

OCCASIONAL PAPERS

THE MUSEUM

TEXAS TECH UNIVERSITY

NUMBER 24

24 MAY 1974

ELECTROPHORETIC ANALYSIS OF PEROMYSCUS COMANCHE BLAIR, WITH COMMENTS ON ITS SYSTEMATIC STATUS

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Recently, with refined electrophoretic techniques, it has become possible to estimate degrees of heterozygosity in populations (Lewontin and Hubby, 1966, Prakash *et al.*, 1969, on *Drosophila*; Selander and Yang, 1969, 1970, Selander *et al.*, 1969*b*, on *Mus*; Selander *et al.*, 1970, on *Limulus*) and thus compare them with respect to selected genetic characters. This assumes *a priori* that the loci controlling proteins examined are representative of the entire genome (Hubby and Lewontin, 1966; Selander *et al.*, 1969*a*). Following this approach, Selander *et al.* (1971) investigated protein polymorphism in the old-field mouse (*Peromyscus polionotus*). They analyzed geographic patterns of variation in allele frequencies, assessed levels of heterozygosity, and determined the genetic relationships of previously defined subspecies.

Populations of the Palo Duro mouse (*Peromyscus comanche*) in Texas have been regarded as affiliated with the *truei* group (Hall and Kelson, 1959). However, because these populations in Texas are geographically isolated and their taxonomy in question (Hooper, 1968; Lee *et al.*, 1972), we undertook an investigation of protein polymorphism in *P. comanche* and other geographically proximal members of the *Peromyscus truei* group (*P. difficilis nasutus*, *P. d. griseus*, and *P. truei truei*). The principal objective was to reveal the degree of genetic relationships between these species and subspecies. The results are compared to the current taxonomy.

Blair (1943) described *P. comanche* from northwestern Texas and suggested that its systematic affinities were with the *truei* group, even

though it was allopatric from other members of the group. Hoffmeister (1951) regarded *P. comanche* as a subspecies of *P. nasutus*. Hoffmeister and de la Torre (1961) considered the differences between *nasutus* and *difficilis* a result of intraspecific clinal variation and synonymized *P. difficilis* (Allen, 1891) with *P. nasutus* (Allen, 1891)—*P. difficilis* had page priority.

Although other workers (Dice, 1952; Tamsitt, 1961*a*, 1961*b*, 1961*c*, 1961*d*), utilizing various methods, have investigated the *truei* group, little conclusive information exists regarding the relationship of *P. comanche* with the other species of the *truei* group. Lee *et al.* (1972) concluded that the karyotype of *P. comanche* was indistinguishable from that of *P. t. truei*. Schmidley (1973) regarded *comanche* as a subspecies of *P. truei* based on karyotypic and morphological features.

We wish to thank Drs. Robert K. Selander of the University of Texas, Austin, and Michael H. Smith of the Savannah River Ecology Laboratory, South Carolina, for their technical advice and assistance. Kenneth Andersen of the University of New Mexico provided some of the specimens used in this study. Financial support was provided by a National Science Foundation Training Grant for Biology, GZ-2144.

METHODS AND MATERIALS

A total of 85 adult mice was used in this study. They were collected in the autumn of 1970 and in the spring and summer of 1971. Specimens of *P. comanche* were collected from Ceta Canyon, Randall Co., Texas, and Los Lingos Canyon, Briscoe Co., Texas. *P. t. truei* was obtained from 2½ mi. S Tijeras, Bernalillo Co., New Mexico, and 33 mi. S, 27 mi. W Socorro, Socorro Co., New Mexico. *P. d. nasutus* was collected from both east and west slopes of the Sandia Mountains, Bernalillo Co., New Mexico, from Suwanee, 40 mi. W Albuquerque, Valencia Co., New Mexico, and at Bonito Lake, Lincoln Co., New Mexico. *P. d. griseus* was trapped along the periphery of Valley of the Fires State Park and 5 mi. W Carrizozo, Lincoln Co., New Mexico.

Mice were collected in Sherman or Longworth live traps baited with mixed grain. Live animals were brought to the laboratory where they were maintained in plastic cages and provided with Purina Laboratory Chow, grain, and water. They were retained in the laboratory for at least one week (and for as long as two months) before processing for electrophoretic studies.

