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Genetic variation in populations of Peromyscus maniculatus in northwestern Montana

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GENETIC VARIATION
IN POPULATIONS OF *PEROMYSCUS MANICULATUS*
IN NORTHWESTERN MONTANA

By

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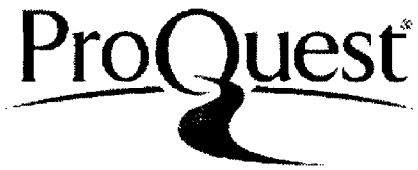

ABSTRACT

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Genetic variation in populations of Peromyscus maniculatus in northwestern Montana (80pp.)

Director: Fred W. Allendorf

The genetic relationships among populations of two subspecies of the deer mouse, Peromyscus maniculatus, were investigated using electrophoresis. Examination of 18 biochemical loci in 300 mice revealed high levels of protein variability (average heterozygosity equal to 11.6 per cent). Genetic similarity values among populations are within a range common for conspecifics. Analysis of the pattern of genetic variation reveals two main groups which are not identical with the subspecific designations. The genetic similarity pattern, however, does correlate well with the pattern of one morphological trait, tail length. The origin of the differentiation is presumed to be glacial isolation. This pattern persists today presumably due to recent separation and restrictions in gene flow.

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CHAPTER I

INTRODUCTION

The goal of evolutionary biology is to understand the differentiation of populations, both the process itself and the resulting differences among groups of organisms. Delineation of taxonomic relationships is an exercise in the detection and measurement of this differentiation. Understanding the nature of the evolutionary processes involved in the creation and maintenance of these differences, however, is a separate endeavor. The science of systematic zoology combines these two goals by seeking to understand the origin and maintenance of differentiation while describing particular taxonomic relationships.

Most taxonomic work prior to 1950 was based upon morphological characteristics. Thus, the bulk of our classification relies upon these criteria. Only recently have other criteria been incorporated in the taxonomic process [e.g., behavioral, physiological, ecological, cytological, and biochemical parameters (Sneath and Sokal, 1973)].

Application of electrophoresis to the field of population genetics allows use of another taxonomic tool. This biochemical technique separates specific structural proteins which exhibit differential movement in an electric field due to differences in charge and shape of the molecule. Variation in migration indicates differences in protein structure which often have a simple genetic basis. Thus, by electrophoretic separation of proteins, variation at many genetic loci can be directly examined.

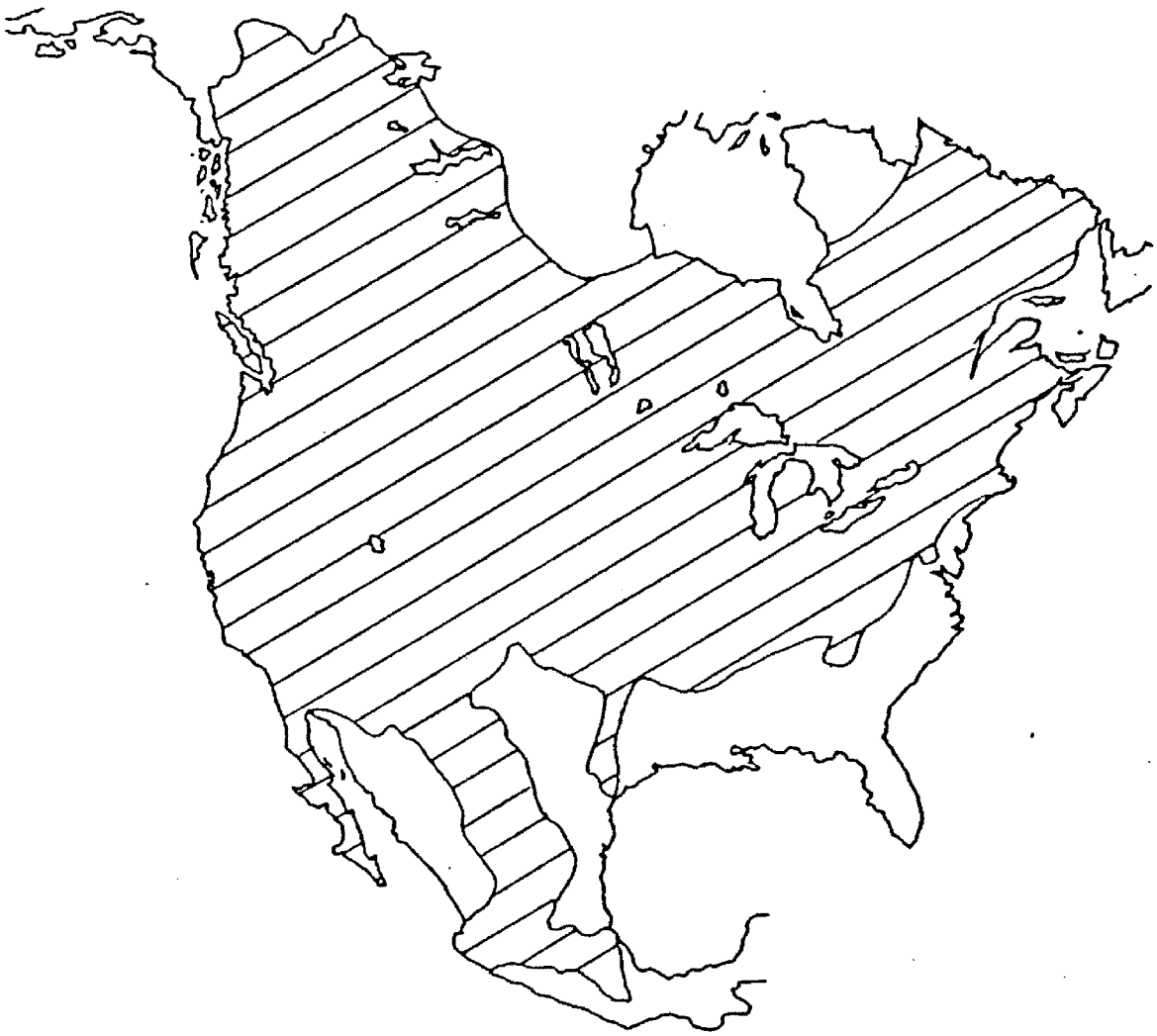
Knowledge of the variation present at particular loci in an organism's genome has certain advantages over more conventional taxonomic criteria: 1) designation of differential protein migration is an objective procedure, 2) most of the loci currently employed by biochemical systematists do not exhibit age or sex specific variation, which is a common problem with other taxonomic parameter, and 3) knowledge of the genetic basis of the variation observed is important and is often lacking for morphological, behavioral, and physiological data (Avise, 1975). This knowledge of genetic variation through space is potentially powerful in describing the current patterns of gene flow between groups.

