ELECTROPHORETIC VARIATION AMONG NATURAL POPULATIONS OF *PEROMYSCUS MANICULATUS*

by

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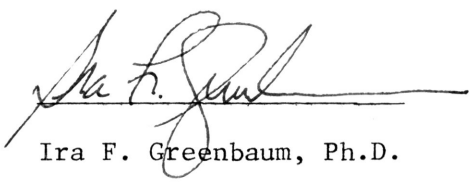

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ABSTRACT

Electrophoretic Variation Among Natural

Populations of Peromyscus maniculatus.

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Allozymic variation at 20 loci determined by isozyme electrophoresis was examined for 10 populations of Peromyscus maniculatus including a total of 159 specimens representing 3 subspecies and 5 ecotypes ranging from Baja California to Oregon. Electrophoretic data reveal that although populations of P. maniculatus exhibit considerable genic polymorphism, there is a high degree of genetic similarity between all populations. This high degree of similarity suggests that gene flow between the populations is not inhibited.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Speciation, the process by which one species becomes two, is the critical step in the evolutionary process. It is through the attainment of reproductive isolation that a group of once genetically homogeneous populations become genetically and evolutionarily distinct. Although speciation is of necessity a genetic phenomenon, little is known about the genetic processes which result in speciation. This paucity of information is primarily the result of the difficulty in identifying groups which are actively undergoing speciation (Baker, 1979). As a result, most genetic data relevant to this question result from studies of recently speciated groups. Studies of conspecific populations which display karyotypic polymorphisms potentially provide unique situations in which to study the genetics of speciation (Baker, 1979; Greenbaum, 1980).

As detailed in the following literature review, Peromyscus maniculatus displays inter- and intrapopulational chromosomal variability which suggests that speciation may be ongoing and that genetic investigations of various chromosomal and ecological races of this species may provide valuable insight into the genetic mechanisms of speciation.

Ten populations of the Deer Mouse, Peromyscus maniculatus, ranging from Baja California to Oregon were examined and analyzed electrophoretically to investigate genetic differences. These

populations represent 5 ecologically different habitats. Three populations represent a desert habitat, two from the Desierto de Altar and one from the Mohave Desert. A coniferous forest habitat is represented by three poulations, one from the San Pedro Martir Mountains and two from the San Bernadino Mountains. Two populations are from northern temperate regions and represent a coastal deciduous forest habitat. The two remaining populations represent a northern dry grassland and a northern wet grassland, respectively.

REVIEW OF LITERATURE

Peromyscus has been widely utilized as a model for study of evolutionary and genetic processes in mammals. This genus provides a potentially valuble biological situation in which to study the genetic processes of speciation. *Peromyscus* is a very speciose group consisting of about 50 species (Hooper, 1968). Speciation has therefore been a common phenomenon of this genus. *Peromyscus* also displays wide geographic and ecological ranges.

Peromyscus maniculatus is probably the most widespread and abundant small mammal in North America. Its populations range from Southern Mexico to the Northwest Territories of Canada and from the Pacific to the Atlantic seaboards (Hall and Kelson, 1959). This species occupies unusually diverse habitats ranging from arid grasslands and deserts to humid deciduous and coniferous forests. Morphological differences are also profound with more than 65 subspecies described (Hall and Kelson, 1959). In addition to the extreme morphological differences, *P. maniculatus* exhibit extreme chromosomal polymorphism. Even though a diploid number of 48 remains constant for all *Peromyscus*, the number of biarmed autosomes ranges from 16 to 88 (Bradshaw and Hsu, 1972; Greenbaum *et al.*, 1978).

Avise *et al.* (1979) examined allozymic variation at 22 loci for scattered populations of *P. maniculatus* from across their range. These authors report little genetic divergence among populations from widely separated geographic localities. In their analysis, some