The laboratory techniques used were the same as those outlined by Selander *et al.* (1971) and Smith *et al.* (1973). Hemolysate and

plasma fractions of the blood as well as kidney and liver extracts were utilized. Electrostar, Lot 171 (Electrostar Co., Madison, Wisconsin) was prepared at a concentration of 11.75 per cent (47 grams per 400 milliliters buffer).

The following proteins were demonstrated: 1) alcohol dehydrogenase (ADH); 2) esterases (ES); 3) albumin (ALB), transferrins (TRF), and other general proteins; 4) glucose-6-phosphate dehydrogenase (G-6-P); 5) glutamate oxalate transaminases (GOT); 6) alpha-glycerophosphate dehydrogenase (GPD); 7) indophenol oxidase (IPO); 8) isocitrate dehydrogenases (IDH); 9) lactate dehydrogenases (LDH); 10) malate dehydrogenases (MDH); 11) phosphoglucomutases (PGM); 12) 6-phosphogluconate dehydrogenases (PGD); 13) phosphoglucose isomerase (PGI); and 14) sorbitol dehydrogenase (SDH).

Representative mice of each kind used in this study were prepared as museum specimens and deposited in the collection of mammals of The Museum, Texas Tech University, as follows: *Peromyscus truei truei*, TTU 13699-13700; *Peromyscus difficilis nasutus*, TTU 13701-13702; *Peromyscus difficilis griseus*, TTU 13703-13704; *Peromyscus comanche*, TTU 13705-13706.

RESULTS

Approximately 40 proteins were recognized in this study, but only 25 appeared with sufficient constancy and clarity to be scored (that is, recognized as a distinct band or bands). Of these 25 protein systems, 12 were determined to be polymorphic by the presence of bands of differing mobility. The allele or band designations (*a*, *b*, *c*, and so on) used here are our own.

Polymorphic Proteins

The tissue(s) from which phenotypes of a given protein type were scored are indicated in parentheses following the name of the protein:

1) *Esterase-1* (hemolysate, liver, and kidney).—Hemolysate electrophoresed with a tris-HCl buffer (Selander *et al.*, 1971—Buffer System 1) revealed a prominent, easily scored system migrating just anodal to the hemoglobin (Fig. 1). This system exhibited three bands of differing mobility in the material studied. The heterozygotes had two bands, indicating that the protein molecule is a monomer (Shaw, 1965). The alleles, listed in decreasing order of speed of migration of their bands, are *Es-1a*, *Es-1b*, and *Es-1c*.

Esterase-1 also appeared prominently in kidney and liver extracts. However, because of similarity of mobility to other esterase systems,

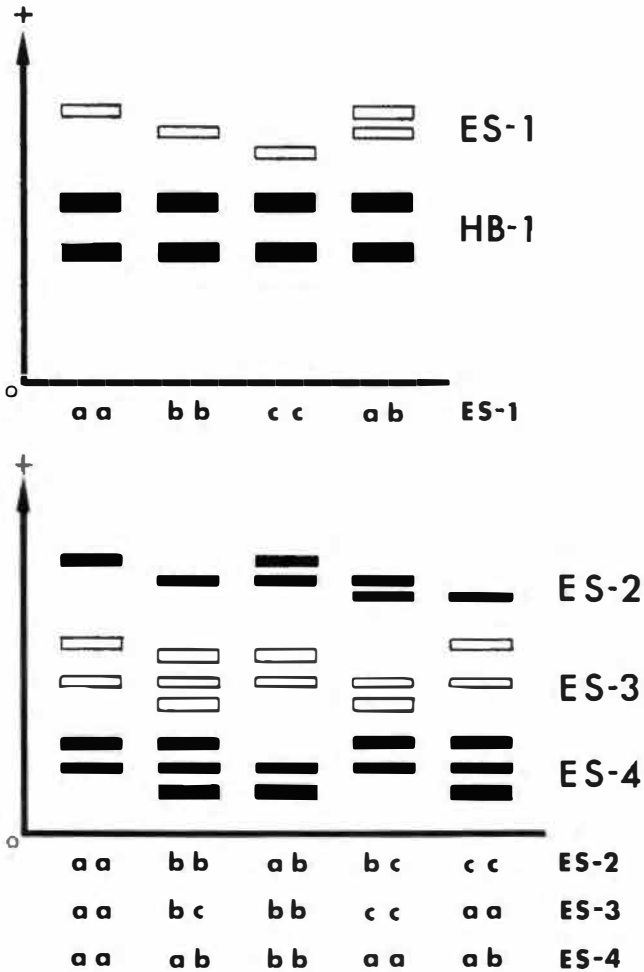


FIG. 1.—Electrophoretic variation among all members of the *Peromyscus truei* group studied. Above: variation in esterase-1 from hemolysate. Below: variation in esterase-2, esterase-3, and esterase-4 in liver extracts. Genotypes are listed below the origin (0) for each phenotype.

this system was difficult to score. Esterase-1 is completely inhibited by eserine.

