogenase were separated from the hemolysed in a continuous buffer system consisting of an electrode buffer of 0.1 M tris - 0.1 M maleic acid - 0.01 M EDTA - 0.01 M magnesium chloride, pH 7.4 (12.1 g tris, 11.6 g maleic acid, 3.72 g disodium salt of EDTA, and 2.03 g magnesium chloride (hexahydrate), diluted to 1 l, and pH adjusted to 7.4 with 1.0 M sodium hydroxide) and a gel of a 1:9 dilution of the electrode buffer of the same pH (Selander et al., 1971). A potential of 100 v was applied for 5 h. to provide sufficient separation.

Most serum proteins (transferrins and esterases) and liver and kidney esterases were separated in a system consisting of a gel of a 1:9 mixture of stock solutions A and B as follows: stock solution A was a 0.03 M lithium hydroxide - 0.19 M boric acid, pH 8.1 (11.81 g boric acid in 900 ml deionized water, adjusted to pH 8.1 with 0.1 M lithium hydroxide and diluted to 1 l) and stock solution B was a 0.05 M tris - 8 mM citric acid, pH 8.4 (6.2 g tris in 900 ml deionized water, pH adjusted to 8.4 with 0.1 M citric acid, and diluted to 1 l). The electrode buffer consisted of stock solution A (Selander et al., 1971). Optimum separation was

obtained at 25 ma per gel with the voltage not exceeding 350 v for a period of 2.5 h. Albumins were separated from the sera in a discontinuous system consisting of a tris - citrate gel (0.004 M citric acid), pH 6.0 (0.84 g monohydrate citric acid in 900 ml deionized water, pH adjusted to 6.0 with 0.1 M tris, and diluted to 1 l). The electrode buffer was a 0.3 M sodium borate solution, pH 6.5 (18.55 g boric acid in 900 ml of deionized water, pH adjusted to 6.5 with 1.0 M sodium hydroxide, and diluted to 1 l) (Jensen and Rasmussen, 1971). Optimum separation was obtained at 25 ma per gel with a maximum potential of 300 v until the borate boundary had migrated 8 cm from the origin (approximately 3 h.).

Glutamate oxalate transaminase was separated from liver or kidney extracts in a continuous buffer system consisting of a gel buffer of 22.89 mM tris - 5.22 mM citric acid, pH 8.0 (2.77 g tris in 900 ml of deionized water, pH adjusted to 8.0 with 0.1 M citric acid, and diluted to 1 l), and an electrode buffer of 0.687 M tris - 0.157 M citric acid, pH 8.0 (83.2 g tris and 29 g monohydrate citric acid, pH adjusted to 8.0 with 0.1 M citric acid, and diluted to 1 l) (Selander et al., 1971). A potential of 100 v was applied for 4 h. for sufficient separation.

Lactate dehydrogenase was separated from kidney and testicular extracts in a discontinuous buffer system consisting of a gel buffer of 0.076 M tris - 0.005 M citric acid,

pH 8.7 (9.21 g tris in 900 ml of deionized water, pH adjusted to 8.7 with 1.0 M citric acid, and diluted to 1 l) and an electrode buffer of 0.3 sodium borate, pH 8.2 (18.55 g boric acid in 900 ml of deionized water, pH adjusted to 8.2 with 1.0 M sodium hydroxide, and diluted to 1 l) (Selander et al., 1971). Sufficient separation was obtained at 25 ma per gel with a maximum potential of 250 v for 3 h.

Malate dehydrogenase and malic enzyme were separated from kidney extracts in a continuous buffer system consisting of a gel buffer of 8 mM tris - 3 mM citric acid, pH 6.7 (0.97 g tris and 0.63 g monohydrate citric acid, diluted to 1 l, and pH adjusted to 6.7 with 1.0 M sodium hydroxide) and an electrode buffer of 0.223 M tris - 0.086 M citric acid, pH 6.3 (27.0 g tris and 18.07 g monohydrate citric acid, diluted to 1 l, and pH adjusted to 6.3 with 1.0 M sodium hydroxide) (Selander et al., 1971). A potential of 170 v was applied for 3 h. to produce desired separation.

After electrophoresis, gels were allowed to cool for a few minutes and sliced in 2 mm horizontal sheets. This was accomplished first with a microtome blade fitted on a plexiglass stand 2 mm above the glass plate and later, and more efficiently, with a 0.2 mm wire stretched tightly across a frame.

Identification of Proteins

Hemoglobins, albumins, and transferrins were stained

with a general protein stain of 2% solution of Buffalo Black NBR (naphthol blue black) for 20 min. in a 5:5:1 mixture of methanol, deionized water, and glacial acetic acid. The gels were destained and fixed with repeated washes in 5:5:1 mixture of methanol, deionized water, and glacial acetic acid.

Enzymes were identified by using specific biochemical staining techniques. Glutamate oxalate transaminase was identified by the staining technique of Delorenzo and Ruddle (1970) consisting of a stain of 50 ml 0.2 M tris hydrochloric acid buffer, pH 8.0 (24.2 g tris, diluted to 1 l, and pH adjusted to 8.0 with 12 N hydrochloric acid); 0.5 mg pyridoxal-5'-phosphate; 200 mg α-aspartic acid; 100 mg α-ketoglutaric acid; and 150 mg Fast Blue RR Salt. Stain was prepared prior to use each time and gels were stained in the dark for 30 min. at 37°C.

Lactate dehydrogenase was detected by a technique modified from Markert and Massaro (1966). The stain consisted of 30 ml of deionized water, 20 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0), 9 ml 0.5 M sodium DL-lactate, 20 mg B-nicotinamide adenine dinucleotide, 10 mg MTT tetrazolium, and 8 mg phenazine methosulfate. Gels were stained in the dark at 37°C for 1 to 2 h.

Malate dehydrogenase activity was detected by a technique modified from Shows and Ruddle (1968) using a stain

consisting of 30 ml 0.2 M tris-hydrochloric acid buffer (pH 8.0), 5 ml 2.0 M malate solution (pH adjusted to 7.0 with 1.0 M sodium hydroxide), 10 mg B-nicotinamide adenine dinucleotide, 20 mg MTT tetrazolium, and 5 mg phenazine methosulfate. The gels were stained in the dark at 37°C for 1 h. Malic enzyme activity was demonstrated with the same stain with the substitution of 20 mg nicotinamide adenine dinucleotide phosphate for 10 mg B-nicotinamide adenine dinucleotide, and stained in the dark at 37°C for 2 h.

The enzyme 6-phosphogluconate dehydrogenase was detected by staining technique of Carter et al. (1968). The stain consisted of 7 ml 0.2 M tris hydrochloric acid buffer (pH 8.0), 7 ml 0.1 M magnesium chloride, 3 ml deionized water, 20 mg barium-6-phosphogluconic acid, 1 mg nicotinamide adenine dinucleotide phosphate, 4 mg MTT tetrazolium, and 1 mg phenazine methosulfate. Gels were stained in the dark at 20°C for 1 h.

Esterases were detected by the methods outlined by Selander et al. (1971). Esterases in the sera, and kidney and liver extracts, were stained with a mixture of 1 ml 0.2 M monobasic sodium phosphate, 1 ml 0.2 M dibasic sodium phosphate, 47 ml deionized water, 25 mg Fast Blue RR Salt, 1 ml of a solution of 0.1 g α-naphthyl propionate (liver and kidney extracts) or α-naphthyl butyrate (serum) in 10 ml of acetone. Gels were stained at 37°C, in the dark, for 10 to

30 min. Some esterases of kidney and liver extracts were inhibited by the use of 0.001 M eserine (7 mg eserine in 26.5 ml deionized water, 0.5 ml 0.2 M monobasic sodium phosphate, and 0.5 ml 0.2 M dibasic sodium phosphate). Gels were preincubated at room temperature for 20 min. prior to staining at 37°C in substrate solution.

Erythrocyte esterases were stained with 24 ml 0.2 M monobasic sodium phosphate, 6 ml 0.2 M dibasic sodium phosphate, 20 ml deionized water, 25 mg Fast Garnet GBC Salt, and 1 ml of a solution of 0.1 g α-naphthyl propionate in 10 ml of acetone. Staining was accomplished at 37°C in the dark for a period of 1 to 2 h.

All gels were fixed in the 5:5:1 methanol, deionized water, glacial acetic acid solution for 24 h., scored or photographed, and wrapped in a clear plastic film for storage.

Since heterozygous individuals are identifiable, electrophoretic data allowed direct calculation of allelic and gentoypic frequencies. Allelic frequency was calculated by summing the number of loci at which a given allele occurred within a population and dividing by the total number of loci at which it could have occurred. Genotypic frequency was calculated by summing the number of individuals which possessed a given genotype in a population and dividing by the total number of individuals within that population.

Deviation from Hardy-Weinberg Equilibrium was tested using Chi-square analysis at all polymorphic loci of a population. Significance was tested at the 95% and 99% level using direct calculations of allelic frequencies.

CHAPTER III

RESULTS

Karyological Results

Six distinct chromosomal patterns were observed within the populations of the Peromyscus boylii species group included in this study. All specimens had a diploid number of 48. The major difference in chromosomal patterns was in the number of pairs of large biarmed autosomes.

Peromyscus boylii rowleyi and Peromyscus boylii utahensis

Fifty-nine specimens of P. boylii rowleyi from western Oklahoma, western Texas, New Mexico, and Chihuahua, and two specimens of P. boylii utahensis from southern Utah had a chromosomal pattern identical to that described by Lee et al. (1972) for P. boylii rowleyi, P. boylii utahensis and P. boylii boylii and to that described by Hsu and Arrighi (1968) for P. boylii rowleyi. The autosomal complement (Fig. 2) consisted of one pair of large subtelocentric chromosomes, one pair of small submetacentric chromosomes, one pair of small metacentric chromosomes, and 20 pairs of large to small acrocentric chromosomes. The X chromosome was a large subtelocentric and the Y was a medium-sized metacentric.

Fig. 2--Karyotype of a male Peromyscus boylii rowleyi (Silver City, Grant County, New Mexico).

Fig. 3--Karyotype of a male Peromyscus boylii ambiguus (Cuidad Victoria, Tamaulipas, Mexico).

Peromyscus boylii ambiguus

Six specimens from near Ciudad Victoria, Tamaulipas, had a karyotype consisting of three pairs of large subtelocentric autosomes, one pair of medium-sized submetacentric autosomes, two pairs of small metacentric autosomes, and 17 pairs of large to small acrocentric autosomes (Fig. 3). The X was a large submetacentric chromosome and the Y was a medium-sized metacentric chromosome. Schmidly (personal communication) reported polymorphism within populations of P. boylii ambiguus due to pericentric inversion, with the number of large biarmed elements varying from eight to ten, and a corresponding variation in the large acrocentric elements from 34 to 32.

Peromyscus boylii spicilegus

One specimen from near Coyotes, Durango, had an identical karyotype to that described by Lee et al. (1972) for P. boylii spicilegus. The autosomal complement consisted of three large pairs of subtelocentric chromosomes, two small pairs of metacentric chromosomes, and 18 pairs of large to small acrocentric chromosomes (Fig. 4). The X was a large subtelocentric chromosome and the Y was a medium-sized submetacentric chromosome. The autosomal complement of P. boylii spicilegus was similar to P. attwateri.

A female specimen also from near Coyote, Durango, had a karyotype intermediate to P. boylii rowleyi and P.

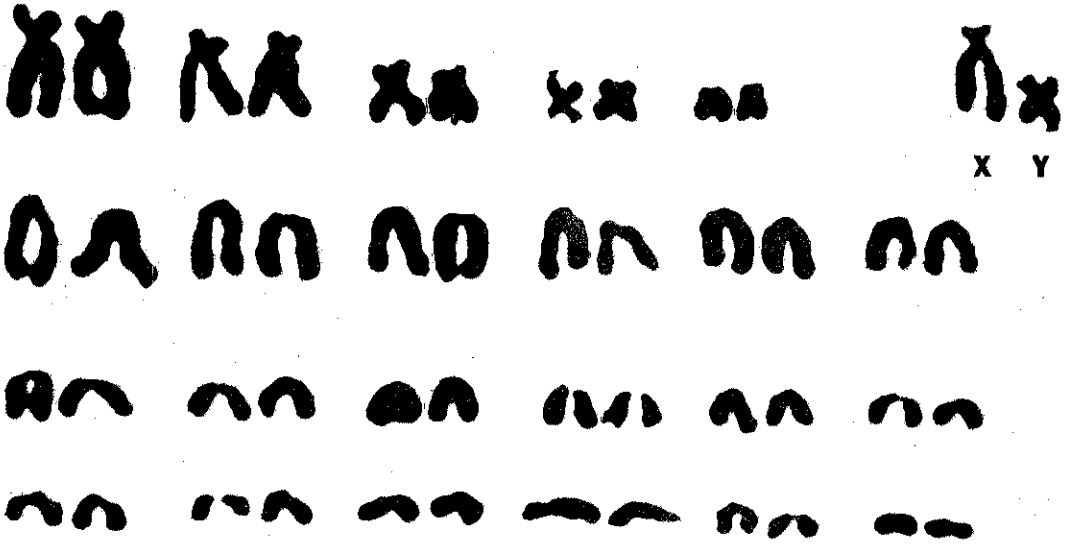


Fig. 4--Karyotype of a male Peromyscus boylii spicilegus from Durango, Mexico.

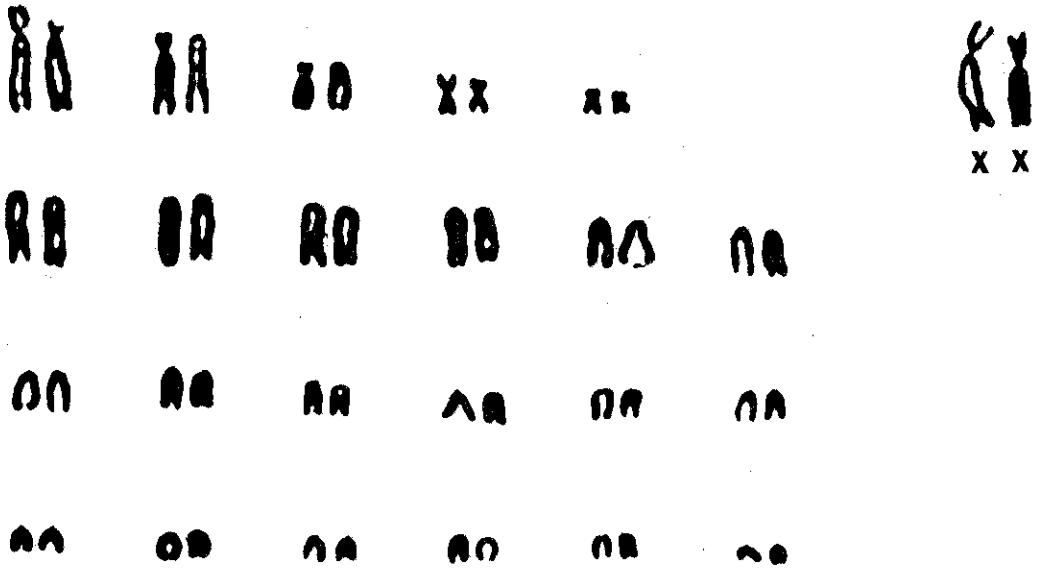


Fig. 5--Karyotype of a female F_1 cross between a Peromyscus boylii rowleyi and Peromyscus boylii spicilegus (Coyote, Durango, Mexico).

boylii spicilegus. The autosomal complement consisted of a large pair of subtelocentric chromosomes, an unmatched pair of large subtelocentric chromosomes, and two pair of small metacentric chromosomes (Fig. 5). Karyologically the specimen appeared to be a first generation offspring from a P. boylii spicilegus and P. boylii rowleyi mating.

Peromyscus attwateri

Sixty-four specimens of P. attwateri from southern Kansas, Oklahoma, and Texas had a karyotype identical to that described by Lee et al. (1972). The autosomal complement consisted of three large pairs of subtelocentric chromosomes, two small pairs of metacentric chromosomes, and 18 large to small pairs of acrocentric chromosomes (Fig. 6). The X was a large submetacentric chromosome and the Y was a small submetacentric chromosome.

Peromyscus pectoralis

Fifty-eight specimens of Peromyscus pectoralis representing populations of all the recognized subspecies from Oklahoma, Texas, Tamaulipas, Neuvo Leon, Coahuila, Durango, Chihuahua, and San Luis Potosi possessed a consistent chromosomal pattern. The autosomal complement consisted of three large pairs of subtelocentric chromosomes, one pair of large submetacentric chromosomes, two pairs of small metacentric chromosomes, and 17 pairs of large to small acrocentric chromosomes (Fig. 7). This pattern was identical to that

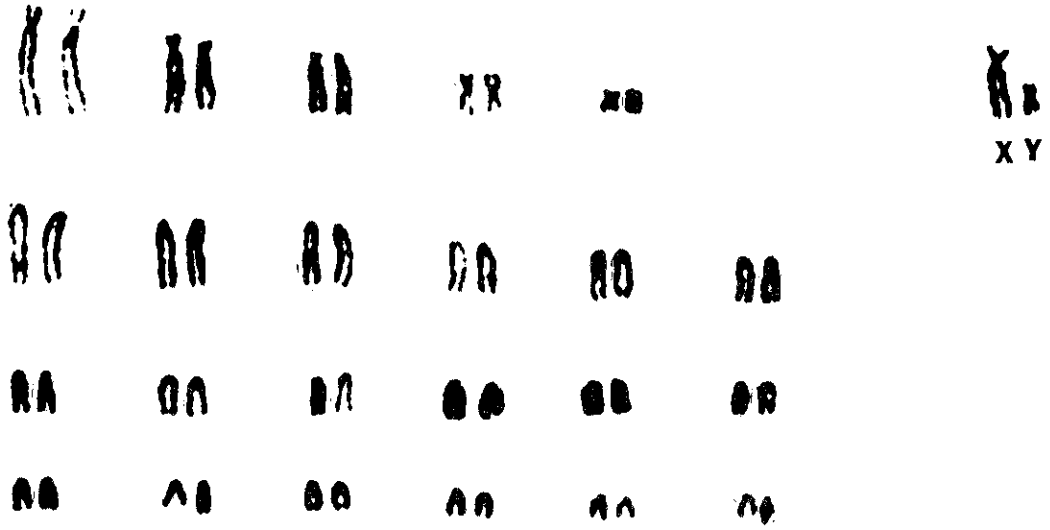


Fig. 6--Karyotype of a male Peromyscus attwateri (Elgin, Chautauqua County, Kansas).

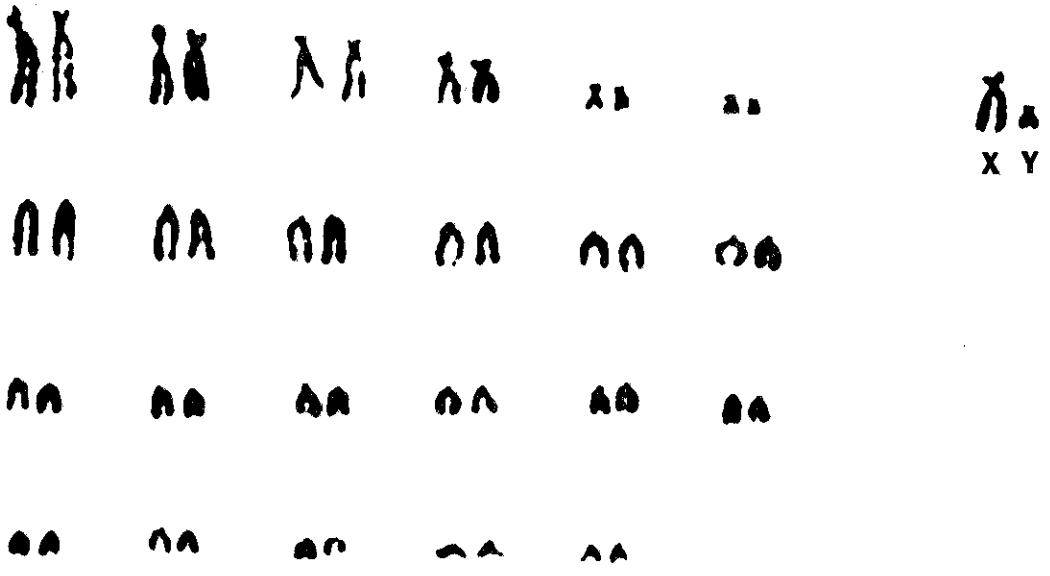


Fig. 7--Karyotype of a male Peromyscus pectoralis (Bermejillo, Durango, Mexico).

described for P. pectoralis by Hsu and Arrighi (1968) and Schmidly (1971). The X chromosome was a large submetacentric, and the Y was a small metacentric chromosome.