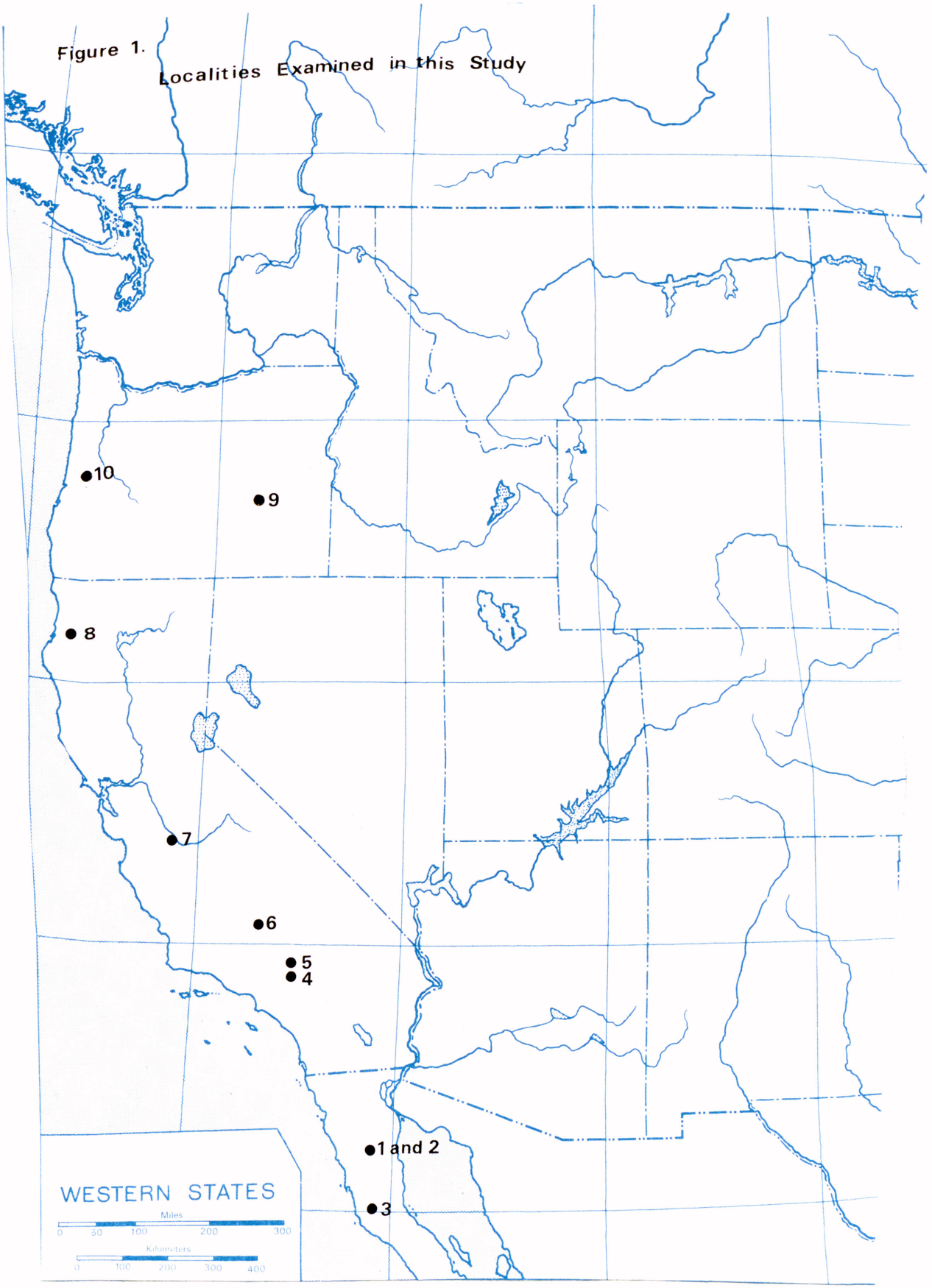
of the subspecies were pooled without regard to morphological or chromosomal differences. This study is designed to detect possibly cryptic genetic differences among populations of P. maniculatus from the Pacific coast. These data will be particularly relevant in light of recently advanced theories regarding the population gentic parameters effecting chromosomal changes over the course of time (Bush, 1975; Bush et al., 1977; Wilson et al., 1975; Wilson et al., 1977).