In samples of *P. d. nasutus* and *P. d. griseus* studied, Es-1c was fixed in both groups (see Table 1). Es-1a and Es-1b were found in approximately the same proportions in both *P. comanche* and *P. truei*, with Es-1b being the common band.

2) *Esterase-2* (liver).—Because esterase-1 and esterase-2 have similar mobilities, it was necessary to inhibit the ES-1 system with

TABLE 1.—Allele frequencies at ES-1 and ES-2 loci among members of the *Peromyscus truei* group studied.

Sample population	Number of animals	ES-1			Number of animals	ES-2		
		Es-1a	Es-1b	Es-1c		Es-2a	Es-2b	Es-2c
<i>P. comanche</i>	20	0.33	0.67	—	12	0.86	0.14	—
<i>P. d. griseus</i>	25	—	—	1.00	13	—	0.75	0.25
<i>P. d. nasutus</i>	14	—	—	1.00	13	—	0.86	0.14
<i>P. truei</i>	17	0.27	0.73	—	4	1.00	—	—

eserine in order to score successfully esterase-2. Three alleles were recognized and the heterozygotes had two bands (Fig. 1).

Es-2b and Es-2c appeared in *P. d. nasutus* and *P. d. griseus*, with Es-2b being the predominant allele (Table 1). Es-2a and Es-2b were found in *P. comanche* and *P. truei*, with Es-2a the predominant allele.

3) *Esterase-3* (liver).—The esterase system found migrating cathodal to esterase-2 was designated esterase-3 (Fig. 1). This system was determined to have one band common to all phenotypes. Therefore, homozygotes had two bands and heterozygotes three. Selander *et al.* (1971) also found this in a similar esterase system in *Peromyscus polionotus*.

Es-3a was fixed in both *P. d. nasutus* and *P. d. griseus* (Table 2). Es-3b and Es-3c occurred exclusively in *P. comanche* and *P. truei*.

4) *Esterase-4* (liver).—This system, the most slowly migrating esterase system, also contained a band common to all phenotypes (Fig. 2). Both alleles, Es-4a and Es-4b, were shared by all groups studied, but Es-4b appeared with greater frequency in *P. d. griseus* and *P. truei* (Table 2).

5) *Esterase-6* (hemolysate).—The most anodally-migrating erythrocytic esterase (ES-6) on tris-maleic gels was polymorphic (Fig. 2). Es-6a was fixed in *P. d. nasutus* and *P. d. griseus*, and Es-6b was fixed in *P. comanche* and *P. truei* (Table 3).

6) *Isocitrate dehydrogenases* (kidney).—Tris-citrate (pH 6.7) gels revealed two forms of IDH that migrated differently (both were NADP-dependent). The mitochondrial form (IDH-1) migrated cathodally and seemed polymorphic, but did not appear with sufficient constancy and clarity to be scored. The supernatant form (IDH-2) migrated anodally (Fig. 2). Though there was considerable subbanding, this system was polymorphic and readily scored. Idh-2a was fixed in *P. comanche* and *P. truei* (Table 3). Idh-2b and Idh-2c were found in *P. d. nasutus* and *P. d. griseus*.