Peromyscus polius

Five specimens of this rare species (39 previously known specimens) from near Santa Barbara, Chihuahua, have a karyotype which was markedly distinct from any member of the boylii group. The autosomal complement consisted of one large pair of subtelocentric chromosomes, six pairs of medium-sized submetacentric chromosomes, seven pairs of medium-sized metacentric chromosomes, one to two pairs of small metacentric chromosomes, and seven to eight pairs of medium-sized to small acrocentric chromosomes (Fig. 8). Two of the specimens possessed a small unmatched acrocentric and a small unmatched metacentric chromosome (Fig. 9). The other three specimens all had two small acrocentrics, and lack the smallest metacentric. The polymorphism observed in this population was the result of a pericentric inversion, involving the smallest pair of metacentric chromosomes. The X was a large submetacentric chromosome and the Y was a small submetacentric chromosome. The mice from this location were referable to Peromyscus polius based on morphological characters presented by Anderson (1972), and were verified by Dr. Sydney Anderson of the American Museum of Natural

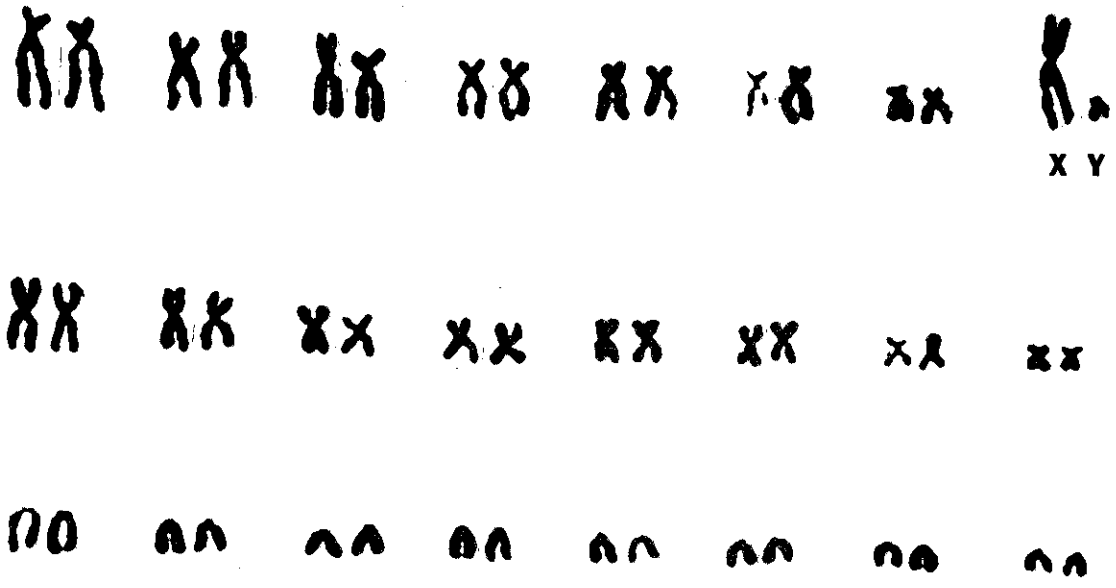


Fig. 8--Karyotype of a male Peromyscus polius (Santa Barbara, Chihuahua, Mexico).

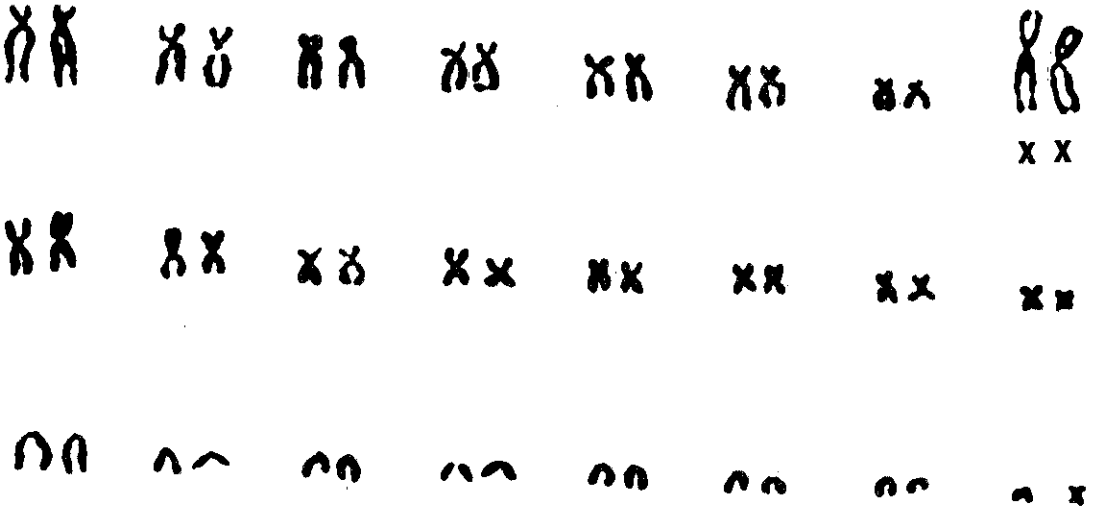


Fig. 9--Karyotype of a female Peromyscus polius (Santa Barbara, Chihuahua, Mexico).

History, New York, New York, and Dr. Emmet T. Hooper, Museum of Zoology, University of Michigan, Ann Arbor, Michigan.

Electrophoretic Results

Fourteen proteins were identified from 368 mice representing 45 populations of the Peromyscus boylii species group. Five loci were monomorphic in all populations and demonstrated no interspecific variation, while four loci were monomorphic in most populations but demonstrated interspecific or intersubspecific variation. A total of 10 loci were polymorphic, but three of these failed to demonstrate interspecific variation. The two loci controlling production of malic enzyme were excluded from analysis due to weak staining. The electrohporetic pattern of three esterases were not scorable, so interpretation of genetic mechanisms was not possible, and these were excluded from analysis. A total of 11 remaining proteins encoded by 19 loci were analyzed. No more than five alleles were found to segregate at any single locus.

Sexual Variation

No evidence was found to suggest the linkage of the 17 structural loci encoding the 11 proteins examined with genetic material of the X chromosome. Both males and females were found to be heterozygous for all loci for which heterozygous phenotypes were observed. In addition, there were no significant sexual differences in frequencies of alleles or

genotypes for any of the polymorphic loci in samples with seven or more individuals (Table 1), and sexual differences observed in frequencies in small populations were not significant when data were pooled for all samples of the species.

Although sexual variation in frequency of transferrin alleles has been reported for voles, Microtus ochrogaster and M. pennsylvanicus (Tamarin and Krebs, 1969), sexual variation has not been detected in most rodents including Dipodomys (Johnson and Selander, 1971), Sigmodon (Johnson et al., 1972), P. polionotus (Selander et al., 1971) or Mus (Selander and Yang, 1969, 1970). Electrophoretic analysis did not detect significant sexual differences in the boylii species group; however, this does not exclude the possibility of some sexual variation in small populations or of seasonal variation. Since conspicuous sexual differences were not observed, data from males and females have been combined in the following analyses.

Scorable Proteins

6-Phosphogluconate Dehydrogenase. --Three electrophoretically single band patterns were demonstrated within the boylii species group (Figure 10). The b allele was predominant or was fixed in most populations of P. pectoralis, and the other two alleles were also demonstrated within populations of this species (Table 2). The d allele was fixed in a population of P. pectoralis collinus from El Salto Falls,

Table 1. - Sexual variation in allele frequency and genotype frequency at albumin locus

	Sex	n	X^2	Genotype aa	ab	bb	Freq. a Allele
P. boylii rowleyi							
Bernalillo Co., N.M.	M	7	0.02	0.57	0.43	0.00	0.75
	F	4		0.50	0.50	0.00	0.75
Grant Co., N.M.	M	9	0.21	0.11	0.34	0.55	0.28
	F	6		0.17	0.17	0.66	0.25
Jeff Davis Co., Tex.	M	2	0.45	1.00	0.00	0.00	1.00
	F	6		0.67	0.33	0.00	0.84
Buena Ventura, Chi.	M	6	0.49	0.83	0.17	0.00	0.92
	F	5		0.80	0.20	0.00	0.90
Celulosa, Chi.	M	13	0.07	0.46	0.54	0.00	0.77
	F	9		0.56	0.44	0.00	0.78
Santa Barbara, Chi.	M	7	0.74	0.86	0.14	0.00	0.93
	F	5		1.00	0.00	0.00	1.00
P. pectoralis laceianus							
Love Co., Okla.	M	6	1.00	0.16	0.33	0.50	0.33
	F	5		0.00	0.80	0.20	0.40
Cooke Co., Tex.	M	4	0.78	0.25	0.75	0.00	0.63
	F	3		0.33	0.00	0.67	0.33

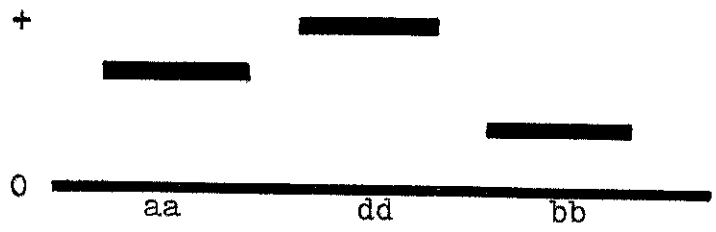


Fig. 10--Electrophoretic variation in 6-phosphogluconate dehydrogenase.

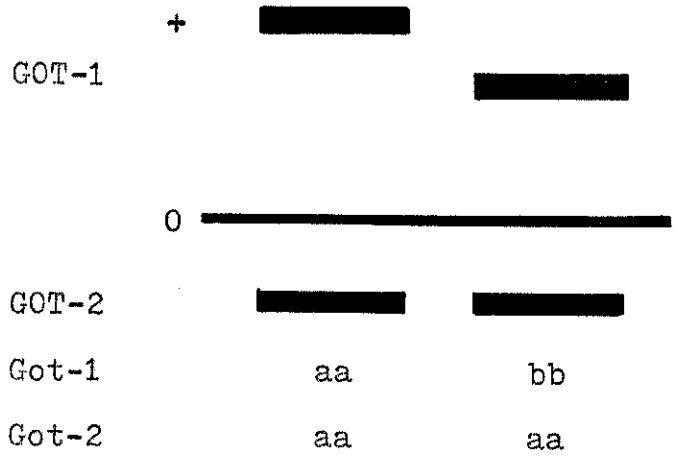


Fig. 11--Electrophoretic variation in glutamate oxalate transaminase.

San Luis Potosi and was present in one of two specimens from 7 mi. E Buena Ventura, Chihuahua. The third allele, a, was present in the homozygous condition in a population of P. pectoralis collinus from 19 mi. SW Jaumave, Tamaulipas.

The a allele was predominant or was fixed in most populations of P. boylii and was fixed in all populations of P. attwateri. The d allele was present in two populations of P. boylii rowleyi from 9 mi. SSE Celulosa, Chihuahua and 7 mi. E Buena Ventura, Chihuahua. Both the d and a alleles were present in a homozygous condition in the population of P. polius. This enzyme apparently is a dimer (Parr, 1966; Carter et al., 1968) with three banded heterozygotes, but no individual was found with more than a single band.

Glutamate Oxaloacetate Transaminase. --Two forms of glutamate oxaloacetate transaminase are present in all rodents that have been examined, including members of the families Heteromyidae (Johnson and Selander, 1971), Geomyidae (Patton et al., 1972), Muridae (Chapman and Ruddle, 1972; Selander et al., 1971; Johnson et al., 1972; and Smith et al., 1973). The mitochondrial, cathodally migrating form, GOT-2, was monomorphic and demonstrated no geographic or interspecific variation within the P. boylii species group, however the supernate form, GOT-1, demonstrated considerable interspecific variation (Figure 11).

Table 2. - Allelic frequency and genetic variability of the 6-phosphogluconate dehydrogenase and the glutamate oxalate transaminase loci of the Peromyscus boylii species group

	6-Phosphogluconate Dehydrogenase			Glutamate Oxalate Transaminase			
				GOT-1			GOT-2
	a	b	c	a	b	c	a
Peromyscus attwateri							
Chautauqua Co., Kan. (56)	1.00	----	----	1.00	----	----	1.00
Comanche Co., Okla. (7)	1.00	----	----	1.00	----	----	1.00
Montague Co., Tex. (2)	1.00	----	----	1.00	----	----	1.00
Cottle Co., Tex. (48)	1.00	----	----	1.00	----	----	1.00
Knox Co., Tex. (3)	1.00	----	----	1.00	----	----	1.00
Johnson Co., Tex. (2)	1.00	----	----	1.00	----	----	1.00
Garza Co., Tex. (8)	1.00	----	----	1.00	----	----	1.00
Kerr Co., Tex. (3)	1.00	----	----	1.00	----	----	1.00
Peromyscus boylii utahensis							
Garfield Co., Utah (7)	1.00	----	----	1.00	----	----	1.00
Peromyscus boylii rowleyi							
Cimmaron Co., Okla. (4)	1.00	----	----	1.00	----	----	1.00
Bernalillo Co., N.M. (12)	1.00	----	----	1.00	----	----	1.00
Catron Co., N.M. (4)	1.00	----	----	1.00	----	----	1.00
Grant Co., N.M. (21)	1.00	----	----	1.00	----	----	1.00
Jeff Davis Co., Tex. (13)	1.00	----	----	1.00	----	----	1.00
Buena Ventura, Chi. (15)	0.91	----	0.09	1.00	----	----	1.00
Celulosa, Chi. (23)	0.83	----	0.17	0.80	0.20	----	1.00
Santa Barbara, Chi. (12)	1.00	----	----	1.00	----	----	1.00

Table 2. - (continued)

	6-Phosphogluconate Dehydrogenase			Glutamate Oxalate Transaminase			
	a	b	c	GOT-1 a	GOT-1 b	GOT-1 c	GOT-2 a
Peromyscus boylii ambiguus							
Cd. Victoria, Tam. (6)	1.00	----	----	1.00	----	----	1.00
Peromyscus boylii spicilegus							
Coyotes, Durango (4)	1.00	----	----	1.00	----	----	1.00
Peromyscus boylii levipes							
Metepec, Hidalgo (3)	1.00	----	----	1.00	----	----	1.00
Las Vigas, Veracruz (3)	1.00	----	----	1.00	----	----	1.00
Pinal de Amoles, Que. (3)	1.00	----	----	1.00	----	----	1.00
Peromyscus polius							
Santa Barbara, Chi. (5)	0.60	----	0.40	----	----	1.00	1.00
Peromyscus pectoralis laceianus							
Love Co., Okla. (11)	----	1.00	----	0.25	0.75	----	1.00
Cooke Co., Tex. (7)	----	1.00	----	----	1.00	----	1.00
Eastland Co., Tex. (10)	----	1.00	----	----	1.00	----	1.00
Throckmorton Co.Tex(12)	----	1.00	----	----	1.00	----	1.00
Mason Co., Tex. (3)	----	1.00	----	----	1.00	----	1.00
Kerr Co., Tex. (2)	----	1.00	----	----	1.00	----	1.00
Uvalde Co., Tex. (2)	----	1.00	----	----	1.00	----	1.00
Jeff Davis Co., Tex. (6)	----	1.00	----	----	1.00	----	1.00
Jeff Davis Co., Tex. (3)	----	1.00	----	0.40	0.60	----	1.00
Buena Ventura, Chi. (2)	----	0.50	0.50	0.50	0.50	----	1.00
Ocampo, Coa. (4)	----	1.00	----	----	1.00	----	1.00

Table 2. - (continued)

	6-Phosphogluconate Dehydrogenase			Glutamate Oxalate Transaminase			
	a	b	c	GOT-1 a	GOT-1 b	GOT-1 c	GOT-2 a
42 mi. N. Saltillo, Coa. (1)	----	1.00	----	----	1.00	----	1.00
12 mi. N. Saltillo, Coa. (6)	----	1.00	----	----	1.00	----	1.00
20 mi. SE Saltillo, Coa. (4)	----	1.00	----	----	1.00	----	1.00
Linares, Nuevo Leon (4)	----	1.00	----	----	1.00	----	1.00
Peromyscus pectoralis pectoralis							
Bermejillo, Durango (4)	----	1.00	----	----	1.00	----	1.00
Hacienda, Que. (4)	----	1.00	----	----	1.00	----	1.00
Arroyo Seca, Que. (2)	----	1.00	----	----	1.00	----	1.00
Peromyscus pectoralis collinus							
Jaumave, Tam. (3)	0.67	0.33	----	----	1.00	----	1.00
El Salto, San Luis Potosi (4)	----	----	1.00	----	1.00	----	1.00
Cd. Valles, San Luis Potosi (4)	----	1.00	----	----	1.00	----	1.00

The a allele was predominant or fixed in all populations of P. boylii rowleyi and fixed in P. boylii spicilegus, P. boylii ambiguus and P. attwateri (Table 1). A single population of P. boylii rowleyi from 9 mi. SSE Celulosa, Chihuahua was polymorphic with the b allele occurring at a frequency of 0.20. The b allele was predominant or fixed in populations of P. pectoralis. Three populations from 4 mi. W Marietta, Love Co., Oklahoma, 10 mi. WSW Ft. Davis, Jeff Davis Co., Texas and 7 mi. E Buena Ventura, Chihuahua were polymorphic with the a allele occurring at a frequency of 0.25, 0.40 and 0.50, respectively. Heterozygotes are three banded in Mus (Delorenzo and Ruddle, 1970) and P. polionotus (Selander et al., 1971), but no three-banded phenotypes were observed within the boylii species group. The d allele was fixed in the single population of P. polius.

Albumins. --Polymorphism of albumins has been reported in populations of P. boylii by Jensen and Rasmussen (1971) and Jensen (1970). They observed two albumins in populations of P. boylii rowleyi and P. boylii utahensis and indicated that populations sampled had a wide range of allelic frequencies with either allele being common. Data presented by Welser et al. (1965) indicate that the serum albumins in P. maniculatus and P. polionotus were phenotypic expressions of codominant autosomal alleles at a single locus. Jensen and Rasmussen (1971) agreed with this inheritance of serum albumins in P. boylii.

Two albumin alleles, undoubtedly corresponding to the m and n of Jensen and Rasmussen (1971), were represented in a sample of the P. boylii species group (Table 3); with a (=m), producing a fast-migrating band, and b (=n), producing a slow-migrating band (Figure 12). Polymorphism was observed in populations of P. pectoralis from north-central Texas and southern Oklahoma, and P. boylii rowleyi from Chihuahua, New Mexico, and the Davis Mts. of western Texas. The a allele was fixed in populations of P. attwateri, P. boylii spicilegus and P. pectoralis from Mexico and the Davis Mts. of Texas. The b allele was fixed in the single populations of P. polius and P. boylii ambiguus sampled.

Samples from three populations of P. boylii levipes had the a allele fixed in two populations and the b allele fixed in the other.

Transferrin. --Serum transferrin polymorphism has been observed in natural populations of P. boylii, P. eremicus, P. crinitus, and P. maniculatus from Arizona (Rasmussen and Koehn, 1966). This protein has been observed to be highly polymorphic in populations of several groups of rodents; Sigmodon arizonae (Johnson et al., 1972), Dipodomys (polymorphic in eight of the 12 species analyzed) (Johnson and Selander, 1971), and Microtus ochrogaster (Gaines and Krebs, 1971), P. polionotus (Selander et al., 1971; Biggers and Dawson, 1971), and P. floridanus (Smith et al., 1973). Only

four populations sampled from the P. boylii species group demonstrated polymorphism at this locus. A total of four alleles were observed (Figure 12), and interspecific and intersubspecific variation was demonstrated at this locus (Table 3).

The d allele, produced the slowest migrating band, and was fixed in all populations of P. attwateri. This allele was also present in the gene pool of the single population of P. polius sampled. In this population, polymorphism of the transferrin locus was observed with the d allele occurring in equal frequencies with the c allele. The c allele was also found in the single population of P. boylii spicilegus. One specimen from the same location as the specimens of P. boylii spicilegus was heterozygous with a and c alleles present at the transferrin locus. The c allele was predominant or fixed in populations of P. pectoralis in Queretaro, and also occurred in two populations of P. boylii levipes from 13 mi. NE Metepec, Hidalgo, and 1 mi. N Pinal de Amoles, Queretaro, in an equal frequency with the b allele, which was fixed in all other populations of levipes sampled. This allele (b) was also fixed in the population of P. boylii ambiguus and in most populations of P. pectoralis. The forth allele a, produced the fastest migrating electrophoretic band, and was fixed in all populations of P. boylii rowleyi.