Since 1966, electrophoretic data have been accumulating for a variety of organisms. There is a high correlation between the taxonomic groupings generated by other criteria and those defined by analysis of electrophoretic data. This fact, plus the advantages already mentioned, indicate that electrophoresis is a valuable systematic tool (see review by Avise, 1975).

The deer mouse, Peromyscus maniculatus, is widespread over North America (Fig. 1) and occurs in a wide variety of habitats. This species exhibits a great deal of geographic variation, in fact, 66 subspecies of the deer mouse (including insular forms) were recognized by Hall and Kelson (1959).

Among these subspecies there are predominatly two morphological forms whose ranges are contiguous or overlapping. These two forms of P. maniculatus include a large-bodied, long-tailed group of forest dwellers and a smaller, short-tailed group that lives primarily on the

Fig. 1. -- Distribution of *Peromyscus maniculatus* in North America (modified from Hall and Kelson, 1959).



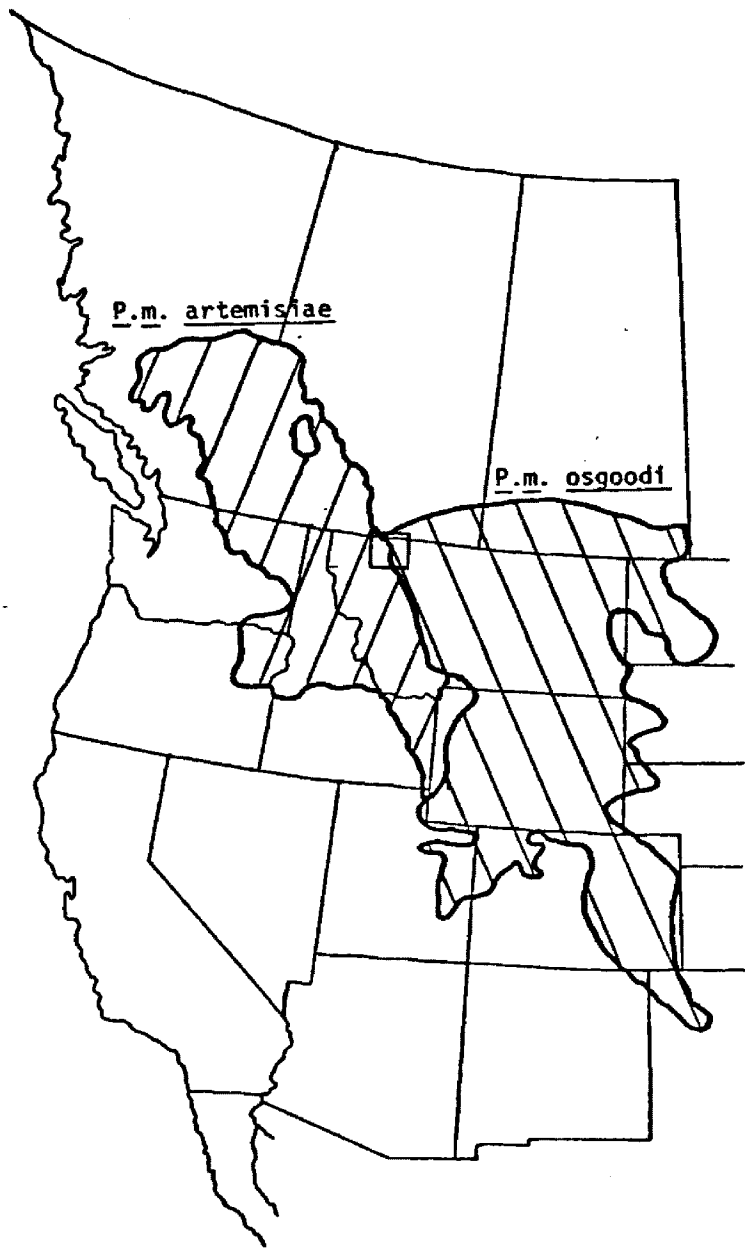
prairie and in other open habitats (Hooper, 1968). The long-tailed form occupies an area extending from the Rocky Mountains and coastal forests of the western United States, eastward across Canada, and southward in the Appalachian Mountains, while the short-tailed form occupies the central grasslands and western deserts (Blair, 1950; Hooper, 1968).