7) *6-Phosphogluconate dehydrogenase* (hemolysate).—This enzyme is apparently a dimer with three-banded heterozygotes (Parr,

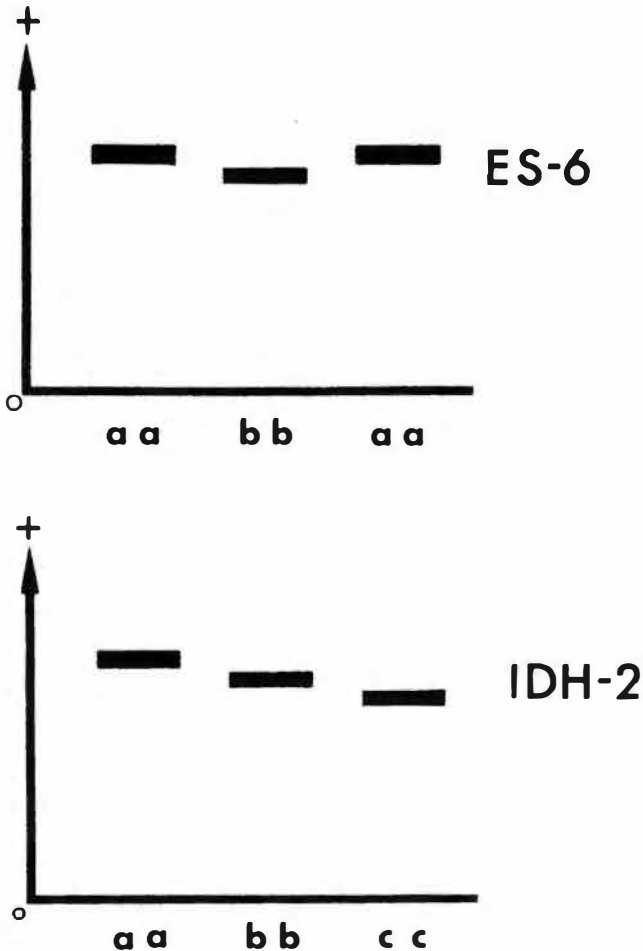


FIG. 2.—Electrophoretic variation among all members of the *Peromyscus truei* group studied. Above: variation in esterase-6 from hemolysate. Below: variation in isocitrate dehydrogenase-2 from kidney extracts.

1966). The results of this study revealed four alleles and two-banded heterozygotes (Fig. 3). This inconsistency may be a function of the resolution abilities of the techniques utilized.

Pgd-1a and Pgd-1b were found exclusively in *P. d. nasutus* and *P. d. griseus*, with Pgd-1b being fixed in *griseus* and the predominant allele in *nasutus* (Table 4). Pgd-1c and Pgd-1d were found in *P. comanche* and *P. truei*, with Pgd-1c being the predominant allele.

8) *Alpha-Glycerophosphate dehydrogenase* (liver).—Gpd-1a was fixed in all groups studies except *P. d. comanche* (Table 4). In this

TABLE 2.—Allele frequencies at ES-3 and ES-4 loci among members of the *Peromyscus truei* group studied.

Sample population	Number of animals	ES-3			Number of animals	ES-4	
		Es-3a	Es-3b	Es-3c		Es-4a	Es-4b
<i>P. comanche</i>	14	—	0.54	0.46	14	0.73	0.27
<i>P. d. griseus</i>	13	1.00	—	—	14	0.61	0.39
<i>P. d. nasutus</i>	13	1.00	—	—	14	0.56	0.44
<i>P. truei</i>	5	—	0.67	0.33	5	0.71	0.29

group, Gpd-1b also appeared, with heterozygotes having two bands (Fig. 3).

9) *Phosphoglucosmutases* (kidney, liver).—Three PGM systems appeared and were designated, in order of increasing anodal mobility, PGM-1, PGM-2, and PGM-3 (Fig. 4). PGM-1 and PGM-2 from liver were similar in mobility, but could be scored from the kidney extracts. PGM-1 was monomorphic in all groups, and PGM-2 and PGM-3 were polymorphic.

Both Pgm-2a and Pgm-2b were present in all groups, but there was a difference in frequency (Table 5). This was characteristic also in PGM-3 where two alleles, Pgm-3a and Pgm-3b, were present in all groups, but in different frequencies.

10) *Transferrin* (plasma).—Selander *et al.* (1971) and Rasmussen and Koehn (1966) reported this protein as highly polymorphic. Our results are similar. The gels were scored by first treating the plasma with Rivanol (Sutton and Karp, 1965), which precipitates proteins other than transferrin. Four alleles were noted and the heterozygotes had two bands (Fig. 5). The Trf-1a allele was fixed in *P. comanche* and *P. truei* samples (Table 6). Trf-1a, Trf-1b, and Trf-1d were found in *P. d. nasutus* with Trf-1b being the predominant allele. All four alleles were found in *P. d. griseus*, with Trf-1b the predominant allele.