Table 3. - Allelic frequency and genetic variation of the albumin and transferrin loci within populations of the Peromyscus boylii species group

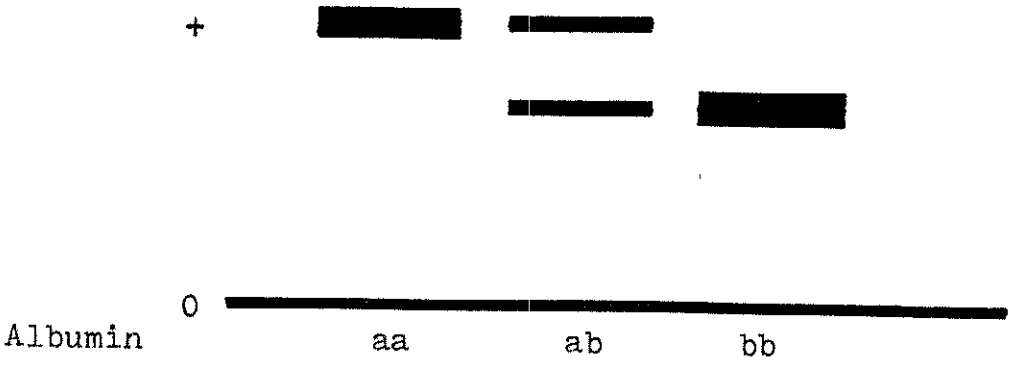
	Albumin		Transferrin			
	a	b	a	b	c	d
Peromyscus attwateri						
Chautauqua Co., Kan. (56)	1.00	----	----	----	----	1.00
Comanche Co., Okla. (7)	1.00	----	----	----	----	1.00
Montague Co., Tex. (2)	1.00	----	----	----	----	1.00
Cottle Co., Tex. (48)	1.00	----	----	----	----	1.00
Knox Co., Tex. (3)	1.00	----	----	----	----	1.00
Johnson Co., Tex. (2)	1.00	----	----	----	----	1.00
Garza Co., Tex. (8)	1.00	----	----	----	----	1.00
Kerr Co., Tex. (3)	1.00	----	----	----	----	1.00
Peromyscus boylii utahensis						
Garfield Co., Utah (7)	----	1.00	1.00	----	----	----
Peromyscus boylii rowleyi						
Cimmaron Co., Okla. (4)	1.00	----	1.00	----	----	----
Bernalillo Co., N.M. (12)	0.75	0.25	1.00	----	----	----
Catron Co., N.M. (4)	0.50	0.50	1.00	----	----	----
Grant Co., N.M. (21)	0.27	0.73	1.00	----	----	----
Jeff Davis Co., Tex. (13)	0.88	0.12	1.00	----	----	----
Buena Ventura, Chi. (15)	0.91	0.09	1.00	----	----	----
Celulosa, Chi. (23)	0.76	0.24	1.00	----	----	----
Santa Barbara, Chi. (12)	0.96	0.04	1.00	----	----	----

Table 3. - (continued)

	Albumin		Transferrin			
	a	b	a	b	c	d
Peromyscus boylii ambiguus						
Cd. Victoria, Tam. (6)	----	1.00	----	1.00	----	----
Peromyscus boylii spicilegus						
Coyote, Durango (4)	1.00	----	0.12	----	0.88	----
Peromyscus boylii levipes						
Metepec, Hidalgo (3)	1.00	----	----	0.50	0.50	----
Las Vigas, Veracruz (3)	1.00	----	----	1.00	----	----
Pinal de Amoles, Que. (3)	----	1.00	----	0.50	0.50	----
Peromyscus polius						
Santa Barbara, Chi. (5)	----	1.00	----	----	0.50	0.50
Peromyscus pectoralis laceianus						
Love Co., Okla. (11)	0.36	0.64	----	1.00	----	----
Cooke Co., Tex. (7)	0.50	0.50	----	1.00	----	----
Eastland Co., Tex. (10)	0.50	0.50	----	1.00	----	----
Throckmorton Co.Tex (12)	0.50	0.50	----	1.00	----	----
Mason Co., Tex. (3)	1.00	----	----	1.00	----	----
Kerr Co., Tex. (3)	0.50	0.50	----	1.00	----	----
Uvalde Co., Tex. (2)	1.00	----	----	1.00	----	----
Jeff Davis Co., Tex. (6)	1.00	----	----	1.00	----	----
Jeff Davis Co., Tex. (3)	1.00	----	----	1.00	----	----
Buena Ventura, Chi. (2)	1.00	----	----	1.00	----	----
Ocampo, Coa. (4)	1.00	----	----	1.00	----	----

Table 3. - (continued)

	Albumin		Transferrin			
	a	b	a	b	c	d
42 mi. N. Saltillo, Coa. (1)	1.00	----	----	1.00	----	----
12 mi. N. Saltillo, Coa. (6)	1.00	----	----	1.00	----	----
20 mi. SE Saltillo, Coa. (4)	1.00	----	----	1.00	----	----
Linares, Nuevo Leon (4)	1.00	----	----	1.00	----	----
Peromyscus pectoralis pectoralis						
Bermejillo, Durango (4)	1.00	----	----	1.00	----	----
Hacienda, Que. (4)	1.00	----	----	----	1.00	----
Arroyo Seca, Que. (2)	1.00	----	----	0.25	0.75	----
Peromyscus pectoralis collinus						
Jaumave, Tam. (3)	1.00	----	----	1.00	----	----
El Salto, San Luis Potosi (4)	1.00	----	----	1.00	----	----
Cd. Valles, San Luis Potosi (4)	1.00	----	----	1.00	----	----



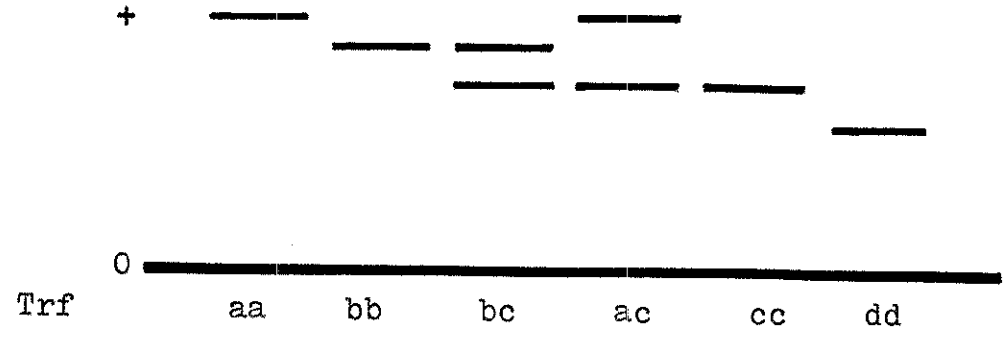


Fig. 12--Electrophoretic variation in albumin and transferrin.

Hemoglobins. --Hemoglobin polymorphism has been reported in natural populations of several species of murid rodents; Mus musculus (Selander et al., 1969), P. gossypinus (Foreman, 1964, 1966), P. polionotus (Selander et al., 1971), P. floridanus (Smith et al., 1973), and P. maniculatus and P. boylii (Rasmussen et al., 1968). The loci controlling the production of hemoglobin demonstrated both polymorphism and interspecific variation within the P. boylii species group (Table 4).

Phenotypic variation in hemoglobin in P. pectoralis is similar to that described for P. gossypinus by Foreman (1966). Electrophoresis of hemoglobin, presumably dimers of an alpha and beta polypeptide chain, has revealed six distinct patterns (Figure 13). Individual mice differ in the combination and amounts of three electrophoretically separable bands, but only one animal has been found, so far, with fewer than two bands.

Although progeny data are not available due to the failure of P. pectoralis to breed in the laboratory, some interpretation of the genetic mechanism is possible. It seems probable that either the alpha or beta chain is identical among the three electrophoretically separable bands. If there were differences in both polypeptide chains, more than three bands would be expected.

Table 4. - Genetic variation and allelic frequencies at the hemoglobin loci of mice of the Peromyscus boylii species group

Population	Hemoglobin a	b^1	b^2	b^3
Peromyscus attwateri				
Chautauqua Co., Kan. (56)	1.00	1.00	----	1.00
Comanche Co., Okla. (7)	1.00	1.00	----	1.00
Montague Co., Tex. (2)	1.00	1.00	----	1.00
Cottle Co., Tex. (48)	1.00	1.00	----	1.00
Knox Co., Tex. (3)	1.00	1.00	----	1.00
Johnson Co., Tex. (2)	1.00	1.00	----	1.00
Garza Co., Tex. (8)	1.00	1.00	----	1.00
Kerr Co., Tex. (3)	1.00	1.00	----	1.00
Peromyscus boylii utahensis				
Garfield Co., Utah (7)	1.00	----	1.00	1.00
Peromyscus boylii rowleyi				
Cimmaron Co., Okla. (4)	1.00	----	1.00	1.00
Bernalillo Co., N.M. (12)	1.00	----	1.00	1.00
Catron Co., N.M. (4)	1.00	----	1.00	1.00
Grant Co., N.M. (21)	1.00	----	1.00	1.00
Jeff Davis Co., Tex. (13)	1.00	----	1.00	1.00
Buena Ventura, Chi. (15)	1.00	----	1.00	1.00
Celulosa, Chi. (23)	1.00	----	1.00	1.00
Santa Barbara, Chi. (12)	1.00	----	1.00	1.00

Table 4. - (continued)

Population	Hemoglobin a	b[1]	b[2]	b[3]
Peromyscus boylii ambiguus				
Cd. Victoria, Tam. (16)	1.00	----	1.00	1.00
Peromyscus boylii spicilegus				
Coyotes, Durango (4)	1.00	----	1.00	1.00
Peromyscus boylii levipes				
Metepec, Hidalgo (3)	1.00	----	1.00	1.00
Las Vigas, Veracruz (3)	1.00	----	1.00	1.00
Pinal de Amoles, Que. (3)	1.00	----	1.00	1.00
Peromyscus polius				
Santa Barbara, Chi. (5)	1.00	----	1.00	1.00
Peromyscus pectoralis laceianus				
Love Co., Okla. (11)	1.00	1.00	1.00	----
Cooke Co., Tex. (7)	1.00	1.00	1.00	----
Eastland Co., Tex. (10)	1.00	1.00	1.00	----
Throckmorton Co., Tex. (12)	1.00	1.00	1.00	----
Mason Co., Tex. (3)	1.00	1.00	1.00	----
Kerr Co., Tex. (2)	1.00	1.00	1.00	----
Uvalde Co., Tex. (2)	1.00	1.00	1.00	----
Jeff Davis Co., Tex. (6)	1.00	1.00	1.00	----
Jeff Davis Co., Tex. (3)	1.00	1.00	1.00	----
Buena Ventura, Chi. (2)	1.00	1.00	----	1.00
Ocampo, Coa. (4)	1.00	1.00	0.12	0.88

Table 4. - (continued)

Population	Hemoglobin			
	a	b^1	b^2	b^3
42 mi. N. Saltillo, Coa. (1)	1.00	1.00	----	1.00
12 mi. N. Saltillo, Coa. (6)	1.00	1.00	----	1.00
20 mi. SE Saltillo, Coa. (4)	1.00	1.00	0.12	0.88
Linares, Nuevo Leon (4)	1.00	1.00	0.12	0.88
Peromyscus pectoralis pectoralis				
Bermejillo, Durango (4)	1.00	0.75	0.50	0.75
Hacienda, Que. (4)	1.00	1.00	----	1.00
Arroyo Seca, Que. (2)	1.00	1.00	----	1.00
Peromyscus pectoralis collinus				
Jaumave, Tam. (3)	1.00	1.00	0.50	0.50
El Salto, San Luis Potosi (4)	1.00	1.00	0.63	0.37
Cd. Valles, San Luis Potosi (4)	1.00	1.00	----	1.00

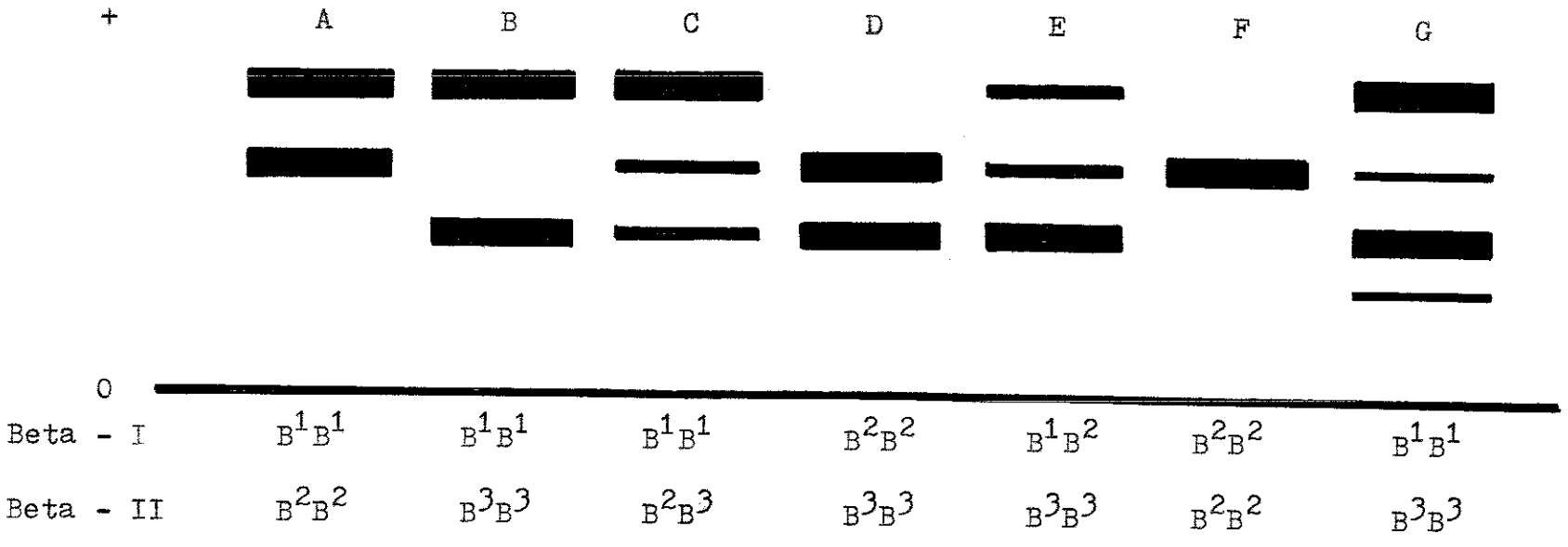


Fig. 13 -- Electrophoretic variation in hemoglobin of mice of the boylii group

Other studies of hemoglobin polymorphism in rodents (Foreman, 1966; Gluecksohn-Waesch, 1960; Jensen,1970) have suggested gene duplication to produce multiple loci for the production of one of the polypeptides of hemoglobin. In P. pectoralis, there appear to be at least two loci for the production of one of the subunits. There are no data to suggest which subunit has had genetic modification, but the beta chain has been tentatively selected.

Three patterns occurred in greatest frequency in P. pectoralis. Pattern A consisted of a fast-migrating band (b^1b^1) and an intermediate-migrating band (b^2b^2). The second pattern (B) consisted of the fast-migrating band (b^1b^1) and slow-migrating band (b^3b^3). The third common pattern (C) was the pattern which would be expected from the cross of a parent with pattern A and a parent with pattern B. This three banded phenotype consisted of a fast-migrating band of typical intensity (b^1b^1) and an intermediate band and a slow band of lesser intensity. Each of these light bands represents the expression of a single codominant allele (b^2 and b^3).

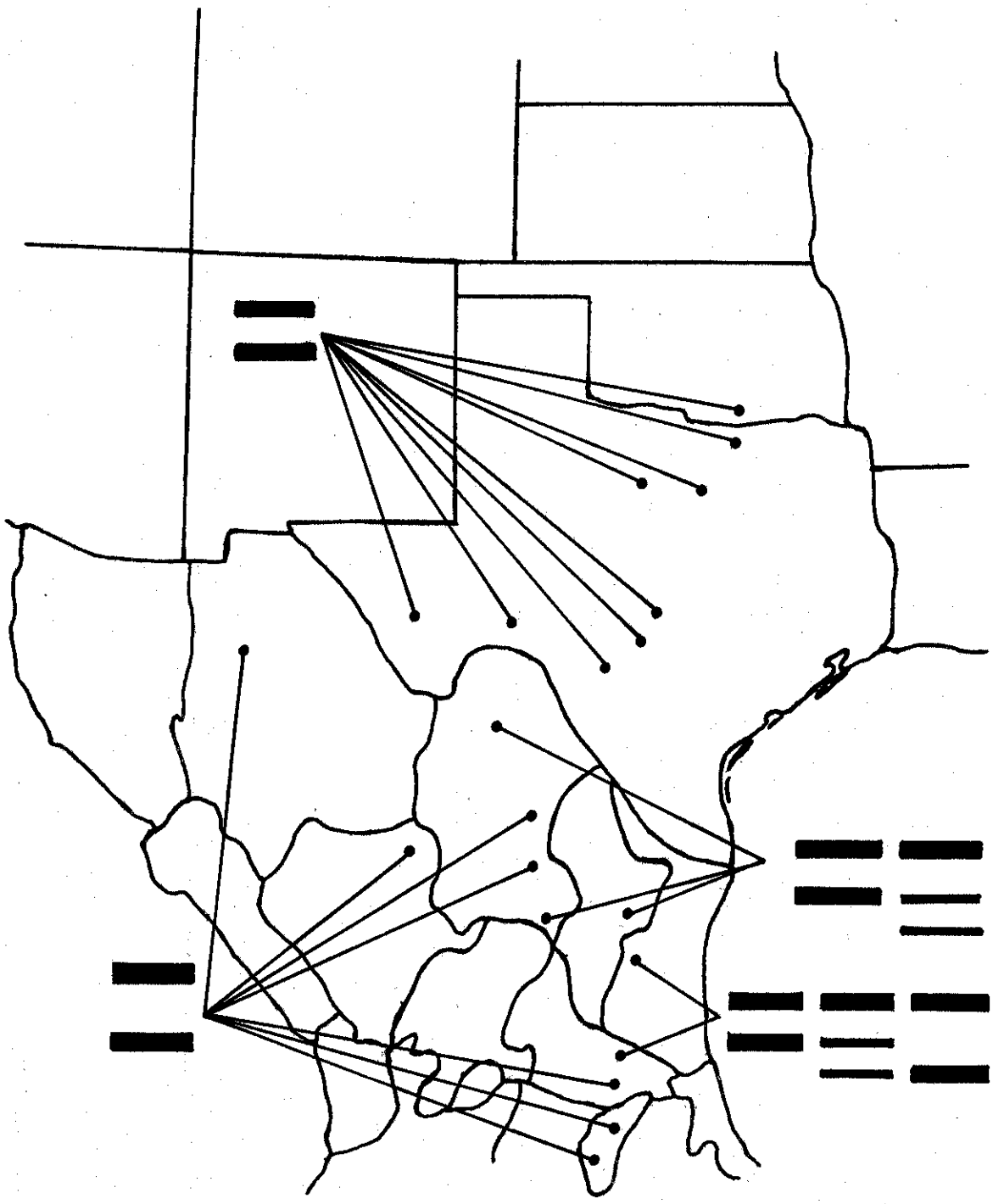
The other three patterns were each represented by a single specimen. Pattern D consisted of the intermediate-migrating band (b^2b^2) and the slow-migrating band (b^3b^3) and was found in a specimen from 20 mi. SE Saltillo, Coahuila. The fifth pattern (E) consisted of three bands, an intense, slow-migrating band (b^3b^3), a light intermediate-migrating

band (b^2), and a light fast-migrating band (b^3) and was found in a specimen from El Salto Falls, San Luis Potosi. The difference in intensity of bands suggests a genetic dosage effect. This would be the expected pattern produced from a mouse with a D pattern crossed with a mouse with a B pattern. The sixth pattern (F) consisted of a single intermediate-migrating band (b^2b^2) and was observed in a specimen from 1 mi. W Bermejillo, Durango. This pattern could be explained by a series of crosses and back-crosses, but the intermediate forms were not observed.

Attempts to separate the quaternary structure of the hemoglobin into alpha and beta subunits by dialysis in 8.0 M urea and 35% acetic acid or electrophoresis on an acid gel, pH 1.6 (Rasmussen et al., 1968), have been unsuccessful. Rasmussen et al., (1968) reported identical patterns of two bands on acid gels for three hemoglobin phenotypes in P. maniculatus. He suggested that the multiple hemoglobins are polymers of tetrameric molecules as reported in the house mouse (Riggs, 1965), or the subunits exhibit similar migration properties at pH 1.6 but form tetramers or dimers which migrate differently.

The distribution of hemoglobins in P. pectoralis (Fig. 14) illustrate a pattern of fixation of the hemoglobin pattern (A) in populations from southern Oklahoma and Texas. The B pattern was fixed in populations from southern and western

Fig. 14--Geographic variation of hemoglobin phenotypes in populations of Peromyscus pectoralis.



Mexico, while five populations from northeastern Mexico were polymorphic at one or more of the hemoglobin loci (Table 5). The distribution of hemoglobin patterns suggests gene flow between northern and southern populations through northeastern Mexico.

Two other electrophoretic patterns were observed within the boylii species group. The first pattern (Figure 13; D) was fixed in all populations of P. boylii and the population of P. polius. This pattern consisted of the intermediate-migrating band (b^2b^2) and the slow-migrating band (b^3b^3). A single specimen of P. pectoralis was also observed with this phenotype.

Jensen (1970) reported a total of five hemoglobin phenotypes in populations of P. boylii. Polymorphism was described in only one population from Dos Cabezas Mountains in Arizona, with the presence of a distinctive phenotype only in this population, which allowed positive detection of heterozygotes. The two other phenotypes were observed in one individual from the Kaibab Plateau of northern Arizona and in two offspring from a female trapped near Flagstaff. Jensen (1970) postulated that at least four loci, three of which may be segregating, were necessary to explain the phenotypes he observed in the populations of P. boylii rowleyi. Polymorphism was not observed in the populations of P. boylii sampled, and hemoglobins of this species could be encoded by a minimum of three loci, two of which may be segregating.