METHODS AND MATERIALS

Allozymic variation was examined and analyzed for 159 specimens of Peromyscus maniculatus representing 10 populations and 3 subspecies. The geographic localities of these populations are listed below and shown in Figure 1. The number in parentheses refer to sample designations used in the tables and figures. P. maniculatus gambelii-(1) Baja California: 16mi. S, 5mi. E, Valle de Trinidad, n=19; (2) Baja California: 8mi. S, 9mi. E, Valle de Trinidad, n=14; (3) Baja California: Laguna Hanson, n=21; (7) California: Fresno, San Joaquin Experimental Range, n=21. P. maniculatus sonoriansis- (4) California: San Bernadino, Heart Bar Campground, n= 25; (5) California: 2.1mi. S, Boulder Bay, n=3; (6) California: 9mi.NNE, Johannesburg, n=5; (9) Oregon: 28mi. S, 6mi. E, Burns, n=20. P maniculatus rubidus- (8) California: 8mi. N, 5mi. E, Arcata, n=10; (10) Oregon: 9mi. S, Alcea, n=20.

Mice were live trapped and karyotyped by the procedures described by Baker (1970). The heart, liver, kidneyand eyes of all the specimens were extracted immediately after sacrifice and stored in liquid nitrogen. The specimens were prepared for morphological analysis and deposit in The Museum of Southwestern Biology, University of New Mexico. Preparation of tissues for electrophoretic analysis, described by Selander (1971), entailed homogenation in an EDTA buffer, high speed centrifugation, and reclamation of the tissue supernatant. Heart and kidney samples were processed together and

Figure 1. Localities Examined in this Study



homogenates were stored at 90°C. Techniques of electrophoresis and enzymatic and non-enzymatic staining were similar to those described by Selander (1971) and Greenbaum and Baker (1976). The specific buffer systems and biochemical stains used are listed in Table 1. Gels were made using 50% Sigma Starch #S-4501 and 50% Electrostarch Lot. 392 Otto Hiller, Madison, Wisconsin.

At each locus the allele occuring in the highest frequency in P. maniculatus received a designation of 100 if migration was anodal or -100 if migration was cathodal. All other alleles were designated numerically as to the percent of migration relative to the 100 (or -100) allele. The numerical designation of "1" was assigned to the isozyme of each system having the greatest mobility. Isozymes with progressively slower migration received progressively higher numerical designations. Direct side-by-side comparisons of allozymic mobility on the same gel were employed in comparing alleles.

Loci were considered to be polymorphic if the frequency of the most common allele was equal to or less than 0.95. Calculations of genic variability (heterozygosities) were determined by direct count.

Three measures of genic distinctness were calculated for all populations: Rogers' coefficient of similarity, S(Rogers,1972), Nei's coefficient of distance, D(Nei,1973), and I, the electrophoretic identity between taxa for a given number of average codon differences per locus (Nei,1973).

Table 1.--Gel types and biochemical staining procedures used in this study.

Gel Type	Tissue	Voltage or Milliamperage	Time (hrs.)	Stains
Continuous Tris-Citrate I (pH=6.7)	Kidney	75 ma	5	Isocitrate dehydrogenase (Idh) Lactate Dehydrogenase (Ldh) Malate Dehydrogenase (Mdh) Tetrazolium Oxidase (To)
Continuous Tris-Citrate II (pH=8.0)	Liver	75 ma	6	a-Glycerophosphate Dehydrogenase (a-Gpd) Alcohol Dehydrogenase (Adh) Glutamic Oxylate Transaminase (Got) Sorbitol Dehydrogenase (Sdh) Glutamate Dehydrogenase (Gdh)
Tris-Maleate Dilute 1:2	Kidney	75 ma	6	Albumin (Alb) Phosphoglucomutase (Pgm) Phosphoglucoisomerase (Pgi)
Discontinuous Tris-Citrate (Poulik)	Kidney	250 V	4	Glucose-6-Phosphate (G6P)
Lithium Hydroxide	Eye	350 V	5	Esterase (Est)

RESULTS

Electrophoretic Patterns of Variability.- Allozymic variation was examined at 20 loci for the 10 localities assayed (Table 2). Twelve loci (60%) were monomorphic for the same allele in all the samples examined as follows: Got-2, Sdh, Gdh, G-6-P, Mdh-1, Mdh-2, Ldh-1, To-1, To-2, Pgm-1, Pgm-2, Pgi. The common "100" allele was the only allele displayed at each monomorphic locus and occured consistently for all ten populations. Eight of the loci (40%) studied exhibited multiple alleles at two or more of the localities except the Ldh-2 locus, which was polymorphic at only on locality. The alleles and their frequencies at each locus are presented for each population in Table 2. Variation from the 100 allele was found in only one population, 8 at the Ldh-2 locus and in two populations, 3 and 10 for the Idh-1 locus, for which the allelic variations were not the same. There were several different minor alleles present at two or more populations for the Est-5 and Adh loci. Idh-2, Got-1 and Est-5 exhibited the largest amount of polymorphism. At the Got-1 and Idh-2 loci, the minor allele was present in every population at relatively constant frequencies.