TABLE 3.—Allele frequencies at ES-6 and IDH-2 loci among members of the *Peromyscus truei* group studied.

Sample population	Number of animals	ES-6		Number of animals	IDH-2	
		Es-6a	Es-6b		Idh-2a	Idh-2b
<i>P. comanche</i>	18	—	1.00	16	1.00	—
<i>P. d. griseus</i>	11	1.00	—	12	—	1.00
<i>P. d. nasutus</i>	16	1.00	—	17	—	1.00
<i>P. truei</i>	11	—	1.00	11	1.00	—

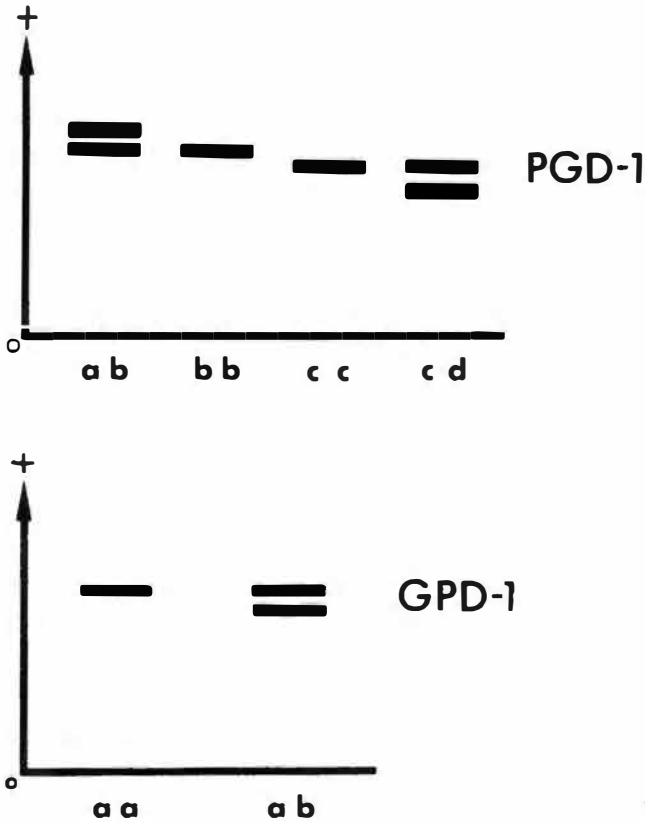


FIG. 3.—Electrophoretic variation among all members of the *Peromyscus truei* group studied. Above: variation in 6-phosphogluconate dehydrogenase-1 from hemolysate. Below: variation in alpha-glycerophosphate dehydrogenase-1 from liver extract.

11) *Albumin* (liver, plasma).—The bands of this protein were scored best from liver; they were narrower and sharper than in the plasma. Two albumin alleles were represented in the samples used in this study (Fig. 5). Alb-1a was fixed in *P. d. nasutus* and *P. d. griseus* (Table 6).

Brown and Welser (1968) found two forms of albumin in *Peromyscus difficilis*. Their results were based on two animals from laboratory stock whose progenitors were originally from Jacala, Hidalgo (*P. d. saxicola*). In this study, Brown and Welser found two albumin variants in seven specimens of *Peromyscus truei* from southwestern Colorado. The lack of albumin polymorphism in the mice in our study may result from restricted trapping localities.

TABLE 4.—*Allele frequencies at PGD-1 and GPD-1 loci among members of the Peromyscus truei group studied.*

Sample population	Number of animals	PGD-1				Number of animals	GPD-1	
		Pgd-1a	Pgd-1b	Pgd-1c	Pgd-1d		Gpd-1a	Gpd-1b
<i>P. comanche</i>	15	—	—	0.78	0.22	21	0.09	0.10
<i>P. d. griseus</i>	17	—	1.00	—	—	24	1.00	—
<i>P. d. nasutus</i>	12	0.07	0.93	—	—	13	1.00	—
<i>P. truei</i>	17	—	—	0.84	0.16	17	1.00	—

Monomorphic Proteins

The following proteins exhibited no variation in the samples used in this study:

1) *Esterase-7* (hemolysate).—This lightly staining system migrated only a short distance anodal to the origin on tris-maleic gels.