Table 5. - Hemoglobin genotypic frequencies of polymorphic population of Peromyscus pectoralis

Population	Beta-I			Beta-II		
	B^1B^1	B^1B^2	B^2B^2	B^2B^2	B^2B^3	B^3B^3
25 mi. SE Ocampo, Coa.	1.00	----	----	----	0.25	0.75
20 mi. SE Saltillo, Coa.	0.75	----	0.25	----	0.25	0.75
14 mi. W Linares, N.L.	1.00	----	----	----	0.25	0.75
1 mi. W Bermejillo, Dur.	0.75	----	0.25	0.25	----	0.75
19 mi. SE Jaumave, Tam.	1.00	----	----	0.33	0.33	0.33
El Salto, S.L.P.	0.75	0.25	----	0.50	----	0.50

A final pattern (G) was observed to be fixed in all populations of P. attwateri. This pattern consisted of four electrophoretic bands, two major and two minor. The major bands corresponded to the fast-migrating band (b^1b^1) and the slow-migrating band (b^3b^3) of P. pectoralis. The presence of minor bands was observed consistently only in populations of P. attwateri. Whether these bands function during embryological development, as do trace hemoglobins in humans, is currently under study.

Malate Dehydrogenase. --Two forms of this enzyme have been demonstrated in various rodents including heteromyids (Johnson and Selander, 1971), geomyids (Patton et al., 1972) and murids (Selander et al., 1971; Smith et al., 1973; Johnson et al., 1972; Thorne, 1960; Shows et al., 1970). Both forms are monomorphic and demonstrate no variation either geographically or interspecifically in the P. boylii species group.

Lactate Dehydrogenase. --Variation in lactate dehydrogenase in rodents is controlled by three loci, A and B loci expressed in most tissues, and a C locus expressed only in the testes (Goldberg and Hawtrey, 1967). The loci are designated Ldh-1, Ldh-2, and Ldh-3, respectively. Polypeptides produced by the Ldh-1 and Ldh-2 loci combine to form a tetramere to produce a five-banded electrophoretic pattern in

rodents as well as other vertebrates (Markert, 1968). The Ldh-3 polypeptides also form tetramers, but do not combine with polypeptides produced by Ldh-1 or Ldh-2 (Selander, 1971).

The a allele of the Ldh-1 locus (Fig. 15), which produced a slow-migrating electrophoretic band, was the most common allele of this group, and was fixed in all populations of P. boylii, P. pectoralis, and P. attwateri sampled (Table 6). The b allele was fixed in the population of P. polius. The Ldh-2 locus was monomorphic in all populations and demonstrated no variation either geographic or interspecific. A total of four alleles were observed at the Ldh-3 locus (Fig. 19). The a allele was fixed in all populations of P. boylii. This allele also occurred in low or equal frequencies in several populations of P. pectoralis, where the b allele was generally predominant (Table 6). The b allele was fixed in most populations of P. pectoralis. Polymorphism was observed in only five populations with the heterozygote pattern consisting of five electrophoretic bands (Fig. 15). The c allele was fixed in populations of P. attwateri sampled. In the population of P. polius the d allele was fixed.

Esterase.--Considerable variation was observed in the esterases; however, due to difficulties in establishing homologies between species analysis was limited to four loci.

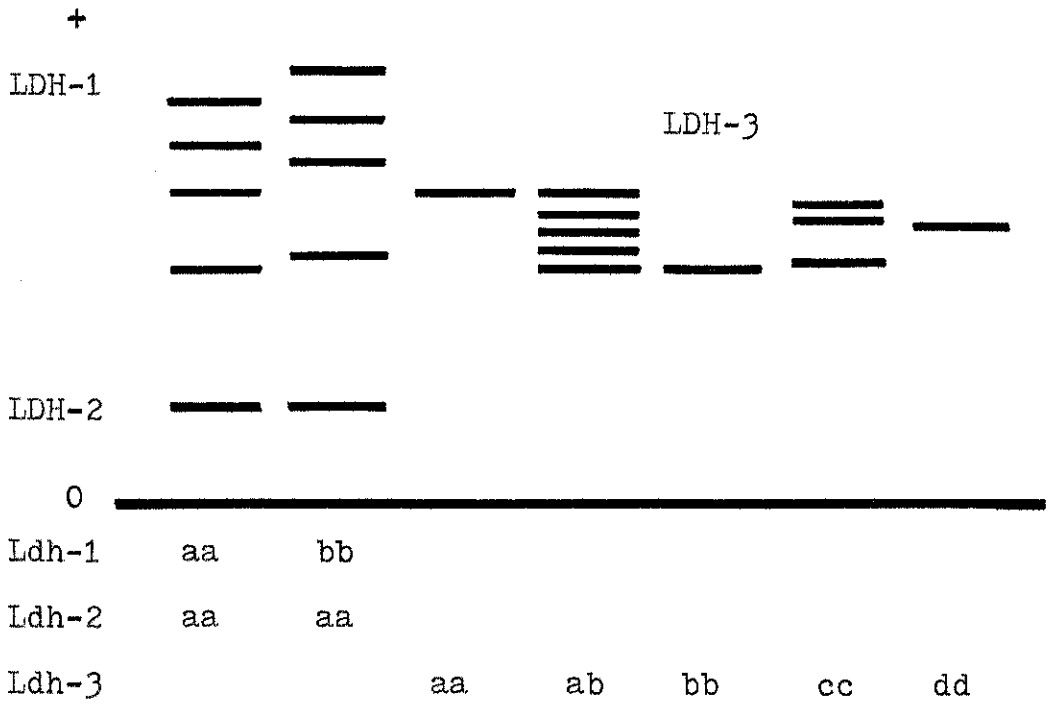


Fig. 15--Electrophoretic variation in lactate dehydrogenase in kidney (LDH-1 and LDH-2) and testis (LDH-3) extracts of the boylii species group.

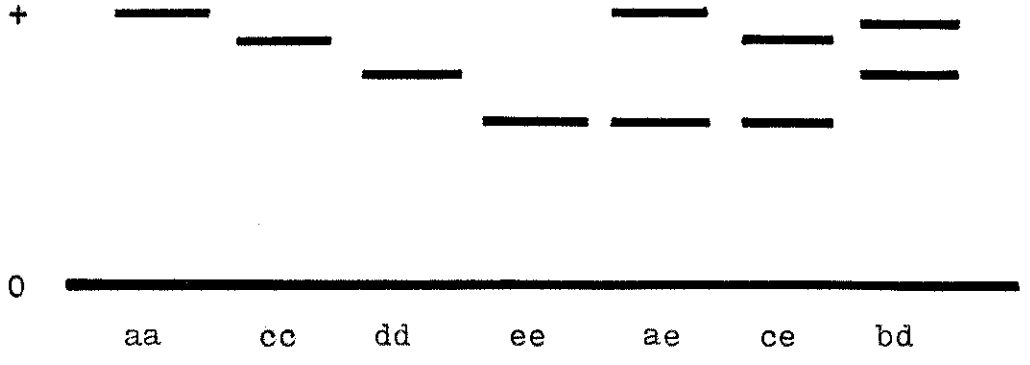


Fig. 16--Electrophoretic variation of the hemolysate esterase (Es-1).

Table 6. - Allelic frequency and genetic variation of the lactate dehydrogenase loci in populations of the Peromyscus boylii species group

	Lactate Dehydrogenase						
	LDH-1		LDH-2		LDH-3		
	a	b	a	a	b	c	d
Peromyscus attwateri							
Chautauqua Co., Kan. (56)	1.00	----	1.00	----	----	1.00	----
Comanche Co., Okla. (7)	1.00	----	1.00	----	----	1.00	----
Montague Co., Tex. (2)	1.00	----	1.00	----	----	1.00	----
Cottle Co., Tex. (48)	1.00	----	1.00	----	----	1.00	----
Knox Co., Tex. (3)	1.00	----	1.00	----	----	1.00	----
Johnson Co., Tex. (2)	1.00	----	1.00	----	----	1.00	----
Garza Co., Tex. (8)	1.00	----	1.00	----	----	1.00	----
Kerr Co., Tex. (3)	1.00	----	1.00	----	----	1.00	----
Peromyscus boylii utahensis							
Garfield Co., Utah (7)	1.00	----	1.00	1.00	----	----	----
Peromyscus boylii rowleyi							
Cimmaron Co., Okla. (4)	1.00	----	1.00	1.00	----	----	----
Bernalillo Co., N.M. (12)	1.00	----	1.00	1.00	----	----	----
Catron Co., N.M. (4)	1.00	----	1.00	1.00	----	----	----
Grant Co., N.M. (21)	1.00	----	1.00	1.00	----	----	----
Jeff Davis Co., Tex. (13)	1.00	----	1.00	1.00	----	----	----
Buena Ventura, Chi. (15)	1.00	----	1.00	1.00	----	----	----
Celulosa, Chi. (23)	1.00	----	1.00	1.00	----	----	----
Santa Barbara, Chi. (12)	1.00	----	1.00	1.00	----	----	----

Table 6.- (Continued)

	Lactate Dehydrogenase						
	LDH-1		LDH-2		LDH-3		
	a	b	a	a	b	c	d
Peromyscus boylii ambiguus							
Cd. Victoria, Tam. (6)	1.00	----	1.00	1.00	----	----	----
Peromyscus boylii spicilegus							
Coyote, Durango (4)	1.00	----	1.00	1.00	----	----	----
Peromyscus boylii levipes							
Metepec, Hidalgo (3)	1.00	----	1.00	1.00	----	----	----
Las Vigas, Veracruz (3)	1.00	----	1.00	1.00	----	----	----
Pinal de Amoles, Que.(3)	1.00	----	1.00	1.00	----	----	----
Peromyscus polius							
Santa Barbara, Que. (5)	----	1.00	1.00	----	----	----	1.00
Peromyscus pectoralis laceianus							
Love Co., Okla. (11)	1.00	----	1.00	0.25	0.75	----	----
Cooke Co., Tex. (7)	1.00	----	1.00	----	1.00	----	----
Eastland Co., Tex. (10)	1.00	----	1.00	----	1.00	----	----
Throckmorton Co., Tex. (12)	1.00	----	1.00	----	1.00	----	----
Mason Co., Tex. (3)	1.00	----	1.00	----	1.00	----	----
Kerr Co., Tex. (2)	1.00	----	1.00	----	1.00	----	----
Uvalde Co., Tex. (2)	1.00	----	1.00	----	1.00	----	----
Jeff Davis Co., Tex. (6)	1.00	----	1.00	0.50	0.50	----	----
Jeff Davis Co., Tex. (3)	1.00	----	1.00	0.25	0.75	----	----
Buena Ventura, Chi. (2)	1.00	----	1.00	----	1.00	----	----
Ocampo, Coa. (4)	1.00	----	1.00	----	1.00	----	----

Table 6. - (Continued)

	Lactate Dehydrogenase						
	LDH-1		LDH-2		Ldh-3		
	a	b	a	a	b	c	d
42 mi. N. Saltillo, Coa. (1)	1.00	----	1.00	0.50	0.50	----	----
12 mi. N. Saltillo, Coa. (6)	1.00	----	1.00	0.50	0.50	----	----
20 mi. SE Saltillo, Coa. (4)	1.00	----	1.00	----	1.00	----	----
Linares, Nuevo Leon (4)	1.00	----	1.00	----	1.00	----	----
Peromyscus pectoralis pectoralis							
Bermejillo, Durango (4)	1.00	----	1.00	----	1.00	----	----
Hacienda, Que. (4)	1.00	----	1.00	----	1.00	----	----
Arroyo Seca, Que. (2)	1.00	----	1.00	----	1.00	----	----
Peromyscus pectoralis collinus							
Jaumave, Tam. (3)	1.00	----	1.00	----	1.00	----	----
El Salto, San Luis Potosi (4)	1.00	----	1.00	----	1.00	----	----
Cd. Valles, San Luis Potosi (4)	1.00	----	1.00	----	1.00	----	----

Esterase-1.--Selander et al. (1971) described two prominent esterase systems which were demonstrated regularly from hemolysates electrophoresed on a tris-hydrochloric gel buffer in P. polionotus. The polymorphism of Es-1 was interpreted as the product of five alleles segregating at a single locus in P. polionotus. Heterozygotes have two bands, suggesting that the protein is a monomer.

In a study of erythrocyte esterase in P. maniculatus gracilis from Michigan, Randerson (1965) described polymorphism and interpreted variation as the product of three alleles segregating at a single locus. One of the alleles described in the Michigan study was a "null" or "silent" allele which did not produce an electrophoretic band, and progeny tests confirmed this interpretation. Selander et al. (1971) suggested that the locus controlling the production of Es-1 in P. polionotus is homologous to that described by Randerson (1965) for P. maniculatus. No evidence for a "silent" allele was found in P. polionotus by Selander et al. (1971).

In the P. boylii species group, polymorphism, geographic variation, and interspecific variation were observed at the Es-1 locus (Table 7). This variation was interpreted as the product of five alleles segregating at a single locus with the presence of a "silent" allele (Fig. 16). This allele, f, appears to be fixed in populations of P. boylii P. attwateri, and P. polius, with no esterase bands

Table 7. - Genetic variation and allelic frequency of the hemolysate esterase-1 locus in the Peromyscus boylii species group.

Population	Hemolysate Esterases Esterase-1 a	b	c	d	e	f
Peromyscus attwateri						
Chautauqua Co., Kan. (56)	----	----	----	----	----	1.00
Comanche Co., Okla. (7)	----	----	----	----	----	1.00
Montague Co., Tex. (2)	----	----	----	----	----	1.00
Cottle Co., Tex. (48)	----	----	----	----	----	1.00
Knox Co., Tex. (3)	----	----	----	----	----	1.00
Johnson Co., Tex. (2)	----	----	----	----	----	1.00
Garza Co., Tex. (8)	----	----	----	----	----	1.00
Kerr Co., Tex. (3)	----	----	----	----	----	1.00
Peromyscus boylii utahensis						
Garfield Co., Utah (7)	----	----	----	----	----	1.00
Peromyscus boylii rowleyi						
Cimmaron Co., Okla. (4)	----	----	----	----	----	1.00
Bernalillo Co., N.M. (12)	----	----	----	----	----	1.00
Catron Co., N.M. (4)	----	----	----	----	----	1.00
Grant Co., N.M. (21)	----	----	----	----	----	1.00
Jeff Davis Co., Tex. (13)	----	----	----	----	----	1.00
Buena Ventura, Chi. (15)	----	----	----	----	----	1.00
Celulosa, Chi. (23)	----	----	----	----	----	1.00
Santa Barbara, Chi. (12)	----	----	----	----	----	1.00

Table 7. - (continued)

	Hemolysate Esterases Esterase-1					
	a	b	c	d	e	f
Peromyscus boylii ambiguus						
Cd. Victoria, Ta. (6)	----	----	----	----	----	1.00
Peromyscus boylii spicilegus						
Coyote, Durango (4)	----	----	----	----	----	1.00
Peromyscus boylii levipes						
Metepec, Hidalgo (3)	----	----	----	----	----	1.00
Las Vigas, Veracruz (3)	----	----	----	----	----	1.00
Pinal de Amoles, Que. (3)	----	----	----	----	----	1.00
Peromyscus polius						
Santa Barbara, Chi. (5)	----	----	----	----	----	1.00
Peromyscus pectoralis laceianus						
Love Co., Okla. (11)	----	----	----	1.00	----	----
Cooke Co., Tex. (7)	----	0.17	----	0.83	----	----
Eastland Co., Tex. (10)	----	0.10	----	0.90	----	----
Throckmorton Co., Tex. (12)	----	----	----	1.00	----	----
Mason Co., Tex. (3)	1.00	----	----	----	----	----
Kerr Co., Tex. (2)	1.00	----	----	----	----	----
Uvalde Co., Tex. (2)	1.00	----	----	----	----	----
Jeff Davis Co., Tex. (6)	0.40	----	----	----	0.60	----
Jeff Davis Co., Tex. (3)	0.33	----	----	----	0.67	----
Buena Ventura, Chi. (2)	----	----	----	----	----	1.00
Ocampo, Coa. (4)	----	----	0.25	----	----	0.75

Table 7.- (continued)

	Hemolysate Esterases Esterase-1					
	a	b	c	d	e	f
42 mi. N. Saltillo, Coa. (1)	----	----	1.00	----	----	----
12 mi. N. Saltillo, Coa. (6)	----	----	0.80	----	0.20	----
20 mi. SE Saltillo, Coa. (4)	----	----	0.25	----	----	0.75
Linares, Nuevo Leon (4)	----	----	0.50	----	----	0.50
Peromyscus pectoralis pectoralis						
Bermejillo, Durango (4)	----	----	0.75	----	0.25	----
Hacienda, Que. (4)	----	----	1.00	----	----	----
Arroyo Seca, Que. (2)	----	----	1.00	----	----	----
Peromyscus pectoralis collinus						
Jaumave, Tam. (3)	----	----	0.33	----	----	0.67
El Salto, San Luis Potosi (4)	----	----	1.00	----	----	----
Cd. Valles, San Luis Potosi (4)	----	----	1.00	----	----	----

staining anodally to the hemoglobin. A compound band, consisting of a major band and two or three sub-bands, stained in most specimens of P. boylii and P. attwateri in the area of the fast hemoglobin band. No interpretation was made of this band other than exclusion from control by the Es-1 locus.

In populations of P. pectoralis considerable allelic variation (Table 7) and polymorphism (Table 8) were observed at the Es-1 locus. The a allele, producing the fastest migrating band (Fig. 16), was predominant or fixed in populations of the Edwards Plateau and the Davis Mts. of Texas. The c allele was common or fixed in most Mexican populations of P. pectoralis and was present in all but one Mexican population (Buena Ventura, Chihuahua). The d allele was predominant or fixed in populations of P. pectoralis from north-central Texas and southern Oklahoma. The e allele was found only in populations in the Davis Mts. of Texas and western Mexico. The f allele, the silent allele, occurred in a small sample from Chihuahua and several populations in north-central Texas.

Esterase-5. --The most anodal migrating esterase (Es-5) (Selander et al., 1971) in the plasma was observed to be polymorphic in a few populations of P. boylii and P. pectoralis. Three alleles segregate at this locus (Figure 17), with the b allele being the most predominant. This allele was fixed in populations of P. attwateri, P. boylii utahensis, P. boylii spicilegus, and P. boylii levipes. The a allele

Table 8. - Polymorphism and geographic variation at the esterase-1 locus in twenty-one populations of Peromyscus pectoralis

Population	aa	cc	dd	ee	bd	ae	ce	ff
Peromyscus pectoralis laceianus								
4. Love Co., Okla. (11)	----	----	1.00	----	----	----	----	----
5. Cooke Co., Tex. (7)	----	----	0.67	----	0.33	----	----	----
7. Eastland Co., Tex. (10)	----	----	0.80	----	0.20	----	----	----
20. Throckmorton Co., Tex. (12)	----	----	1.00	----	----	----	----	----
11. Mason Co., Tex. (3)	1.00	----	----	----	----	----	----	----
12. Kerr Co., Tex. (3)	1.00	----	----	----	----	----	----	----
13. Uvalde Co., Tex. (2)	1.00	----	----	----	----	----	----	----
22. Jeff Davis Co., Tex. (5)	0.17	----	----	0.50	----	0.33	----	----
23. Jeff Davis Co., Tex. (3)	----	----	----	0.33	----	0.67	----	----
28. Buena Ventura, Chi. (2)	----	----	----	----	----	----	----	1.00
33. Ocampo, Coa. (4)	----	0.25	----	----	----	----	----	0.75
34. 42 mi. N. Saltillo, Coa. (1)	----	1.00	----	----	----	----	----	----
35. 12 mi. N. Saltillo, Coa. (5)	----	0.60	----	----	----	----	0.40	----
36. 20 mi. SE Saltillo, Coa.(4)	----	0.25	----	----	----	----	----	0.75
37. Linares, Nuevo Leon (4)	----	0.50	----	----	----	----	----	0.50

Table 8. - (continued)

Population	aa	cc	dd	ee	bd	ae	ce	ff
Peromyscus pectoralis pectoralis								
31. Bermejillo, Durango (4)	----	0.75	----	0.25	----	----	----	----
42. Hacienda, Queretaro (4)	----	1.00	----	----	----	----	----	----
43. Arroyo Seca, Queretaro (2)	----	1.00	----	----	----	----	----	----
Peromyscus pectoralis collinus								
38. Jaumave, Tam. (3)	----	0.33	----	----	----	----	----	0.67
40. El Salto, San Luis Potosi (4)	----	1.00	----	----	----	----	----	----
41. Cd. Valles, San Luis Potosi (4)	----	1.00	----	----	----	----	----	----

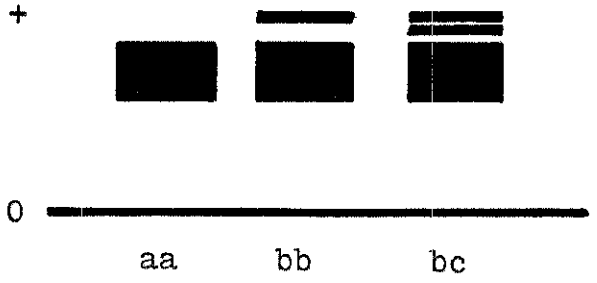


Fig. 17--Electrophoretic variation of serum esterase (Es-5).