Genic Variablity.-Calculations of genic variability per population are presented in Table 3. The mean number of loci heterozygous per individual at each locality ($\bar{H}$) ranges from .044 to .083 and averages .067. These values are in the upper range of those noted for mammals (Avise et al., 1971).

Table 2.--Alleles and frequencies for polymorphic loci examined.

Population		1	2	3	4	5	6	7	8	9	10
Locus	Allele										
Alb	100	.868	.929	.875	1.0	1.0	1.0	.976	.950	.875	.975
	108	.132	.071	.075				.024	.050	.125	.025
	84			.050							
Est-5	100	.789	.500	.675	.860	.833	.700	.762	.800	.825	.925
	127	.211	.500	.275	.060	.167	.200	.214		.125	.075
	118							.024			
	74			.050	.080		.100		.100	.050	
	51								.100		
Adh	-100	1.0	1.0	.950	.960	1.0	1.0	.929	.900	.900	.850
	-117									.025	
	-97			.025	.040			.048	.050		.100
	-82							.023			.025
	-77								.050	.075	.025
	-51			.025							
a-Gpd	100	.974	1.0	.950	.960	1.0	1.0	1.0	1.0	.950	.950
	117	.026		.050	.040					.050	.050

Table 2.--(continued).

Population		1	2	3	4	5	6	7	8	9	10
Locus	Allele										
Ldh-2	100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.900	1.0	1.0
	52								.100		
Idh-1	100	1.0	1.0	.900	1.0	1.0	1.0	1.0	1.0	1.0	.850
	123			.100							
	91										.150
Idh-2	-100	.711	.607	.725	.800	.833	.600	.714	.722	.850	.875
	-52	.289	.393	.275	.200	.167	.400	.286	.278	.150	.125
Got-1	100	.737	.857	.700	.800	.500	.700	.571	.500	.675	.550
		.263	.143	.300	.200	.500	.300	.429	.500	.325	.450

Table 3.--Heterozygosity estimates ($\bar{H}$) calculated as the mean number of loci heterozygous per individual at each locality.

Population	$\bar{H}$	Population	$\bar{H}$
1	.081	6	.064
2	.077	7	.060
3	.074	8	.070
4	.044	9	.073
5	.083	10	.045

Genic Similarity and Distance.-Coefficients of genic similarity, S and distance, D were calculated for paired combinations of all localities based on the 20 loci examined in this study (Table 4). Nei's identity coefficient (I) was also calculated for all populations and are presented in Table 5. Average similarities ($\bar{S}$), identities ($\bar{I}$), and distances ($\bar{D}$) are presented in Table 6 for all populations. Similarity values ranged from .9830 between populations 5 and 7 to .9312 between populations 2 and 10. Distance values ranged from 0014 between populations 5 and 7 to .0213 between populations 2 and 10. Nei's identity values ranged from .9986 to .9790 between populations 5 and 7, and 2 and 10, respectively. All three measures of genetic distinctness indicate little difference between the ten populations.

Table 4.--Genic similarity (S;Rogers, 1972) above diagonal and genetic distance (D;Nei and Chakraborty, 1973) below for paired combinations of localities studied.

	1	2	3	4	5	6	7	8	9	10
1	----	.9700	.9799	.9766	.9720	.9800	.9804	.9646	.9802	.9551
2	.0061	----	.9622	.9597	.9507	.9750	.9611	.9451	.9514	.9312
3	.0015	.0054	----	.9679	.9609	.9737	.9751	.9611	,9744	.9591
4	.0025	.0114	.0040	----	.9745	.9735	.9726	.9657	.9779	.9662
5	.0048	.0158	.0052	.0055	----	.9724	.9830	.9756	.9748	.9731
6	.0022	.0054	.0026	.0040	.0058	----	.9792	.9642	.9675	.9512
7	.0024	.0094	.0024	.0044	.0014	.0022	----	.9790	.9726	.9698
8	.0059	.0182	.0064	.0061	.0026	.0054	.0025	----	.9666	.9646
9	.0020	.0125	.0029	.0029	.0031	.0055	.0029	.0041	----	.9712
10	.0071	.0213	.0069	.0056	.0029	.0097	.0043	.0044	.0035	----

Table 5.-- Nei's identity coefficient values (I, Nei, 1973).