2) *Lactate dehydrogenase* (kidney).—Two LDH systems were observed on tris-citrate (pH 6.7) gels. As in other vertebrates (Markert, 1968), subunits of LDH-1 and LDH-2 associated in tetramers to form a five-banded pattern. The five, equally spaced anodal bands were uniformly monomorphic in all samples.

3) *Malate dehydrogenase* (kidney).—Two phenotypes, one NADP-dependent, were demonstrated in this study. The mitochondrial fraction of NADP-MDH (MDH-2) migrated cathodally, and, despite some subbanding, was determined to be monomorphic. The supernatant NAD-MDH (MDH-1) migrated anodally and likewise was uniformly monomorphic.

4) *Alcohol dehydrogenase* (liver).—This prominent system migrated cathodally on tris-citrate (pH 8.0) gels.

5) *Sorbitol dehydrogenase* (liver).—Migrating anodally, this monomorphic system appeared just above the origin on tris-citrate (pH 8.0) gels.

TABLE 5.—*Allele frequencies at PGM-2 and PGM-3 loci among members of the Peromyscus truei group studied.*

Sample population	Number of animals	PGM-2		Number of animals	PGM-3	
		Pgm-2a	Pgm-2b		Pgm-3a	Pgm-3b
<i>P. comanche</i>	16	0.67	0.33	19	0.80	0.20
<i>P. d. griseus</i>	17	0.33	0.67	16	0.14	0.86
<i>P. d. nasutus</i>	13	0.30	0.70	17	0.29	0.71
<i>P. truei</i>	13	0.67	0.33	15	0.75	0.25

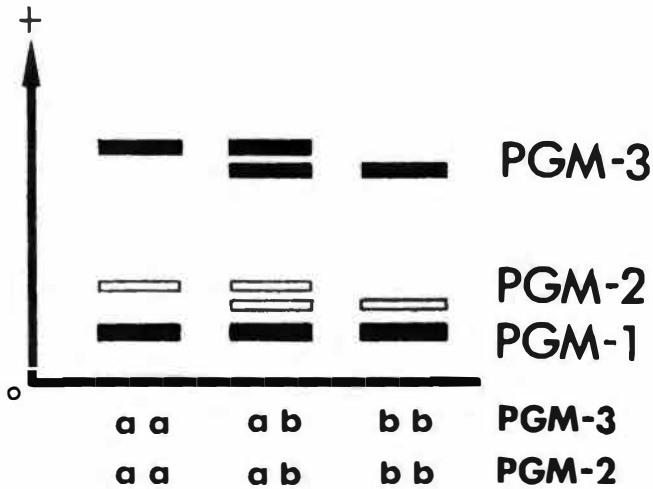


FIG. 4.—Electrophoretic variation among all members of the *Peromyscus truei* group studied. Variation in phosphoglucumutases-3 from liver extract and phosphoglucumutase-2 from kidney extract.

6) *Indophenol oxidase* (liver, kidney).—The pale bands of an IPO system appeared most prominently on Poulik (pH 8.7) gels previously stained for MDH and then left in the light. Only one anodal system was noted.

7) *Phosphoglucose isomerase* (liver).—Selander *et al.* (1971) found four alleles present at this locus in *Peromyscus polionotus*. However,

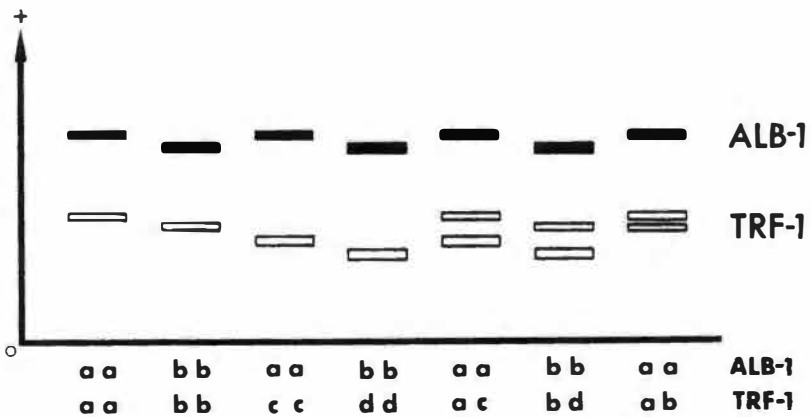


FIG. 5.—Electrophoretic variation among all members of the *Peromyscus truei* group studied. Variation in transferrin-1 from plasma and albumin-1 from liver extract.