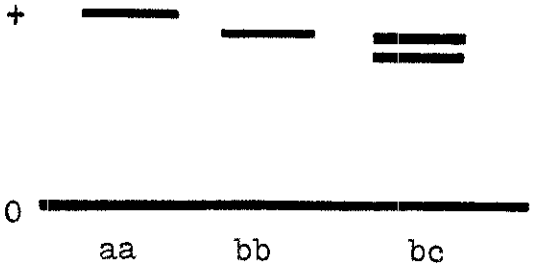


Fig. 18--Electrophoretic variation of esterase-6 within the boylii group.

was observed in eight populations (Table 9) of P. boylii rowleyi, P. boylii ambiguus, and P. pectoralis and was fixed in a single population of P. boylii rowleyi from the Oklahoma panhandle. The c allele was observed in only four populations of the boylii species group (Table 9), including two of P. boylii rowleyi and one each of P. pectoralis and P. polius.

Esterase-6. --The most anodally migrating erythrocytic esterase (Es-6) (Selander et al., 1971) on tris-maleic gels is polymorphic in P. boylii and P. attwateri and monomorphic in P. pectoralis. Three alleles were detected at the Es-6 locus, with the b allele (Fig. 18) being predominant or fixed in most populations (Table 9). The a allele was observed in populations of P. attwateri, P. boylii utahensis and P. boylii rowleyi (Table 9). The c allele was a minor allele in P. polius having a frequency of 0.10. The b allele was fixed in all populations of P. pectoralis.

Esterase-10. --The slowest erythrocytic esterase on tris-maleic gels (Es-10), migrating only a short distance anodally from the origin, was polymorphic in populations of P. boylii and P. attwateri but does not appear in hemolysate from P. pectoralis. This esterase may correspond to the Es-7 but did not stain lightly nor did it appear on Tris-Hcl gels as reported by Selander et al. (1971). Five alleles were detected at the Es-10 locus (Fig. 19) of populations of P. boylii and P. attwateri, with the a

Table 9. - Allelic frequencies and genetic variation at the esterase-5 and 6 loci of mice of the Peromyscus boylii species group

Population	Esterase-5 a	b	c	Esterase-6 a	b	c
Peromyscus attwateri						
Chautauqua Co., Kan. (56)	----	1.00	----	----	1.00	----
Comanche Co., Okla. (7)	----	1.00	----	----	1.00	----
Montague Co., Tex. (2)	----	1.00	----	0.50	0.50	----
Cottle Co., Tex. (48)	----	1.00	----	0.05	0.95	----
Knox Co., Tex. (3)	----	1.00	----	----	1.00	----
Johnson Co., Tex. (2)	----	1.00	----	----	1.00	----
Garza Co., Tex. (8)	----	1.00	----	0.33	0.67	----
Kerr Co., Tex. (3)	----	1.00	----	----	1.00	----
Peromyscus boylii utahensis						
Garfield Co., Utah (7)	----	1.00	----	0.50	0.50	----
Peromyscus boylii rowleyi						
Cimmaron Co., Okla. (4)	1.00	----	----	----	1.00	----
Bernalillo Co., N.M. (12)	----	1.00	----	----	1.00	----
Catron Co., N.M. (4)	----	1.00	----	----	1.00	----
Grant Co., N.M. (21)	0.20	0.73	0.07	0.07	0.93	----
Jeff Davis Co., Tex. (13)	0.12	0.88	----	0.25	0.75	----
Buena Ventura, Chi. (15)	----	1.00	----	----	1.00	----
Celulosa, Chi. (23)	----	1.00	----	----	1.00	----
Santa Barbara, Chi. (12)	0.08	0.88	0.04	0.08	0.92	----

Table 9. - (continued)

	Esterase-5			Esterase-6		
	a	b	c	a	b	c
Peromyscus boylii ambiguus						
Cd. Victoria, Tam (6)	0.08	0.92	----	----	1.00	----
Peromyscus boylii spicilegus						
Coyote, Durango (4)	----	1.00	----	----	1.00	----
Peromyscus boylii levipes						
Metepec, Hidalgo (3)	----	1.00	----	----	1.00	----
Las Vigas, Veracruz (3)	----	1.00	----	----	1.00	----
Pinal de Amoles, Que. (3)	----	1.00	----	----	1.00	----
Peromyscus polius						
Santa Barbara, Chi. (5)	----	0.70	0.30	----	0.90	0.10
Peromyscus pectoralis laceianus						
Love Co., Okla (11)	----	1.00	----	----	1.00	----
Cooke Co., Tex. (7)	----	1.00	----	----	1.00	----
Eastland Co., Tex. (10)	----	1.00	----	----	1.00	----
Throckmorton Co., Tex. (12)	----	1.00	----	----	1.00	----
Mason Co., Tex. (3)	----	1.00	----	----	1.00	----
Kerr Co., Tex. (2)	----	1.00	----	----	1.00	----
Uvalde Co., Tex. (2)	----	1.00	----	----	1.00	----
Jeff Davis Co., Tex. (6)	----	1.00	----	----	1.00	----
Jeff Davis Co., Tex. (3)	----	1.00	----	----	1.00	----
Buena Ventura, Chi. (2)	----	1.00	----	----	1.00	----
Ocampo, Coa. (4)	----	1.00	----	----	1.00	----

Table 9. - (Continued)

	Esterase-5			Esterase-6		
	a	b	c	a	b	c
42 mi. N. Saltillo, Coa. (1)	----	1.00	----	----	1.00	----
12 mi. N. Saltillo, Coa. (6)	0.10	0.90	----	----	1.00	----
20 mi. SE Saltillo, Coa. (4)	0.12	0.88	----	----	1.00	----
Linares, Nuevo Leon (4)	----	1.00	----	----	1.00	----
Peromyscus pectoralis pectoralis						
Bermejillo, Durango (4)	----	0.75	0.25	----	1.00	----
Hacienda, Que. (4)	----	1.00	----	----	1.00	----
Arroyo Seca, Que. (2)	----	1.00	----	----	1.00	----
Peromyscus pectoralis collinus						
Jaumave, Ta. (3)	0.67	0.33	----	----	1.00	----
El Salto, San Luis Potosi (4)	----	1.00	----	----	1.00	----
Cd. Valles, San Luis Potosi (4)	----	1.00	----	----	1.00	----

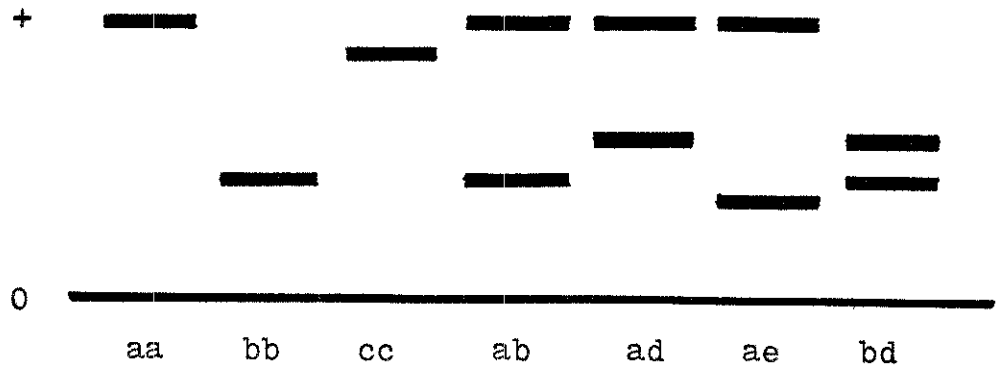


Fig. 19--Electrophoretic variation of esterase-10 within populations of Peromyscus boylii and Peromyscus attwateri.

allele predominant or fixed in most populations (Table 10). The b allele was the second most common allele and the only other allele observed in P. attwateri. The other three alleles (c,d,e) occurred at low frequencies (less than 0.10) in populations of P. boylii rowleyi from Chihuahua (Table 10). The c allele occurred at a frequency of 0.20 in P. polius. This esterase was absent in P. pectoralis and the fixation of the silent allele, f, was assumed for analysis of genetic similarity.

Genetic Variability

Genetic variability within a population can be expressed in several ways: the proportion of populations which are polymorphic for each locus, the total number of alleles detected at each locus, and the mean number of alleles segregating for each polymorphic population. These indicies of genetic variability for the species of the P. boylii group are presented in Tables 11, 12, and 13. Seven of the 17 structural loci were observed to be polymorphic in populations of P. boylii (Table 11), while nine loci were observed to be polymorphic in populations of P. pectoralis (Table 12). A single variant was observed in P. pectoralis at the Got-1 locus, but to prevent undue bias by considering this allele to be established in a species when it may represent a mutation which would not continue in the gene pool, this locus was arbitrarily considered to be monomorphic. Only two of the 17

Table 10. - Allelic frequencies and genetic variation at the esterase-10 locus of mice of the *Peromyscus boylii* species group

Population	Esterase-10 a	b	c	d	e	f
Peromyscus attwateri						
Chautauqua Co., Kan. (56)	1.00	----	----	----	----	----
Comanche Co., Okla. (7)	1.00	----	----	----	----	----
Montague Co., Tex. (2)	0.50	0.50	----	----	----	----
Cottle Co., Tex. (48)	1.00	----	----	----	----	----
Knox Co., Tex. (3)	0.83	0.17	----	----	----	----
Johnson Co., Tex. (2)	1.00	----	----	----	----	----
Garza Co., Tex. (8)	0.50	0.50	----	----	----	----
Kerr Co., Tex. (3)	0.67	0.33	----	----	----	----
Peromyscus boylii utahensis						
Garfield Co., Utah (7)	1.00	----	----	----	----	----
Peromyscus boylii rowleyi						
Cimmaron Co., Okla. (4)	0.75	0.25	----	----	----	----
Bernalillo Co., N.M. (12)	0.68	0.32	----	----	----	----
Catron Co., N.M. (4)	1.00	----	----	----	----	----
Grant Co., N.M. (21)	0.72	0.28	----	----	----	----
Jeff Davis Co., Tex. (13)	0.37	0.63	----	----	----	----
Buena Ventura, Chi. (15)	0.64	0.18	0.09	0.045	0.045	----
Celulosa, Chi. (23)	0.59	0.35	----	0.02	0.04	----
Santa Barbara, Chi. (12)	0.71	0.21	0.08	----	----	----

Table 10. - (continued)

Population	Esterase-10 a	b	c	d	e	f
Peromyscus boylii ambiguus						
Cd. Victoria, Tam (6)	0.75	0.25	----	----	----	----
Peromyscus boylii spicilegus						
Coyote, Durango (4)	1.00	----	----	----	----	----
Peromyscus boylii levipes						
Metepec, Hidalgo (3)	1.00	----	----	----	----	----
Las Vigas, Veracruz (3)	1.00	----	----	----	----	----
Pinal de Amoles, Que. (3)	1.00	----	----	----	----	----
Peromyscus polius						
Santa Barbara, Chi. (5)	0.50	0.30	0.20	----	----	----
Peromyscus pectoralis laceianus						
Love Co., Okla. (11)	----	----	----	----	----	1.00
Cooke Co., Tex. (7)	----	----	----	----	----	1.00
Eastland Co., Tex. (10)	----	----	----	----	----	1.00
Throckmorton Co., Tex. (12)	----	----	----	----	----	1.00
Mason Co., Tex. (3)	----	----	----	----	----	1.00
Kerr Co., Tex. (2)	----	----	----	----	----	1.00
Uvalde Co., Tex. (2)	----	----	----	----	----	1.00
Jeff Davis Co., Tex. (6)	----	----	----	----	----	1.00
Jeff Davis Co., Tex. (3)	----	----	----	----	----	1.00
Buena Ventura, Chi. (2)	----	----	----	----	----	1.00
Ocampo, Coa. (4)	----	----	----	----	----	1.00

Table 10. - (continued)

	Esterase-10					
	a	b	c	d	e	f
42 mi. N. Saltillo, Coa. (1)	----	----	----	----	----	1.00
12 mi. N. Saltillo, Coa. (6)	----	----	----	----	----	1.00
20 mi. SE Saltillo, Coa. (4)	----	----	----	----	----	1.00
Linares, Nuevo Leon (4)	----	----	----	----	----	1.00
Peromyscus pectoralis pectoralis						
Bermejillo, Durango (4)	----	----	----	----	----	1.00
Hacienda, Que. (4)	----	----	----	----	----	1.00
Arroyo Seca, Que. (2)	----	----	----	----	----	1.00
Peromyscus pectoralis collinus						
Jaumave, Tam. (3)	----	----	----	----	----	1.00
El Salto, San Luis Potosi (4)	----	----	----	----	----	1.00
Cd. Valles, San Luis Potosi (4)	----	----	----	----	----	1.00

Table 11. - Genetic variation in proteins of *Peromyscus boylii*

Protein	Phenotypic Variation	Proportion of Population polymorphic	Number of Alleles Total in species	Mean per polymorphic population
Hydrolases				
Esterase-1	Monomorphic		1	
Esterase-5	Polymorphic	0.29	3	2.50
Esterase-6	Polymorphic	0.29	2	2.00
Esterase-10	Polymorphic	0.57	5	2.75
Dehydrogenases				
Lactate Dehydrogenase				
LDH-1	Monomorphic		1	
LDH-2	Monomorphic		1	
LDH-3	Monomorphic		1	
Malate Dehydrogenase				
MDH-1	Monomorphic		1	
MDH-2	Monomorphic		1	
6-Phosphogluconate Dehydrogenase	Polymorphic	0.14	2	2.00
Transaminases				
Glutamate oxalate Transaminase				
GOT-1	Polymorphic	0.14	2	2.00
GOT-2	Monomorphic		1	
Nonenzymatic Proteins				
Hemoglobin				
Alpha	Monomorphic		1	
Beta-1	Monomorphic		1	
Beta-2	Monomorphic		1	
Albumin	Polymorphic	0.43	2	2.00
Transferrin	Polymorphic	0.21	2	2.00

Table 12. - Genetic variation in proteins of *Peromyscus pectoralis*

Protein	Phenotypic Variation	Proportion of Population polymorphic	Number of Alleles: Total in species	Number of Alleles: Mean per polymorphic population
Hydrolases				
Esterase-1	Polymorphic	0.48	6	2.00
Esterase-5	Polymorphic	0.19	3	2.00
Esterase-6	Monomorphic		1	
Esterase-10	Monomorphic		1	
Dehydrogenases				
Lactate Dehydrogenase				
LDH-1	Monomorphic		1	
LDH-2	Monomorphic		1	
LDH-3	Polymorphic	0.37	2	2.00
Malate Dehydrogenase				
MDH-1	Monomorphic		1	
MDH-2	Monomorphic		1	
6-Phosphogluconate Dehydrogenase	Polymorphic	0.10	2	2.00
Transaminases				
Glutamate oxalate Transaminase				
GOT-1	Polymorphic	0.14	2	2.00
GOT-2	Monomorphic		1	
Nonenzymatic Proteins				
Hemoglobin				
Alpha	Monomorphic		1	
Beta-1	Polymorphic	0.05	2	2.00
Beta-2	Polymorphic	0.29	2	2.00
Albumin	Polymorphic	0.24	2	2.00
Transferrin	Polymorphic	0.05	2	2.00

Table 13. - Genetic variation in proteins of *Peromyscus attwateri*

Protein	Phenotypic Variation	Proportion of Population polymorphic	Number of Alleles Total in species	Number of Alleles Mean per polymorphic population
Hydrolases				
Esterase-1	Monomorphic		1	
Esterase-5	Monomorphic		1	
Esterase-6	Polymorphic	0.63	2	2.00
Esterase-10	Polymorphic	0.50	2	2.00
Dehydrogenases				
Lactate Dehydrogenase				
LDH-1	Monomorphic		1	
LDH-2	Monomorphic		1	
LDH-3	Monomorphic		1	
Malate Dehydrogenase				
MDH-1	Monomorphic		1	
MDH-2	Monomorphic		1	
6-Phosphogluconate Dehydrogenase	Monomorphic		1	
Transaminases				
Glutamate oxalate Transaminase				
GOT-1	Monomorphic		1	
GOT-2	Monomorphic		1	
Nonenzymatic Proteins				
Hemoglobin				
Alpha	Monomorphic		1	
Beta-1	Monomorphic		1	
Beta-2	Monomorphic		1	
Albumin	Monomorphic		1	
Transferrin	Monomorphic		1	

loci were observed to be polymorphic in populations of P. attwateri (Table 13), with two large samples (N-46 and 56) being monomorphic at all loci. Five loci were found to be polymorphic in the single population of P. polius. Two alleles segregated at four loci (6-PGD, Trf, Es-5 and Es-6), while at the Esterase-10 locus, three alleles appeared to segregate.

Of the 17 loci, 6 were monomorphic and 11 were considered polymorphic in one or more populations, but no more than 5 loci were polymorphic in any one population ($\bar{x}$ number of polymorphic loci per population = 2.26). The lowest proportion of polymorphic loci (Table 14) was found in P. attwateri (range = 0.00 to 0.118; $\bar{x}$ = 0.059). Slightly higher proportions of polymorphic loci were found in P. boylii and P. pectoralis, ranging from 0.059 to 0.233 with means of 0.145 and 0.137, respectively. The greatest proportion of polymorphic loci was observed in P. polius with a value of 0.294.

Significant variation was demonstrated in the proportion of polymorphic loci among major regions inhabited by P. boylii rowleyi and P. pectoralis (Table 15). In Oklahoma, the average population of P. boylii was polymorphic at the 5.9% level while those from Mexico had a mean polymorphic level of 21.6%. The lowest mean level of polymorphic loci (1.3%) for populations of P. pectoralis was found on the Edwards

Table 14. - Genetic variation in populations of the *boylii* species group

Population	Proportion of Polymorphic Loci	Mean Heterozygosity	
		Allelic Frequencies	Direct Count
Peromyscus attwateri			
Chautauqua Co., Kan.	0.000	0.0000	0.0000
Comanche Co., Okla.	0.000	0.0000	0.0000
Montague Co., Tex.	0.118	0.0588	0.0196
Cottle Co., Tex.	0.059	0.0056	0.0000
Knox Co., Tex.	0.059	0.0166	0.0392
Johnson Co., Tex.	0.059	0.0294	0.0000
Garza Co., Tex.	0.118	0.0554	0.0196
Kerr Co., Tex.	0.059	0.0260	0.0196
Peromyscus boylii rowleyi			
Cimmaron Co., Okla.	0.059	0.0221	0.0294
Bernalillo Co., N.M.	0.118	0.0477	0.0481
Grant Co., N.M.	0.233	0.0794	0.0222
Jeff Davis Co., Tex.	0.233	0.0794	0.0430
Buena Ventura, Chi.	0.177	0.0514	0.0535
Celulosa, Chi.	0.233	0.0879	0.0639
Santa Barbara, Chi.	0.233	0.0522	0.0267
Peromyscus boylii ambiguus			
Cd. Victoria, Tam.	0.118	0.0307	0.0294

Table 14. - (continued)

Population	Proportion of Polymorphic Loci	Mean Heterozygosity: Allelic Frequencies	Mean Heterozygosity: Direct Count
Peromyscus boylii spicilegus			
Coyote, Durango	0.059	0.0124	0.0147
Peromyscus boylii levipes			
Metepec, Hidalgo	0.059	0.0294	0.0196
Pinal de Amoles, Que.	0.059	0.0294	0.0588
Peromyscus pectoralis laceianus			
Love Co., Okla.	0.177	0.0712	0.0614
Cooke Co., Tex.	0.118	0.0460	0.0448
Eastland Co., Tex.	0.118	0.0400	0.0389
Throckmorton Co.,Tex.	0.059	0.0294	0.0358
Kerr Co., Tex.	0.059	0.0294	0.0588
Jeff Davis Co., Tex.	0.177	0.0763	0.1176
Ocampo, Coa.	0.118	0.0344	0.0147
N Saltillo, Coa.	0.177	0.0588	0.0353
SE Saltillo, Coa.	0.177	0.0469	0.0294
Linares, N. L.	0.118	0.0418	0.0147
Peromyscus pectoralis pectoralis			
Bermejillo, Durango	0.233	0.0882	0.0000
Arroyo Seca, Que.	0.059	0.0221	0.0294
Peromyscus pectoralis collinus			
Jaumave, Tam.	0.233	0.1074	0.0196
El Salto, S. L. P.	0.118	0.0495	0.0147

Table 15. - Geographic variation of genetic variation in the boylii species group

Population	Mean Number of Loci Polymorphic per Population	Mean Proportion of Loci Polymorphic per Population	Mean Heterozygosity
P. boylii rowleyi			
Oklahoma	1.00	0.059	0.0294
New Mexico and Davis Mountains	3.33	0.196	0.0378
Chihuahua, Mexico	3.67	0.216	0.0480
P. pectoralis laceianus			
North-central Texas	2.00	0.118	0.0531
Edwards Plateau, Tex.	0.33	0.013	0.0588
Davis Mts., Texas	2.50	0.147	0.1176
Northern Mexico	2.50	0.147	0.0235
P. pectoralis pectoralis			
Western Mexico	2.50	0.147	0.0294
P. pectoralis collinus			
North-eastern Mexico	3.00	0.177	0.0172
P. attwateri			
Kansas	0.00	0.000	0.0000
Oklahoma	0.00	0.000	0.0000
Cross Timbers	1.50	0.089	0.0098
Llano Estacado	1.33	0.079	0.0294
Edwards Plateau	1.00	0.059	0.0196

Plateau. In north-central Texas and southern Oklahoma, the average population was polymorphic at 11.8% of the loci while populations from the Davis Mountains of Texas and populations from northern Mexico had the same mean value of 14.7%. Populations of P. pectoralis collinus from San Luis Potosi had a mean of 17.7% of the loci polymorphic.