	1	2	3	4	5	6	7	8	9	10
1	----	.9939	.9985	.9975	.9952	.9978	.9976	.9941	.9980	.9929
2		----	.9946	.9887	.9844	.9947	.9906	.9820	.9876	.9790
3			----	.9960	.9948	.9974	.9976	.9937	.9971	.9931
4				----	.9945	.9960	.9956	.9939	.9978	.9945
5					----	.9943	.9986	.9974	.9969	.9971
6						----	.9978	.9946	.9946	.9904
7							----	.9975	.9971	.9957
8								----	.9959	.9956
9									----	.9965
10										----

Table 6.--Averages for similarity ($\bar{S}$), distance ($\bar{D}$) and identity ($\bar{I}$) values.

	Values
$\bar{S}$	.9681
$\bar{D}$	.0056
$\bar{I}$	.9700

DISCUSSION

Chromosomal evolution at the subspecies level has commonly been considered as a primary speciation mechanism (White, 1978a; 1978b). Such theories are based on the meiotic disruption which supposedly accompanies heterozygosity for structural rearrangements of chromosomes. Although analysis of chromosomal data for the populations studied here is not complete, the 10 populations examined represent samples from parts of the range of P. maniculatus known to be karyotypically diverse (Bradshaw and Hsu, 1972). Cytogenic evolution in Peromyscus has been reported to involve two types of structural rearrangements, pericentric inversions amd additions of heterochromatin (Arrighi et al., 1974; Greenbaum et al., 1978a, 1978b; Greenbaum and Baker, 1978). Although heterochromatin alterations are not thought to be associated with negative heterosis, crossing over in inverted segments should result in a loss of 50% gamete viability. Within P. maniculatus both kinds of rearrangements have been recorded and G-band analysis has documented an intra-populational inversion polymorphism for a population from Iowa (Greenbaum et al., 1978a).

The genic data presented here indicate that the P. maniculatus, over the range sampled, represent a panmictic population. Effective gene flow among these populations is indicatd by the occurence of the same alleles and cosistent gene frequencies at most loci. Neither

habitat nor cytotype appears to inhibit gene flow among the populations sampled. These findings are most consistent with a hypothesis that chromosomal variation in west coast P. maniculatus is the result of heterochromatin differences. However, considering the extent of reported chromosomal variation and the documented potential for pericentric inversions in this genus, it is unlikely that all of the variation is explained by heterochromatin polymorphisms. The lack of detected gene flow barriers suggests that in P. maniculatus, pericentric inversions may not impose the level of negative meiotic heterosis commonly thought to accompany inversion heterozygosity. These and other recent data (Baker, 1979; Bickham and Baker, 1979; Greenbaum, 1980) suggests that genetic drift and small effective population size may not be prerequisite to chromosomal evolution. Such a deme size dependent model has been recently hypothesized as the primary, and perhaps only mechanism for karyotypic evolution. Considering however, that chromosomal rearrangement fixation may not be inhibited by negative heterosis, then selection may be implicated as the primary affector of this process (Bickham and Baker, 1979; Baker, 1980; Greenbaum, 1980).

The consistency in the genic data suggest that, in this species, sympatric speciation as the result of chromosomal evolution, such as the stasipatric or chain process models described by White (1968. 1976b), is unlikely as such a process is dependent upon the establishment of a chromosomally mediated gene flow barrier. Subsequently, it appears that although karyotypic evolution is likely to be sympatric, speciation may be primarily the result of allopatric separa-

tion. Such a hypothesis is consistent with reports of the speciation process of other maniculatus group species such as P. melanotis, P. leucopus, and P. oreas (King, 1968; Greenbaum et al., 1978a, 1978b; Greenbaum and Baker, 1978).

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