TABLE 6.—*Allele frequencies at TRF-1 and ALB-1 loci among members of the Peromyscus truei group studied.*

Sample population	Number of animals	TRF-1				Number of animals	ALB-1	
		Trf-1a	Trf-1b	Trf-1c	Trf-1d		Alb-1a	Alb-1b
<i>P. comanche</i>	20	1.00	—	—	—	21	1.00	—
<i>P. d. griseus</i>	25	0.06	0.72	0.11	0.11	24	—	1.00
<i>P. d. nasutus</i>	13	0.03	0.94	—	0.03	13	—	1.00
<i>P. truei</i>	17	1.00	—	—	—	17	1.00	—

only one monomorphic, cathodally-migrating band was revealed in this study.

8) *Glutamate oxalate transaminases* (liver).—Both the mitochondrial, cathodally-migrating form of this enzyme (GOT-2), and the supernatant, anodally-migrating form (GOT-1), were determined to be monomorphic in the groups studied. An apparent heterozygote of GOT-2 appeared infrequently in *P. comanche*.

9) *Hemoglobin* (hemolysate).—Two anodally-migrating bands of equal intensity characterized all samples tested.

Genetic Comparisons

The proportion of loci polymorphic per population and heterozygous per individual was calculated for each member of the group studied (Table 7). These estimates of genetic variability were based on 25 loci. Consideration should be given to the fact that the proportion of polymorphic loci is strongly dependent upon sample size. This estimate was equal (22 per cent) in *P. d. nasutus*, *P. d. griseus*, and *P. truei*, and slightly higher (30 per cent) in *P. comanche*.

Individual heterozygosity was highest in *P. d. griseus* (8 per cent) and *P. comanche* (7 per cent). It should be noted that both of these have quite limited ranges and a restricted habitat. *P. d. nasutus* and

TABLE 7.—*Estimates of genetic variability among members of the Peromyscus truei group studied, based on 25 loci.*

Sample population	Number of animals	Proportion of loci	
		Polymorphic per population	Heterozygous per individual
<i>P. comanche</i>	21	0.30	0.07
<i>P. d. nasutus</i>	25	0.22	0.04
<i>P. d. griseus</i>	14	0.22	0.08
<i>P. truei</i>	17	0.22	0.04

TABLE 8.—*Genetic similarity between members of the Peromyscus truei group based on shared alleles at 25 loci.*

Sample population		1	2	3	4
<i>P. comanche</i>	(1)	1.00	0.64	0.64	0.96
<i>P. d. nasutus</i>	(2)	—	1.00	0.88	0.68
<i>P. d. griseus</i>	(3)	—	—	1.00	0.68
<i>P. truei</i>	(4)	—	—	—	1.00

P. truei, which occupy essentially similar habitats, had a mean heterozygosity value of 4 per cent.

An estimate of genetic similarity was computed by comparing the proportion of shared alleles at 25 loci (Table 8). The results show a great similarity between *P. comanche* and *P. truei*, and a comparably high similarity between *P. d. nasutus* and *P. d. griseus*.

DISCUSSION

Peromyscus truei and *Peromyscus difficilis* occur chiefly in montane habitats. They are saxicolous and most commonly associated with piñon-juniper stands in New Mexico and Colorado. However, *P. comanche* is found in canyons and on rocky, juniper-covered slopes on the eastern edge of the Llano Estacado. This escarpment forms an irregular border for some 200 miles on the east and is characterized by the presence of many steep-walled canyons that cut into the flat cap rock. West of the escarpment lies the Llano Estacado, a flat, arid plain approximately 100 miles wide that seemingly serves as an effective ecological barrier between *P. comanche* and its closest relatives in the *Peromyscus truei* group (*Peromyscus truei truei* and *Peromyscus difficilis nasutus*). Tamsitt (1959) reported finding *P. d. nasutus* among piñon pines in Quay County, New Mexico, some 80 miles distant from the nearest known population of *P. comanche* (in Palo Duro Canyon, Randall Co., Texas).

When Blair (1943) described *Peromyscus comanche*, he based his decision for specific distinction primarily on breeding studies, skeletal measurements, and external morphology. He found incomplete fertility in crosses between *P. comanche* and *P. d. nasutus*, due in part to "gross disturbances" in spermatogenesis in F₁ hybrid males. No crosses were made between *nasutus* and *truei*. Blair thought *comanche* resembled *nasutus* more than it did *truei*. He concluded that *comanche* was apparently derived from *nasutus* and, through geographic isolation, diverged into a distinct breeding population.