Although these values represent estimates of genetic variation, the proportion of polymorphic loci is strongly dependent upon sample size and is, therefore, a relatively poor index of the degree of genetic variation in populations. A superior index has been provided by Lewontin and Hubby (1966), utilizing the proportion of loci which are heterozygous in the average individual of the population. This was estimated by calculating the expected frequencies of heterozygotes in a population from the Hardy-Weinberg equation, using direct counts of allele frequencies, summing the expected frequencies of heterozygotes for all loci, and dividing by the total number of loci, including monomorphic ones which contribute no heterozygosity. The heterozygosity of an average individual of a population (H) was also calculated by direct count of observed frequencies of heterozygosities at each locus, summation of the frequencies of heterozygotes, and division by the total number of loci. The number of loci which are polymorphic and the levels of heterozygosity by direct count (H) and calculation from allele frequencies for

35 populations are presented in Table 14. The variation between the two estimates of heterozygosity is probably due to the inability to detect heterozygotes at several polymorphic loci.

CHAPTER IV

DISCUSSION

Chromosomal Variation

Recent reports of chromosomal morphology of the genus Peromyscus (Hsu and Arrighi, 1968; Bradshaw and Hsu, 1972; Lee et al., 1972; Te and Dawson, 1971) indicate only slight variation in the number of biarmed autosomes within most species groups of the subgenus Peromyscus. This suggests a monophyletic origin of each species group, except the maniculatus and boylii species groups, which have 16 to 40 and 6 to 36 biarmed autosomes, respectively. This suggests a polyphyletic origin for members of each of these two groups.

Considerable chromosomal variation has been observed within the boylii species group (Lee et al., 1972 and Schmidly and Schroeter, 1973). The 11 distinct karyotypes can be divided into three major groups on the basis of the number of biarmed autosomes. Two forms have a large number of biarmed autosomes, P. polius (28 to 30) and P. boylii similus from San Blas, Nayarit (36) (Schmidly and Schroeter, 1973). Peromyscus evides and P. oaxacensis are reported to have an intermediate number of biarmed autosomes with 20 and 22, respectively (Schmidly and Schroeter, 1973). The other forms of the boylii species group which have been examined,

including P. pectoralis, P. attwateri and eight subspecies of P. boylii, have a low number of biarmed autosomes ranging from 6 to 14. The three groups formed may represent three evolutionary lines within the boylii species group.

Two species of the boylii group, for which large samples are available, exhibit little or no polymorphism or geographic variation in chromosomes. The 64 specimens of P. attwateri from a three state area demonstrate no obvious chromosomal variation and Lee et al. (1972) reported only a slight variation in the size of the X chromosomes in a single female P. attwateri. Specimens (n=58) of P. pectoralis from a wide area of Texas and northern Mexico exhibit no obvious chromosomal variation. In contrast, four of the six observed chromosomal variants of this study were among populations of P. boylii.

The extent of chromosomal variation between populations of P. boylii is equivalent to that observed between species of Sigmodon (Zimmerman, 1970), Neotoma (Baker and Mascarello, 1969) and other Peromyscus (Hsu and Arrighi, 1968). This would suggest that more than one gene pool or species is represented among populations of P. boylii. In contrast, genetic data obtained suggest the existence of gene flow between populations of P. boylii with markedly different karyotypes.

Genetic Variation

The estimates of heterozygosity for populations of the boylii species group are within the range of the estimates for other rodents (Selander, Hunt, and Yang, 1969; Johnson and Selander, 1971). The average value of H for the four species is 0.0439, indicating that only 4.39% of the 17 loci are heterozygous in an average individual. Heterozygosity varies from a low of 0.00 to 0.0392 in P. attwateri to a maximum of 0.0824 in P. polius.

Peromyscus attwateri exhibits much lower levels of heterozygosity than those reported in other species of Peromyscus. Smith et al. (1973) reported a range of heterozygosity in P. floridanus, a Pleistocene relic, of from 0.046 to 0.064, with a mean 0.053, and suggested P. floridanus to be one of the least variable species in the genus. In mainland populations of P. polionotus Selander et al. (1971) reported heterozygosity ranging from 0.0496 in South Carolina and Georgia to 0.086 in peninsular Florida. In isolated populations of P. polionotus inhabiting small barrier islands and peninsulas of the Gulf Coast of Florida, lower estimates of heterozygosity were obtained, ranging from 0.018 on islands to 0.033 on peninsulas, with a mean of 0.028. The only other rodents which are reported to have levels of heterozygosity lower than P. attwateri are a few species of Dipodomys (Johnson and Selander, 1971). Other reports of heterozygosity which are lower than the mean for P. attwateri are those of troglobitic

populations of the fish Astyanax mexicanus which lack genetic variability, although surface populations of the same species are highly variable with a range of 0.138 to 0.077 (Avise and Selander, 1972). Certain species of lizards of the genus Anolis inhabiting a small island in the Bahamas (South Bimini) have an absence of heterozygosity (A. angusticpes) or a reduction of heterozygosity with a value of 0.009 (A. sagrei) (Webster et al., 1973), while other species of Anolis inhabiting the same island have levels of heterozygosity ranging from 0.031 to 0.064 (Webster et al., 1973). Isolation appears to be one factor which might contribute to low levels of heterozygosity, but pocket gophers, genus Thomomys, which occur in small isolated continental populations, have levels of heterozygosity which are as high as those of P. polionotus and P. floridanus (Patton et al., 1972).

Johnson and Selander (1971) examined several possible relationships affecting degree of genetic variability in kangaroo rats. They observed that the major contribution of heterozygosity to a population came from one or two loci and concluded that sampling error might significantly affect estimates of genetic variability. Since statistical treatment to determine the significance of the observed interspecific variation was not possible, they assumed that much of the variation reflected some significant degree of interspecific variation in levels of heterozygosity. They concluded that

no relationship existed between degree of genetic variability and the extent of geographic range. For example, of the seven species of kangaroo rats exhibiting low levels of heterozygosity, three have small ranges, and four have extensive ranges including D. ordii, the most widely distributed member of the genus.

Although genetic variability cannot be explained on the basis of current geographic range, the past geographic distribution may offer a solution. Peromyscus attwateri presently occurs in disjunct populations in portions of five states (Hall and Kelson, 1959). The past distribution of P. attwateri is not known due to a total absence of fossil records, but the occurrance of P. boylii is reported from two Pleistocene faunas from the Edwards Plateau (Dalquest et al., 1969; Roth, 1973). It seems probable that these specimens are P. attwateri as this species is the only boylii-like form on the Edwards Plateau today. The absence of any form of P. boylii from Pleistocene faunas from sites in northwest Texas where P. attwateri occurs today suggests the isolation of P. attwateri on the Edwards Plateau during the late Pleisotcene (Dalquest, 1965, 1967). The low level of heterozygosity could have become randomly fixed (Sewell Wright Effect) in P. attwateri by isolation during much of the Pleisotcene, and the low heterozygosity was retained with subsequent expansion of the range during recent times.

The main contributors to the level of heterozygosity observed in P. attwateri are two esterase loci (Es-6, Es-10). Geographic distribution of esterase patterns observed indicates a general increase in heterozygosity of these loci with proximity to the Edwards Plateau (Table 15). Selander et al. (1971) noted that the degree of genetic variability in populations of P. polionotus decreased clinally northward, presumably due to northward colonization by small populations in recent times. This pattern would also fit P. attwateri, with high levels of heterozygosity in populations of the Edwards Plateau, and the level of heterozygosity decreasing clinally northward. One exception in this pattern was observed in populations on the escarpments of the Llano Estacado in western Texas, with levels of heterozygosity greater than that observed in populations on the Edwards Plateau (Table 15). This variation in degree of heterozygosity may reflect gene exchange with populations of P. boylii rowleyi during recent times, since the alleles which contribute to the heterozygosity of these populations of P. attwateri are found in the populations of P. boylii rowleyi in New Mexico and the Davis Mountains of Texas.

Another relationship explored by Johnson and Selander (1971) to account for the interspecific variation in degree of heterozygosity was adaptation in terms of habitat distribution and niche width. They reported a positive correlation

between heterozygosity and wide habitat tolerance (Johnson and Selander, 1971).

The low levels of heterozygosity may well explain the narrow habitat tolerance in P. attwateri. With overgrazing of the Edwards Plateau in recent times, P. pectoralis has replaced P. attwateri in many areas (Clark, 1952). The low degree of heterozygosity in P. attwateri and thus a low degree of genetic variability may prevent adaptation to such a change in habitat. Peromyscus pectoralis, with a considerably higher degree of heterozygosity (Table 15) and genetic variability, appears to adapt to such habitat changes and has displaced P. attwateri (Schmidly, 1971). Johnson and Selander (1971) reported species of kangaroo rats with low heterozygosity occurring in sympatry with species with typical levels, but the forms with low heterozygosity being restricted to optimum habitat. Schmidly (1971) reported P. pectoralis and P. attwateri occurring together on the Edwards Plateau primarily in areas with extensive vegetation, with optimum habitats occupied by P. attwateri.

Most populations of P. boylii exhibited ranges of heterozygosity which are consistent with those observed in other species of Peromyscus (Table 14). Three populations demonstrated low levels of heterozygosity, including P. boylii spicilegus from Coyote, Durango; P. boylii levipes from Metepec, Hidalgo; and P. boylii rowleyi from Grant County, New Mexico (Table 14). The reduced heterozygosity

in the populations of P. boylii spicilegus and P. boylii levipes may be the result of small sample size. The low heterozygosity in the population of P. boylii rowleyi from Grant County must reflect the genetic make-up of this population with four loci contributing to the heterozygosity in a sample of 15 mice collected over an 8 month period. The geographic variation in heterozygosity observed in populations of P. boylii rowleyi from Oklahoma, Davis Mountains of Texas, New Mexico and Chihuahua (Table 15) forms a north-south cline of increasing heterozygosity, with the most variable populations occurring in the mountains of Chihuahua, Mexico.

A wide range of heterozygosity was observed in populations of P. pectoralis ranging from 0.0147 to 0.1173 with a mean of 0.0438. The geographic variation in heterozygosity observed (Table 15) also appears to fit a pattern of a north-south cline of increasing heterozygosity, with the least variable populations occurring in the tropics of northeastern Mexico. The extremely high levels of heterozygosity (0.1173), found in populations from the Davis Mountains of Texas, may be the product of gene exchange from two Pleistocene refugia to be discussed later. A higher value of heterozygosity for a rodent (0.1190) has been shown for Mus musculus (Selander and Yang, 1969). However, still higher levels have been reported in Drosophila (Prakash et al.

1969), Astyanax (Avise and Selander, 1972), and Limulus (Selander et al., 1970).

Considerable geographic variation was also observed in the loci which contribute to the heterozygosity in P. pectoralis. The high level of heterozygosity in populations in the Davis Mountains (Table 15) is produced by heterozygotes at the Es-1 and Ldh-3 loci, with the Es-1 and the hemoglobin beta chain loci contributing to the heterozygosity of Mexican populations. The heterozygosity of populations of north-central Texas is produced primarily by heterozygotes at the albumin and Es-1 loci, while only the albumin locus is the major contributor to the heterozygosity of populations of the Edwards Plateau.

The albumin locus is a major contributor to the level of heterozygosity in most northern populations of P. pectoralis and P. boylii. In a regression analysis of the frequency of the albumin b allele against latitude Jensen (1970) observed a positive correlation with a regression coefficient of 0.213. A similar pattern of distribution of alleles was observed for P. boylii rowleyi from Chihuahua and New Mexico (Figure 20). Table 16 shows the gene frequencies observed and calculated for each location of P. boylii rowleyi. Correlation and regression analysis of the albumin b allele with latitude showed a positive correlation (Figure 21). The same general pattern was observed in populations of P. pectoralis (Figure 22). Table 17 shows gene frequencies observed

Fig. 20--Geographic variation of the albumin a allele in populations of Peromyscus boylii rowleyi.

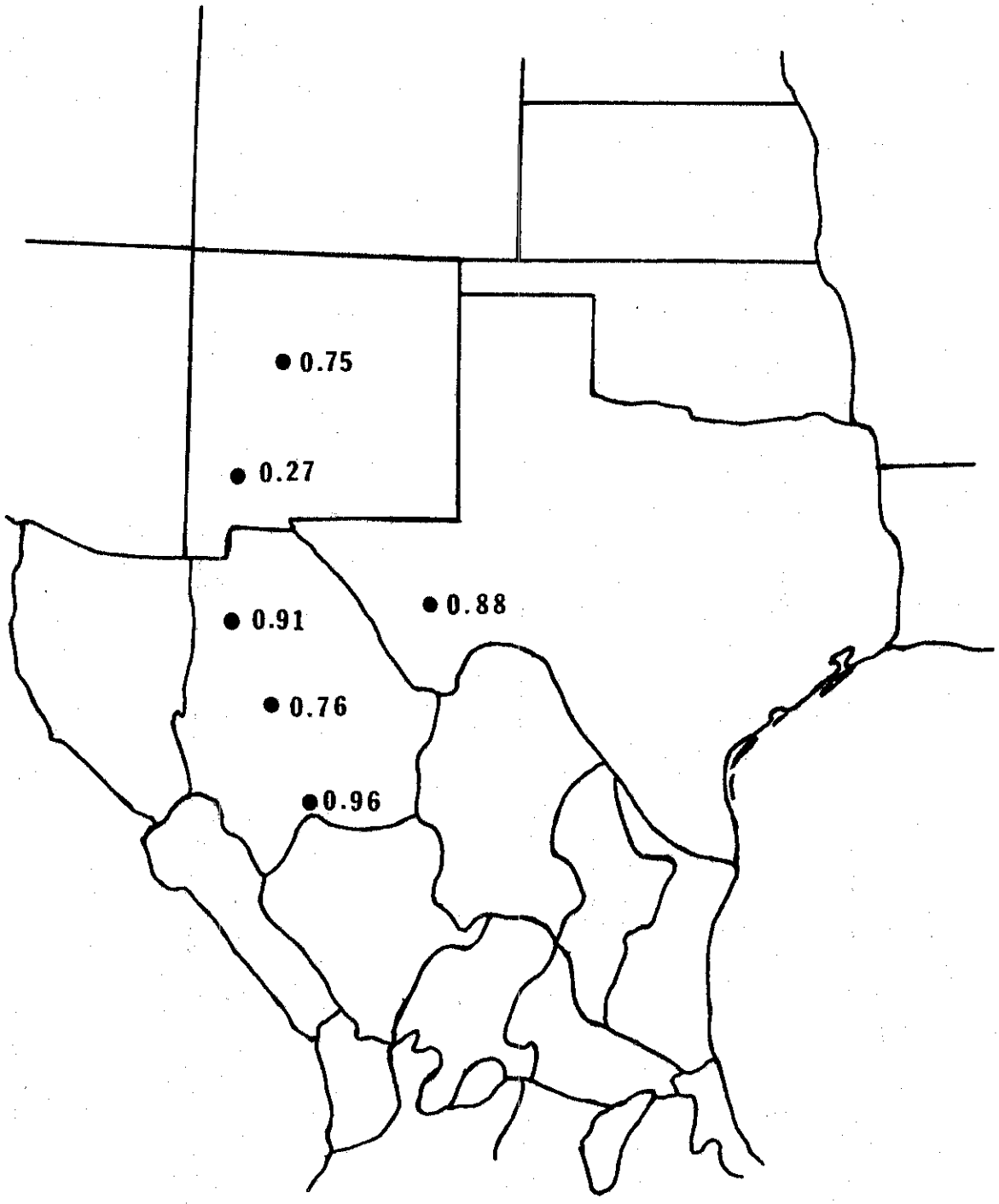


Table 16. - Geographic variation of albumin phenotype in populations of *Peromyscus boylii rowleyi*

Location and Latitude		aa	ab	bb	N	Chi-Square (df=1)		Frequency a	Frequency b
Chihuahua:									
3 mi. SW Santa Barbara (26°48')	Obs.	11	1	0	12	0.023	0.80	0.958	0.042
	(Exp.)	(11.02)	(0.96)	(0.02)					
9 mi. SSE Celulosa (28°24')	Obs.	12	11	0	23	2.27	0.20	0.76	0.24
	(Exp.)	(13.3)	(8.4)	(1.3)					
7 mi. E Buena Ventura (29°55')	Obs.	9	2	0	11	0.11	0.70	0.91	0.09
	(Exp.)	(9.09)	(1.82)	(0.09)					
Texas:									
Davis Mts. Jeff Davis Co. (30°18')	Obs.	10	3	0	13	0.22	0.50	0.88	0.12
	(Exp.)	(1.017)	(2.65)	(0.17)					
New Mexico:									
Albuquerque, Bernalillo Co. (35°6')	Obs.	3	3	0	6	0.66	0.50	0.75	0.25
	(Exp.)	(3.38)	(2.25)	(0.38)					
10 mi. N. Silver City, Grant Co., N.M. (32°48')	Obs.	2	4	0	15	1.52	0.20	0.27	0.73
	(Exp.)	(1.07)	(5.87)	(8.07)					

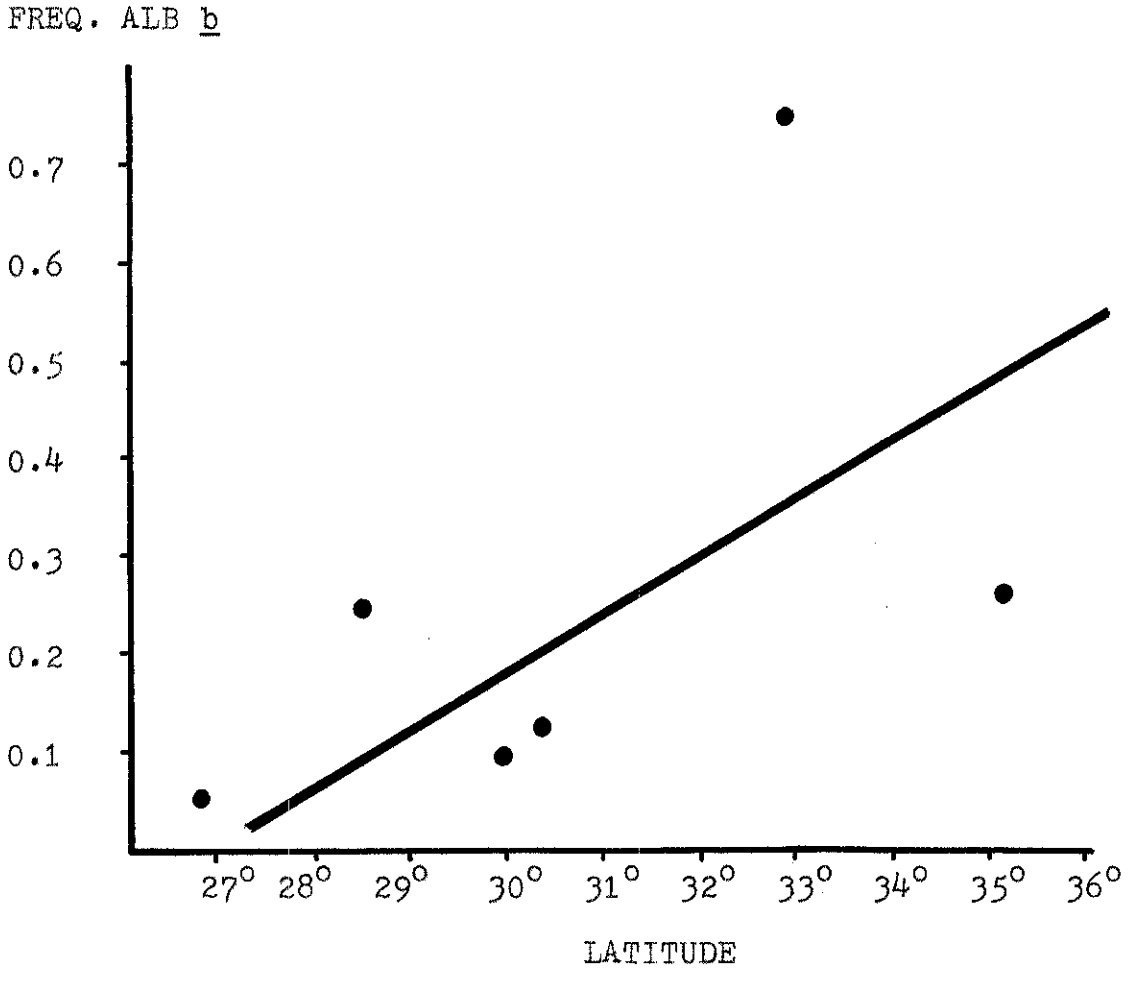


Fig. 21--Regression of frequency of albumin b allele with location latitude. Regression coefficient, b = 0.045, t = 12.01. This t value is significant at the .01 level (5 degrees of freedom).

Fig. 22--Geographic variation of the albumin a allele in populations of Peromyscus pectoralis.

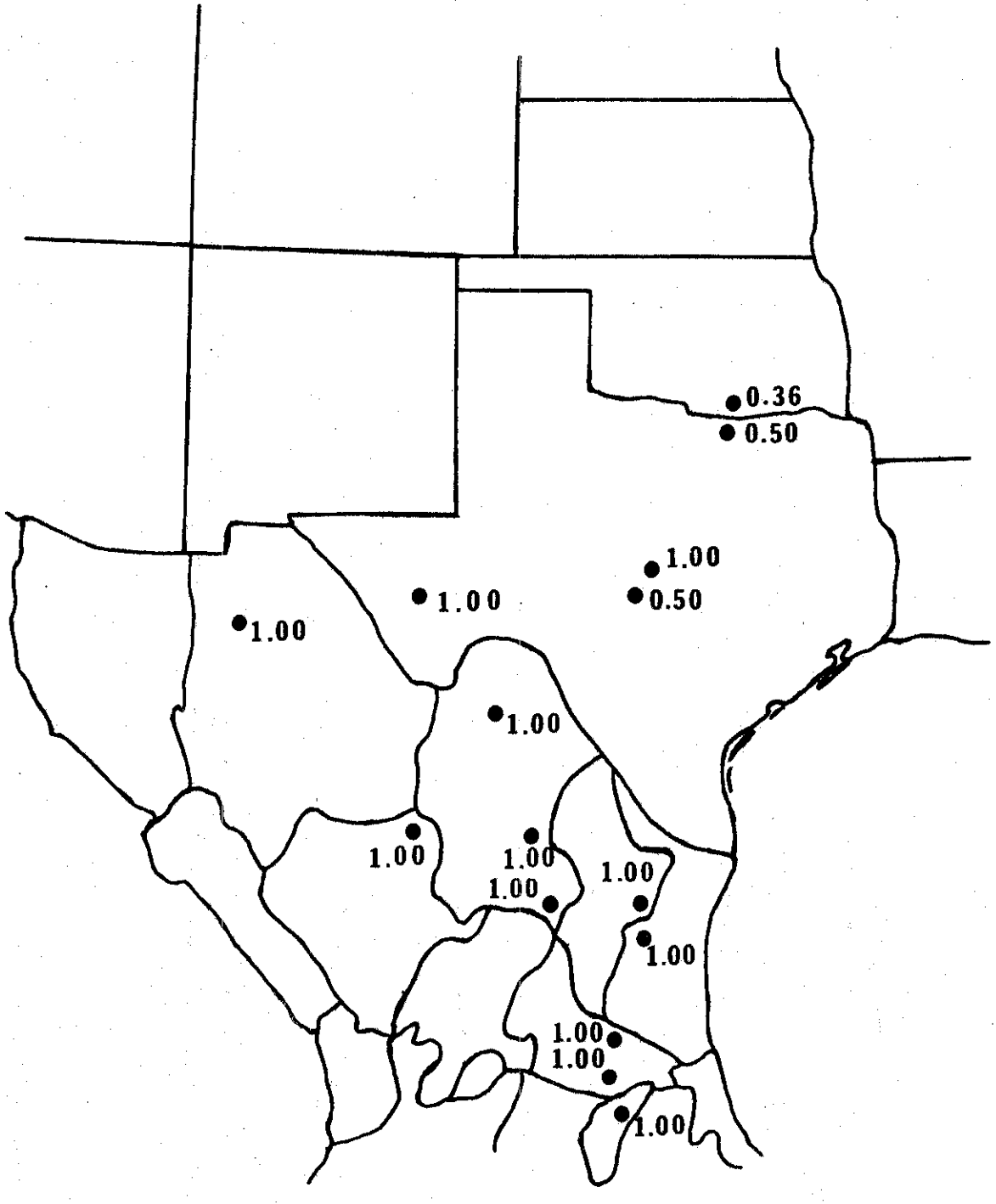


Table 17. - Geographic variation of albumin phenotype in populations of *Peromyscus pectoralis*

Location and Latitude	aa	ab	bb	N	Chi-Square (df=1)	Frequency a	Frequency b
Queretaro							
Hacienda X-Coneo	4	0	0	4		1.0	0.0
San Luis Potosi							
28.5 mi. W Cd. Valles	4	0	0	4		1.0	0.0
El Salto Falls ($22^{o}36'$)	4	0	0	4		1.0	0.0
Tamaulipas							
19 mi. SW Jaumave ($23^{o}18'$)	3	0	0	3		1.0	0.0
Nuevo Leon							
14 mi. W Linares	3	0	0	3		1.0	0.0
Coahuila							
20 mi. SE Saltillo ($25^{o}12'$)	4	0	0	4		1.0	0.0
12 mi. N Saltillo ($25^{o}48'$)	5	0	0	5		1.0	0.0

Table 17. - (continued)

Location and Latitude	aa	ab	bb	N	Chi-Square (df=1)		Frequency a	Frequency b
25 mi. SE Ocampo	4	0	0	4			1.0	0.0
Durango								
1 mi. W Bermejillo	4	0	0	4			1.0	0.0
Chihuahua								
1 mi. W Buena Ventura	2	0	0	2			1.0	0.0
Texas								
Kerrville, Kerr Co. (36°6')	0 (0.5)	2 (1)	0 (0.5)	2	2.0	0.10	0.5	0.5
10 mi. WSW Ft. Davis, Jeff Davis Co. (30°18')	3	0	0	3			1.0	0.0
10 mi. N Mason, Mason Co. (30°48')	3	0	0	3			1.0	0.0
10 mi. NW Gainesville, Cooke Co. (33°48')	2	3	2	7	0.14	0.70	0.5	0.5
Oklahoma								
4 mi. W Marietta, Love Co., (33°52')	1 (1.45)	6 (5.09)	4 (4.45)	11	0.35	0.35	0.36	0.64

and calculated for each location. The analysis of correlation and regression of the albumin b allele with latitude (Figure 23) indicate a positive correlation. This correlation is probably due to some other variable also correlated with latitude, such as length of day, average temperature, or available moisture, rather than latitude per se. Selander et al. (1971) noted a clinal shift in albumin in P. polionotus from southern Florida to South Carolina and Georgia. The significance of clinal shifts in albumin alleles in such a range of species of Peromyscus is, at present, unknown.

Genetic Similarity and Systematics

The probability of genotypic identity (Hedrick, 1971) was employed to measure degrees of genetic similarity among populations, regardless of species. Hedrick (1971) suggested that since selection operates primarily on the organism in the diploid state, estimates of genetic similarity should be based on genotypic frequencies rather than gene frequencies. In practice, Hedrick's index detects more differences between populations due to the wider range in variation of frequencies of genotypes (Richmond, 1973).

Genetic similarities of 37 populations were calculated based on 52 genotypic frequencies of the 17 loci examined. Hedrick's index was calculated for each genotype of a locus by summing the probabilities of drawing identical genotypes from two populations for each genotype of a locus and dividing this by one half the sum of the probabilities of drawing

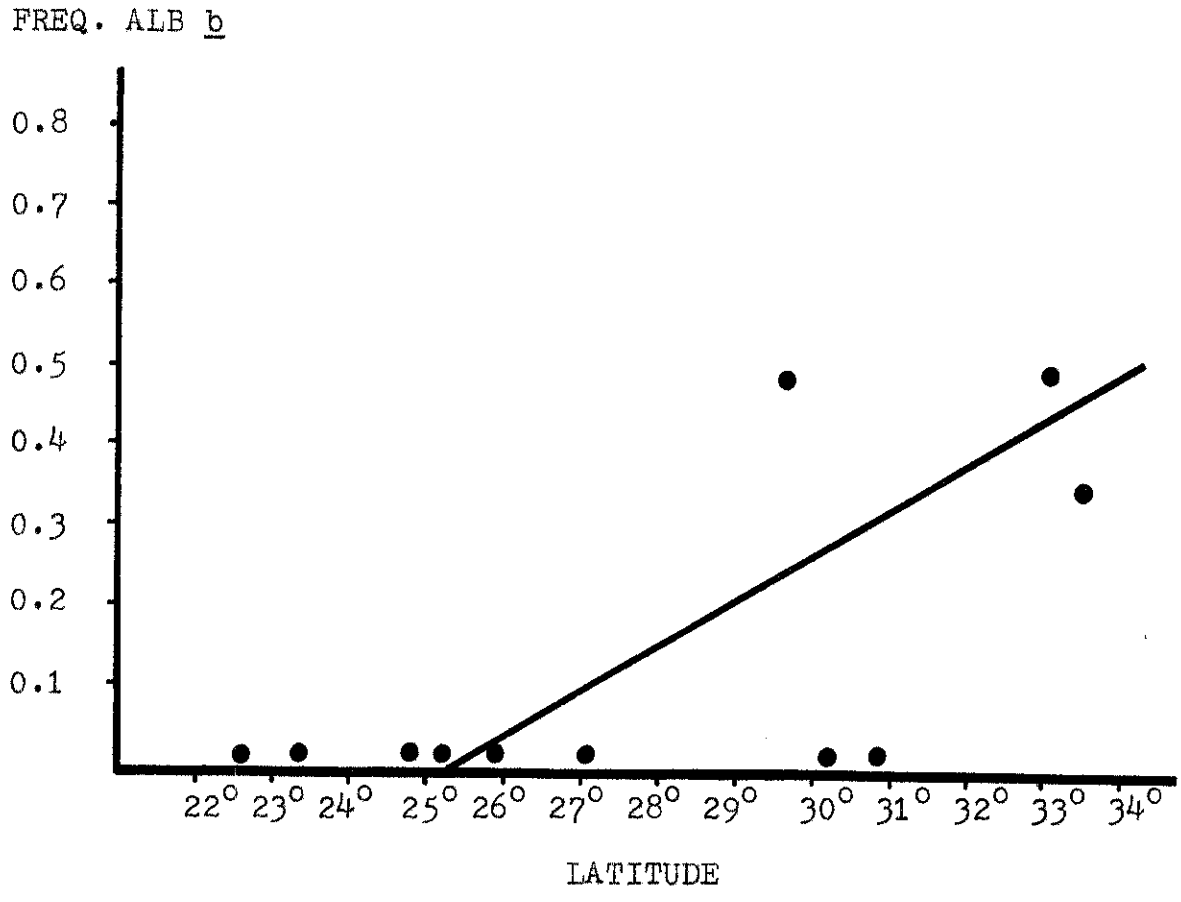


Fig. 23--Regression of frequency of albumin b allele with location latitude of populations of Peromyscus pectoralis. Regression coefficient, b = 0.049, t = 18.27. This t value is significant at the .01 level (10 degrees of freedom).

identical genotypes from the same population on two successive independent draws as shown below:

$$I_{x \cdot y} = \frac{\sum_{j=1}^{n} P_{jx} \cdot P_{jy}}{\frac{1}{2}\left[\sum_{j=1}^{n} P_{jx}^2 + \sum_{j=1}^{n} P_{jy}^2\right]}$$

where Pjx and Pjy are the frequencies of the jth genotype in population x and y and n is the number of genotypes at that locus. The mean genetic similarity between two populations was calculated by summing the probabilities of genotypic identity at each locus and divided by the total number of loci examined. From a matrix of mean genetic similarities for paired combinations of populations, a cluster analysis was performed on an IBM 360 computer by the weighted pair group method (Sokal and Sneath, 1965) to produce a phenogram (Figure 24).

Paired combinations of populations of P. pectoralis laceianus have a mean coefficient of genetic identity of 0.824 with a range of 1.00 to 0.760, while those for populations of P. pectoralis collinus and P. pectoralis pectoralis have mean coefficients of genetic similarity of 0.955 and 0.898, respectively. The low degree of genetic similarity within recognized subspecies of P. pectoralis suggests that many of these populations do not represent members of the same gene pool. The past distribution of P. pectoralis

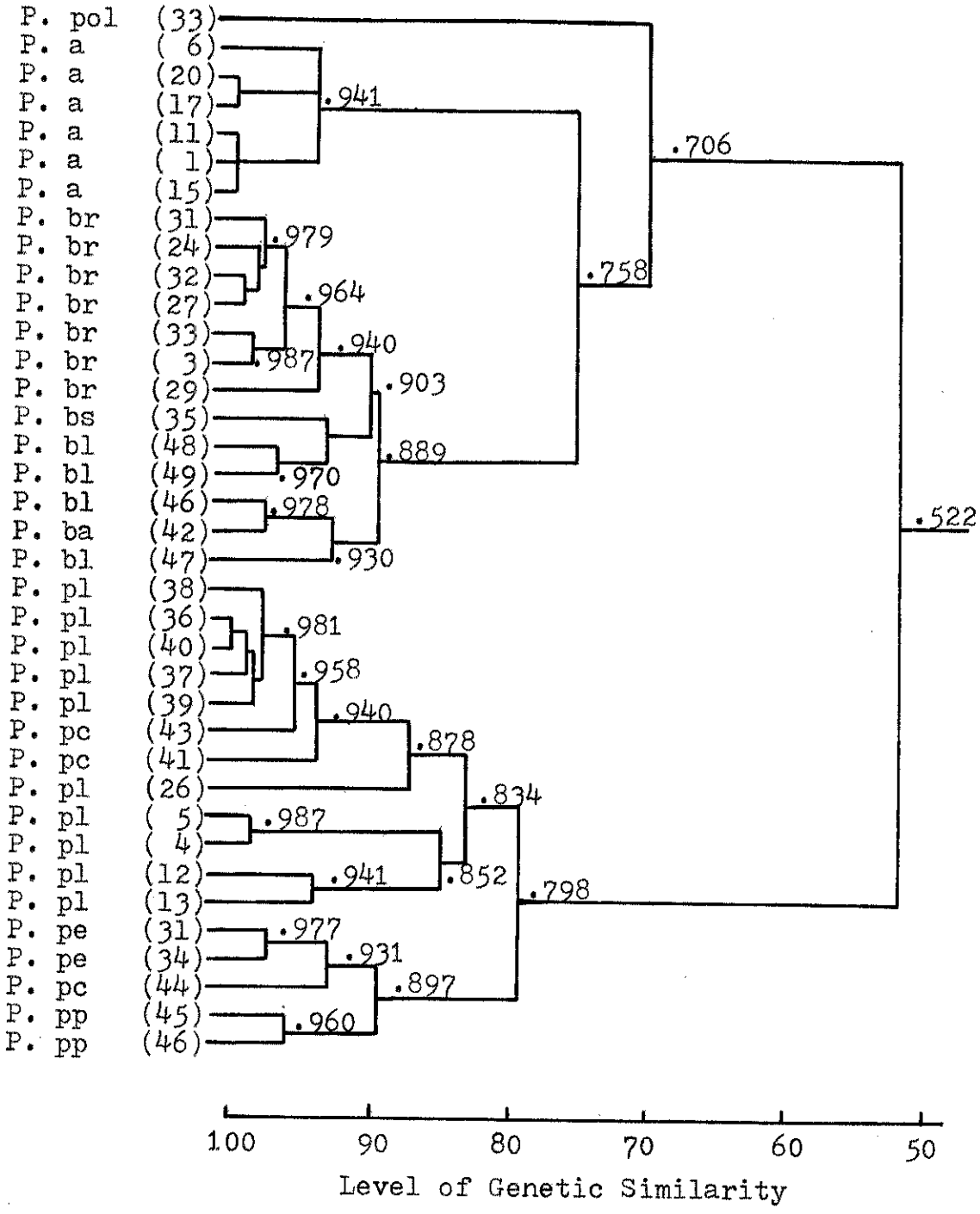


Fig. 24--Genetic similarity phenogram for 37 populations of the boylii species group. Numbers in parenthesis correspond with locations given in appendix.

in the United States and Mexico may explain the low degree of genetic similarity observed.

The distribution of P. pectoralis during the Pleistocene can be developed on the basis of limited fossil records and reports of the displacement of associated plant and animal communities during Pleistocene glaciation. Schmidly (1971) suggested that the displacement of biotic communities during the Wisconsin period could have isolated populations of P. pectoralis into three refugia located on the Edwards Plateau of Texas, the tropics of northeastern Mexico, and the desert regions of the Central Plateau of Mexico. Schmidly (1971) suggested the products of this isolation are the three currently recognized subspecies of P. pectoralis.

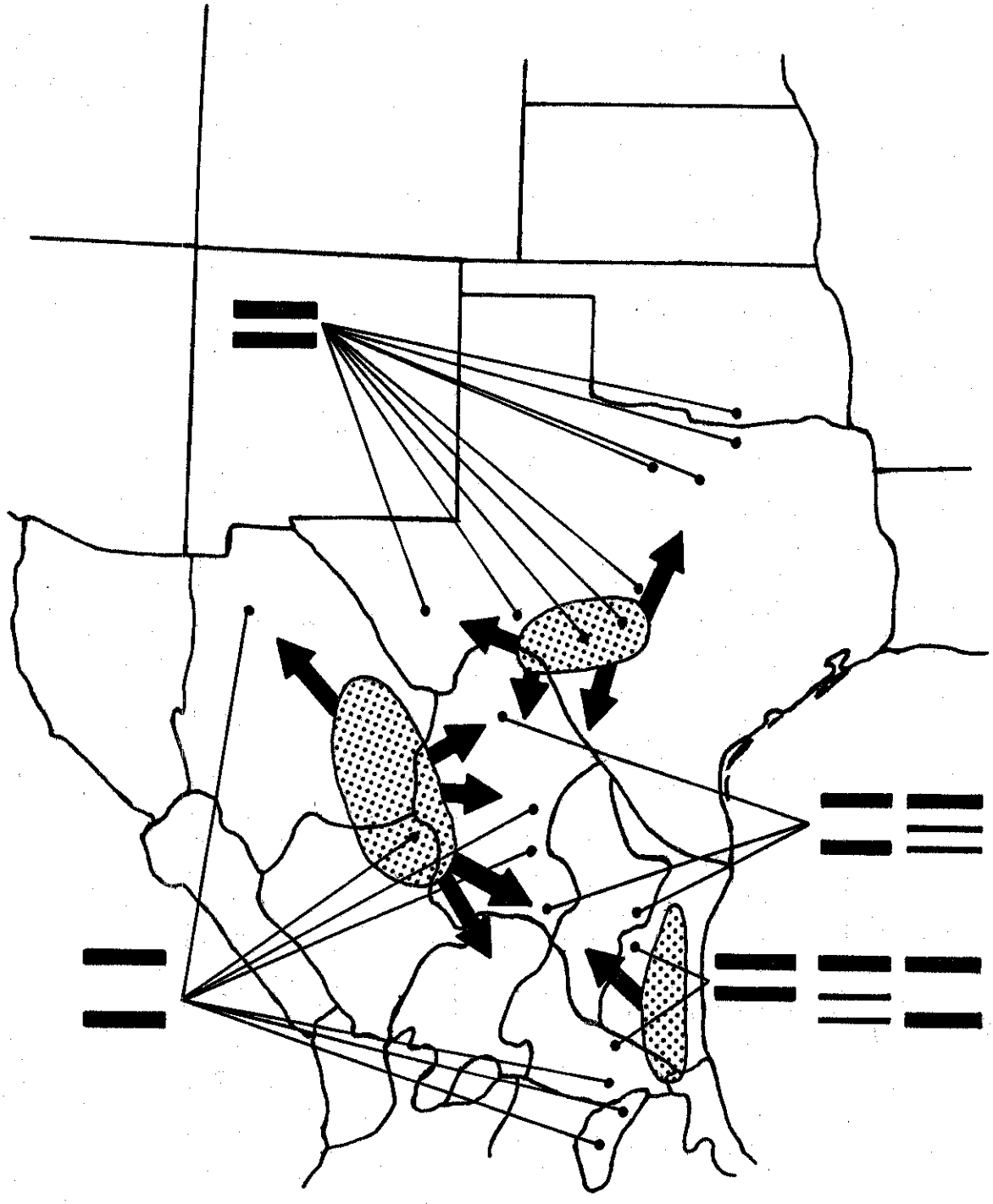
Two of the refugia can be substantiated, in part, by fossil evidence. The tropical refuge of P. pectoralis has been verified by reports from a late Pleisotcene fauna from Cueva de Abra in the tropics of southern Tamaulipas (Dalquest and Roth, 1970). The associated fauna was primarily tropical, much like the modern fauna, but contains mammals now isolated to the west by lowlands and to the north in arid brushlands (Dalquest and Roth, 1970). The age of the Cueva de Abra fauna is questionable, but the fauna existed during the Wisconsin or immediately following it.

Dalquest et al. (1969) and Roth (1973) reported P. pectoralis from the Edwards Plateau refugium in Pleistocene

faunas from Schulze Cave and Klein Cave. On the basis of the associated fauna, Dalquest et al. (1969) postulated that alpine meadow conditions were present on the high plains during late Wisconsin time and extended southward to a point west of the Edwards Plateau, and that the woodlands of eastern Texas were continuous to the Edwards Plateau, isolating the latter. Peromyscus pectoralis was not present in the late Pleistocene Freisenhahn Cave fauna near San Antonio, Texas (Martin, 1968), or the Longhorn Cavern Fauna in Burnett County, Texas (Seimken, 1961), suggesting that it had a very restricted geographic distribution on the Edwards Plateau during the Pleistocene.