Hoffmeister (1951) suggested that *Peromyscus truei* had a center of dispersal from the southwestern United States, particularly the Colorado Plateau. Other members of the *truei* group differentiated from this parental stock. Perhaps at one time the progenitors of *P. comanche* had a continuous distribution across the plains, at least as far as the present eastern escarpment of the Llano Estacado. Considering the habitat preference of this group, the climate and vegetation must have been quite different then than now.

Blair (1950) thought the continuous distribution of this group across the plains may have existed as recently as late Wisconsin time (10,000 BP), when climatic shifts toward aridity began to occur. Wendorf (1970) described a late Pleistocene climatic event on the Llano Estacado. This event, named the Lubbock Subpluvial, was characterized by cooler summers and more effective precipitation than in that region today. Pollen spectra indicate that a boreal forest of pine and occasional spruce developed on the Llano at that time.

Considering these climatic conditions, it seems plausible that a spread of mice of the *Peromyscus truei* type occurred concurrent with boreal forests. With the onset of warmer temperatures, these forests receded toward the mountains of New Mexico and Colorado, leaving a remnant population associated with juniper along the eastern edge of the escarpment of the Llano Estacado.

The results of our study strongly suggest that *P. comanche* was derived directly from *Peromyscus truei* stock rather than from *P. nasutus* as suggested by Blair (1943). Protein systems of *truei* and *comanche* are similar genetically. In fact, they seem to be as closely related as those of *P. d. nasutus* and *P. d. griseus*. In only one instance (GPD) was an allele found in *comanche* and not in *truei*. Each time (except GPD) a system was polymorphic in *comanche*, that system was also polymorphic in *truei*. The results support the current taxonomy of *nasutus* and *griseus* as subspecies of *Peromyscus difficilis*.

The method of determining genetic similarity in this study is comparatively conservative. However, when compared with the results of Smith *et al.* (1973) and Johnson and Selander (1971), the similarity between *P. comanche* and *P. truei* on the one hand, and between *P. d. nasutus* and *P. d. griseus* on the other, places these groups well within the boundaries of conspecific taxa.

Despite the great similarity between *P. comanche* and *P. truei* revealed in our protein studies, they remain measurably different phenotypically in pelage and skeletal characteristics. If electrophoretic data are used as a supplement to other taxonomic data, we would

conclude that *Peromyscus comanche* is a distinct species and should be grouped with *Peromyscus bullatus*, *Peromyscus difficilis*, and *Peromyscus truei* within the *truei* group. Lee *et al.* (1972) pointed out that the karyotype of *comanche* is indistinguishable from that of *P. t. truei*. Evidently, sufficient time has not yet elapsed to permit significant changes in chromosomes. Nonetheless, the differences revealed in our studies, the results of breeding studies, and the allopatric nature of *comanche*, suggest this case to be that of a borderline insipient species.

We feel the results and conclusions of our study present a realistic biological evaluation of this group. The kind of information derived from this type of analysis would seem a more valid measurement of genetic compatibility and diversity than has hitherto been available.

CONCLUSIONS

1) Analysis of four members of the *Peromyscus truei* species group in Texas and New Mexico revealed approximately 40 electrophoretic protein systems.

2) Twelve of the 25 systems scored were polymorphic in one or more members of the group.

3) Estimates of genetic variability revealed slight variation in both the proportion of loci polymorphic per population and heterozygous per individual, but the significance of this variation was not readily ascertained.

4) Calculations of genetic similarity based on shared alleles at 25 loci revealed a high degree of similarity between *Peromyscus difficilis nasutus* and *P. d. griseus*. An even greater similarity was demonstrated between *Peromyscus comanche* and *Peromyscus truei truei*.

5) The present classification of *griseus* and *nasutus* as subspecies of the same species (*P. difficilis*) is reaffirmed.

6) Based on the genetic similarity, *P. comanche* is suggested to have been derived directly from a parental *Peromyscus truei* stock, perhaps isolated geographically as recently as late Wisconsin time (10,000 BP).

7) Coupling this electrophoretic data with other taxonomic considerations, *P. comanche* is concluded to be a distinct species within the *Peromyscus truei* group.

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