Biochemical data suggest the direction of gene flow from these refugia, with the retreat of uninhabitable biotic communities for P. pectoralis. Present distribution of hemoglobin phenotypes in P. pectoralis (Figure 25) suggests the fixation of the alleles producing the fast and slow migrating bands in populations inhabiting the two refugia on the Central Plateau of Mexico and the tropics of northeastern Mexico. The alleles producing the fast and intermediate bands were fixed in populations of P. pectoralis inhabiting the Edwards Plateau during the late Pleistocene. With the removal of uninhabitable barriers, gene flow from the Edwards Plateau refugium and one or both of the Mexican refugia produced populations inhabiting northern and northeastern Mexico which are highly polymorphic at the

Fig. 25--Distribution of hemoglobin phenotypes of P. pectoralis and hypothetical dispersal of genes from the three Pleistocene refugia (shaded areas).

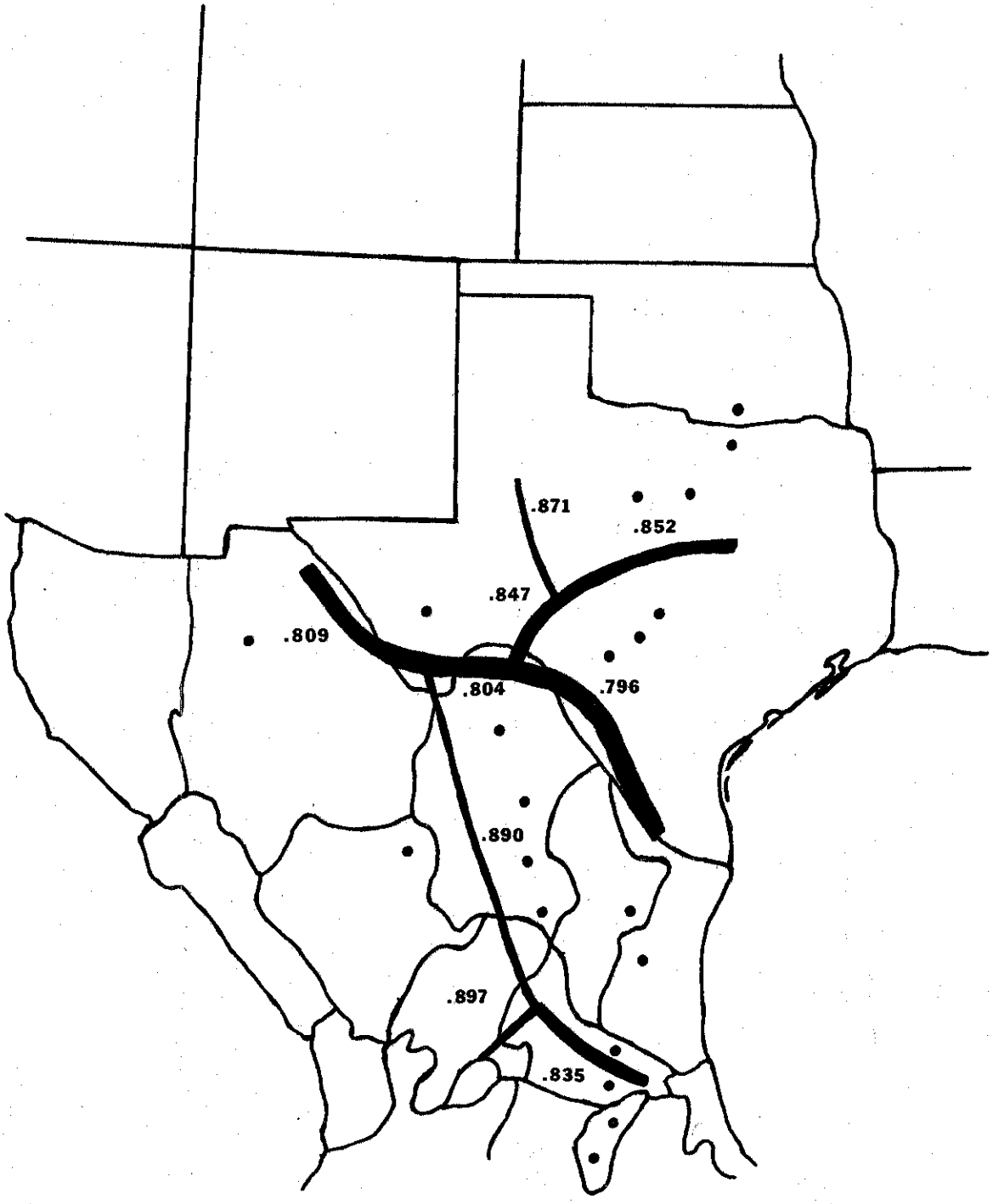


hemoglobin loci. Samples from the populations from northern and northeastern Mexico include hemoglobin phenotypes identical to that observed fixed in populations of the Central Plateau and the Tropics of northeastern Mexico, as well as the pattern which is fixed in populations on the Edwards Plateau. In addition, hemoglobin phenotypes which represent F_1 crosses and back-crosses are found.

The habitat of the Davis Mountains was probably not suitable for P. pectoralis during Wisconsin glaciation (Schmidly, 1971). Based on pollen grain analysis, Martin (1964) indicated this area to be part of an extensive yellow-pine, spruce-fur forest. Biochemical data suggest that the populations of P. pectoralis now inhabiting the Davis Mountains were colonized from two of the refugia. The a allele at the Es-1 locus appears to be fixed on the Edwards Plateau, but occurs in moderate frequency (0.40-0.33) in populations from the Davis Mountains, where the predominant allele at the Es-1 locus is e. This allele (e) also occurs in low frequencies (0.20-0.25) in populations of P. pectoralis from northern Coahuila and Durango.

By mapping the major discontinuities in genetic similarities (Figure 26), P. pectoralis can be divided into six genetic groups including those inhabiting: (1) north-central Texas and southern Oklahoma, (2) the Edwards Plateau, (3) the Trans-Pecos region, (4) north-eastern Mexico, (5) central Mexico, and (6) western Mexico. The discontinuities

Fig. 26--Major discontinuities in genetic similarities of populations of Peromyscus pectoralis.



observed are very similar to those found by Schmidly (1972) utilizing cranial and body measurements. The major difference is in the magnitude of some of the discontinuities. The major shift in biochemical characters occurs between populations of the Trans-Pecos and Edwards Plateau regions of Texas and populations of north-western and north-central Mexico (Figure 26), having coefficients of genetic similarities of 0.809 to 0.796. The differences in predominance or fixation of different alleles at three loci (hemoglobin, albumin, Es-1) produce this character shift.

The populations of north-central Texas and southern Oklahoma were probably colonized from the Edwards Plateau. The a allele of the albumin locus is fixed in most populations of P. pectoralis, but the b allele is found in populations on the Edwards Plateau and in north-central Texas. This would suggest that mutations to produce the b allele occurred during isolation on the Edwards Plateau during the Pleistocene. Dispersal to the north from this refugium resulted in an increase in frequency of the b allele either by selection or founder effect. Dispersal to the west and south and gene flow with populations from the other refugia, contributing only the a allele, decreased the frequency of the b allele, with the ultimate removal of b from these populations.

These same populations may have received some of their genetic make-up from the western Mexico refugium via the

Trans-Pecos. The b allele of the Got-1 locus is predominant or fixed in most populations of P. pectoralis. An a allele is present in populations of western Mexico at a frequency of 0.50, the Davis Mountains at a frequency of 0.40, and in some populations of north-central Texas and southern Oklahoma at frequencies of 0.25 and 0.14, respectively. These are the only populations of P. pectoralis in which the a allele was observed; this would suggest gene flow from the western Mexico refugium to populations of north-central Texas via populations of the Trans-Pecos. Thus, the presence of two unique alleles (b and d) at the Es-1 locus, plus changes in frequencies of the alleles of the albumin and Got-1 loci, contribute to the separation of the populations of north-central Texas and southern Oklahoma from other populations of Texas.

A systematic interpretation of the character shifts in Mexico is not possible due to the absence of sufficient samples from the refugium of tropical regions of northeastern Mexico. Thus, no character shifts were observed to separate populations of P. pectoralis collinus from other subspecies. Re-evaluation of the taxonomic status of this subspecies is not advisable on the basis of the few number of specimens and small number of localities sampled.

The variation between genetic groups is as great or greater than that found between many species of rodents.

However, based on chromosomal uniformity, I believe P. pectoralis is a conspecific unit with locally high degrees of heterozygosity and atypical variation of genetic similarity reflecting gene exchange between the differentiated genomes of the three refugia after retreat of the Wisconsin glaciation.

Biochemical data indicate that P. attwateri is genetically distinct from other members of the boylii group and support the specific status suggested by Lee et al. (1972) for this form. Paired combinations of populations of P. attwateri have a mean coefficient of genetic similarity of 0.957 and a range of 1.00 to 0.941. Paired populations of P. attwateri and P. pectoralis have a mean coefficient of genetic similarity of 0.623 with a range 0.647 to 0.537, while P. attwateri and P. boylii yield a mean coefficient of genetic similarity of 0.774 with a range of 0.824 to 0.704. This indicates that P. attwateri is more similar to P. boylii than to P. pectoralis.

Based primarily on pelage coloration, Osgood (1909) assumed that P. attwateri was more similar to P. boylii rowleyi and P. boylii boylii than to other subspecies of P. boylii, while Schmidly (1971) presented data on the morphology, dental pattern, and karyology which suggested P. attwateri was clearly divergent from the rowleyi-boylii assemblage. The chromosomal compliment of P. attwateri is

very similar to that of P. boylii spicilegus (Lee et al., 1972) and has the closest chromosomal affinities to the boylii forms from northwestern Mexico; however, cranial morphology would suggest this species is more closely allied to boylii forms occurring in northeastern Mexico (Schmidly, 1971).

Of the four subspecies of boylii analyzed in this study, P. attwateri shows the lowest levels of genetic similarity to populations from northeastern Mexico. Paired combinations with P. boylii ambiguus from near Cuidad Victoria, Tamaulipas, yield a mean coefficient of genetic similarity of 0.737 and when paired with P. boylii levipes from Veracruz, Hidalgo, and Queretaro, a mean coefficient of 0.768 was found. A slightly higher degree of genetic similarity was observed with a population of P. boylii spicilegus, with a mean coefficient of genetic similarity of 0.794. Populations of P. attwateri do not appear to be more similar to populations of P. boylii from northeastern or northwestern Mexico than to the populations of P. boylii rowleyi from Texas, New Mexico, and Oklahoma, with a mean genetic similarity of 0.783.

Examination of specific loci for allelic homology would suggest that the affinities of P. attwateri appear to lie with P. boylii. Of the 17 loci examined in populations of P. attwateri, unique alleles were observed at only one locus

(transferrin), and 15 loci were observed to have alleles common to populations of P. boylii, while only one locus (hemoglobin Beta-1) was observed to have alleles common to populations of P. pectoralis. Populations of P. attwateri also appear to have a similar genome to a few specimens of P. difficilis, a member of the truei group, and additional studies are needed to investigate the possible affinities of P. attwateri with this latter group.

With the presence of different karyotypes in allopatric populations of P. boylii, it would appear that all forms of P. boylii may not represent a conspecific unit. However, paired combinations of all populations of P. boylii yield a mean coefficient of genetic similarity of 0.923 with a range of 0.996 to 0.861. Therefore, biochemical data suggest that the four subspecies of boylii examined (rowleyi, spicilegus, levipes and ambiguus), each with a different chromosomal pattern, do form a conspecific unit.

Although not previously shown by other workers, gene exchange does occur between populations of P. boylii rowleyi and P. boylii spicilegus from near Coyote, Durango. Three of the four specimens from this locality have a genotype cc for the transferrin locus and are chromosomally typical of P. boylii spicilegus described by Lee et al. (1972). The fourth specimen is karyotypically an F_1 produced from a cross of P. boylii rowleyi and P. boylii spicilegus and has

a transferrin genotype ac, the a allele being common to P. boylii rowleyi. Schmidly (personal communication) also reports finding karyotypes intermediate between rowleyi and and spicilegus and intermediate between rowleyi and levipes.

The population of P. polius appears to be more closely related to the P. boylii-P. attwateri assemblage than to P. pectoralis (Figure 24). Osgood (1909) suggested the affinities of P. polius lie with the truei group. Hoffmeister (1951) in a revision of the truei group, tentatively placed P. polius in the boylii group but suggested its affinities might lie with the melanophrys group. Paired combinations of P. polius with populations of the boylii group are presented in Table 18. The highest level of genetic similarity is with P. boylii ambiguus with a mean of 0.737. Additional studies are in progress to determine the level of genetic similarity of this species with members of the truei group and the melanophrys group.

Table 18. - Mean Coefficients of Genetic Similarity within the *boylii* Species Group

	P.a.	*P.b.r.*	*P.b.s.*	*P.b.a.*	*P.b.l.*	*P.p.*	*P.pol*
P. attwateri	--						
P. boylii rowleyi	0.780	--					
P. boylii spicilegus	0.794	0.877	--				
P. boylii ambiguus	0.737	0.876	0.940	--			
P. boylii levipes	0.768	0.907	0.895	0.943	--		
P. pectoralis	0.623	0.560	0.572	0.572	0.579	--	
P. polius	0.693	0.686	0.718	0.737	0.693	0.460	--

CHAPTER V

SUMMARY

Six distinct chromosomal patterns were observed within the populations of four of the nine species of the boylii species group. Three species (boylii, attwateri, and pectoralis) had karyotypes consisting of low numbers (6 to 14) of biarmed autosomes. Peromyscus polius had a chromosomal complement consisting of 28 to 30 biarmed elements. The extreme range in number of biarmed elements with no intermediate forms indicates the possibility of a polyphyletic origin of this group.

Little or no chromosomal variation was observed in three of the species, but considerable chromosomal variation occurred among populations of P. boylii. Generally, the distribution of each chromosomal pattern corresponded to the distribution of recognized subspecies of P. boylii. This would suggest that the allopatric populations of P. boylii were not conspecific.

A chromosomal intermediate to P. boylii rowleyi and P. boylii spicilegus was observed in a specimen from near Coyote, Durango, Mexico. Since P. boylii spicilegus was observed at this locality and P. boylii rowleyi has been reported from the vicinity, this chromosomal intermediate was probably the result of gene flow between these two

populations. This indicates that at least these two chromosomal forms of P. boylii are conspecific.

In addition to chromosomal evidence, analysis of biochemical genetic data demonstrated and suggested effective gene flow between the chromosomal forms of P. boylii. Electrophoretically demonstrable variation was analyzed in 11 proteins encoded by 17 autosomal loci in samples from 45 populations of four of the nine species of the boylii species group. Of the 17 structural loci, 11 were polymorphic in one or more populations. No more than five loci were observed to be polymorphic within a single population, with a mean number of polymorphic loci of 2.26.

The level of genetic variability, as measured by the proportion of loci in a heterozygous state in the average individual of a population, for populations of the boylii species group were within the range reported for other rodents. Populations of P. attwateri exhibited levels of heterozygosity considerably lower than those reported for other species of Peromyscus. The low level of genetic variability in P. attwateri was probably the result of founder effect of the original population which was isolated on the Edwards Plateau during the Pleistocene and genetic drift which allowed the fixation of genes within this isolated population.

Geographic variation observed in levels of heterozygosity among populations of P. attwateri indicate a north to south cline of increasing heterozygosity with the most variable populations occurring on the Edwards Plateau and the Llano Estacado of western Texas. The general clinal pattern was interpreted as the result of colonization from the Edwards Plateau population with the increase in homozygosity arising from founder effect and genetic drift in the established disjunct populations. A similar pattern of geographic variation of heterozygosity was observed in P. boylii. The highest levels of heterozygosity observed in P. boylii occurred in populations in Chihuahua, Mexico. The geographic pattern of heterozygosity would appear, at least in part, to be related to the past distribution and subsequent colonization of this species. The high levels of heterozygosity reflect the relic population during the late Pleistocene, while the populations colonized from these have reduced levels of heterozygosity.

A wide range of the levels of heterozygosity were observed among populations of P. pectoralis, but the highest levels were not observed in populations from suspected Pleistocene refugia. Populations of P. pectoralis also exhibited a wide range of genetic similarity (1.00 to 0.706) for paired combination with a mean of 0.824. This variation in levels of heterozygosity and genetic similarity is the

result of colonization and, hence, genetic contribution from three Pleistocene refugia. Differentiation of gene pools occurred in the refugia populations during the period of isolation. With the removal of the primary isolation barriers, gene exchange between populations derived from two or more refugia have produced populations with high levels of heterozygosity. Since colonization from the three refugia was in all direction, existing populations have received their genetic makeup from one to three different gene pools. Thus, populations of P. pectoralis exhibit lower levels of genetic similarity than observed among populations of most species.

Paired combinations of all populations of P. boylii exhibited a mean coefficient of genetic similarity of 0.923 with a range of 0.996 to 0.861. Therefore, biochemical data suggest that the four subspecies of boylii examined (rowleyi, spicilegus, levipes and ambiguus), each with a different chromosomal pattern, do form a conspecific unit.

The populations of P. polius appears to be more closely related to the P. boylii-P. attwateri assemblage than to P. pectoralis. The highest level of genetic similarity with P. polius (0.737) was observed when paired with a population of P. boylii ambiguus from northeastern Mexico.

Biochemical data indicated that P. attwateri was genetically distinct from other members of the boylii group.

Populations of P. attwateri were more similar to populations of P. boylii than to P. pectoralis or P. polius. Examination of specific loci for allelic homology indicated clearly that the affinities of P. attwateri lie with P. boylii, thus, suggesting that P. attwateri arose from genetic divergence of a population of P. boylii isolated on the Edwards Plateau during the late Pleistocene.

APPENDIX

	Collecting Localities	No. Individuals
1.	Kansas: 1 mi. W Elgin, Chautauqua County	56
2.	Oklahoma: Wichita Mountains, Comanche County	7
3.	Oklahoma: 2 mi. SE Kenton, Cimarron County	4
4.	Oklahoma: 4 mi. W Marietta, Love County	11
5.	Texas: Moss Lake, 10 mi. NW Gainesville, Cooke Co.	7
6.	Texas: 4 mi. NE Nocona, Montague County	2
7.	Texas: 10 mi. NW Jacksboro, Jack County	1
8.	Texas: 8 mi. S Ranger, Eastland County	13
9.	Texas: 1 mi. W Palo Pinto, Palo Pinto County	8
10.	Texas: 5 mi. N Palo Pinto, Palo Pinto County	2
11.	Texas: Cleburn State Park, Johnson County	2
12.	Texas: 10 mi. N Mason, Mason County	3
13.	Texas: Kerrville, Kerr County	3
14.	Texas: 2 mi. SE Concan, Uvalde County	2
15.	Texas: 8 mi. S Childress, Cottle County	46
16.	Texas: 15 mi. ESE Paducah, Cottle County	4
17.	Texas: 3 mi. E, 3 mi. S Vera, Knox County	3
18.	Texas: 1 mi. W Post, Garza County	5
19.	Texas: 2 mi. N Post, Garza County	8
20.	Texas: 1 mi. W Justiceburg, Garza County	3
21.	Texas: 18 mi. SE Throckmorton, Throckmorton Co.	12
22.	Texas: Sweetwater Lake, Nolan County	1

Appendix (continued)

23.	Texas: 10 mi. S Sheffield, Terrell County	1
24.	Texas: 14 mi. Ft. Davis, Jeff Davis County	13
25.	Texas: 5 mi. W Ft. Davis, Jeff Davis County	6
26.	Texas: 10 mi. WSW Ft. Davis, Jeff Davis County	3
27.	New Mexico: Albuquerque, Bernalillo County	12
28.	New Mexico: 2 mi. W Mogollon, Catron County	4
29.	New Mexico: 10 mi. N Silver City, Grant County	21
30.	Utah: 7 mi. E Escalante, Garfield County	7
31.	Chihuahua: 7 mi. E Buena Ventura	17
32.	Chihuahua: 9 mi. SSE Celulosa	23
33.	Chihuahua: 3 mi. SW Santa Barbara	17
34.	Durango: 1 mi. W Bermejillo	4
35.	Durango: Coyote	4
36.	Coahuila: 25 mi. SE Ocampo	4
37.	Coahuila: 42 mi. N Saltillo	1
38.	Coahuila: 12 mi. N Saltillo	10
39.	Coahuila: 20 mi. SE Saltillo	4
40.	Nuevo Leon: 14 mi. W Linares	4
41.	Tamaulipas: 19 mi. SE Jaumave	3
42.	Tamaulipas: 16 mi. SW Cd. Victoria	6
43.	San Luis Potosi: El Salto Falls	4
44.	San Luis Potosi: 28.5 mi. W Cd. Valles	4
45.	Queretaro: Hacienda X-Coneo	4
46.	Queretaro: 1 mi. SE Arroyo Seca	2
47.	Queretaro: 1 mi. N Pinal de Amoles	2

Appendix (continued)

48.	Hidalgo: 13 mi. NE Metepec	3
49.	Veracruz: 3 mi. E Las Vigas	3

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