The presence or absence of interbreeding between these two forms was inferred from the pattern of morphological change at contact areas. If there was no suggestion of direct gene exchange from the morphological data (i.e., intergradation) (Blair, 1950), then it was usually postulated to occur by way of a circuitous route through other populations.

Numerous documentations of contact areas between long-tailed and short-tailed forms exist in the literature (Blair, 1950; Dice, 1944, 1949; Glazier, 1971; Hooper, 1942, 1968; Murie, 1933; Osgood, 1909). This study is concerned with a particular site at the junction of the ranges of P. m. artemisiae and P. m. osgoodi in northwestern Montana (Fig. 2). The zone of contact was first documented by Osgood (1909) when he made his revision of the genus Peromyscus. For each subspecies he described the geographic distribution, general habitat associations, and physical characteristics.

Specimens, representing both subspecies, taken from the St. Mary Lake area in Montana were interpreted by Osgood as being distinct morphologically and, therefore, not influenced by past hybridization. Subspecies delineation was retained since Osgood concluded there was intergradation indirectly between the two forms in a "ring of races." This conclusion was founded by the geographical pattern of morphological change observed. He stated that P. m. artemisiae intergraded to the

Fig. 2. -- Distribution of *Peromyscus maniculatus artemisiae* and *P. m. osgoodi* after Hall and Kelson (1959). The square indicates the study area in northwestern Montana.



south with P. m. sonoriensis which intergraded with P. m. rufinus to the east and, finally, with P. m. osgoodi. Mayr (1942) described a zone of contact between both forms and P. m. arcticus, now called P. m. borealis.

Murie (1933) and Glazier (1971) made further investigation of the area of contact at St. Mary Lake in Glacier National Park. In this restricted geographic area, both authors found certain morphometric measurements to be distinctive, with only occasional overlap.

Each of these investigators mentioned a habitat association of P. m. osgoodi, the short-tailed form, with prairie and P. m. artemisiae with forested areas. These habitat preferences may contribute to the apparent reproductive isolation, i.e., the abrupt change in morphological traits, by simply reducing contact and therefore gene flow. However, both Murie (1933) and Glazier (1971) discussed a number of exceptions to this habitat restriction, indicating potential physical contact and interaction of the two forms.

Murie (1933) concluded that gene exchange is nonexistent or extremely limited between the two forms. Admitting that knowledge of other contact areas was lacking, he stated that P. m. osgoodi and P. m. artemisiae at the St. Mary locality could be considered distinct species.

These statements by Murie led to the inclusion of this particular subspecific contact zone by Mayr (1942) in his discussion of evidence for the process of geographic speciation of animals. Mayr stated that, "...the two forms would undoubtedly be considered good species if the

chain of intergrading races now connecting them was broken."

Glazier (1971) came to a similar conclusion about the lack of interbreeding. However, though rigorous analysis was lacking, his morphometric data revealed a few animals with intermediate measurements, suggesting occasional hybridization. Thus, the previous belief of complete reproductive isolation became questionable.

The efforts of these investigators have contributed to the understanding of this particular zone of contact between these two forms; yet many questions remain unanswered. There are apparently two forms of P. maniculatus in northwestern Montana. The contact zone appears to be very narrow, since the morphological change is abrupt. This pattern of long- and short-tailed forms occurs repeatedly in P. maniculatus. By acquiring additional information on the relationships between these two morphological races, the taxonomic status of the deer mouse may be clarified and some insight gained into the nature of evolutionary change.

The pattern of genetic biochemical variation in these two deer mouse subspecies found in a microgeographic area that includes their contact zone was investigated in this study. The objectives were threefold: 1) to describe the pattern of genetic variation within and among populations of these mice, and compare the levels of genetic similarity and variability to those observed in the species as a whole, 2) to reassess a previously defined taxonomic situation using additional criteria, and 3) to explore the possible evolutionary processes involved in the production and maintenance of any differentiation observed.

CHAPTER II

METHODS AND MATERIALS

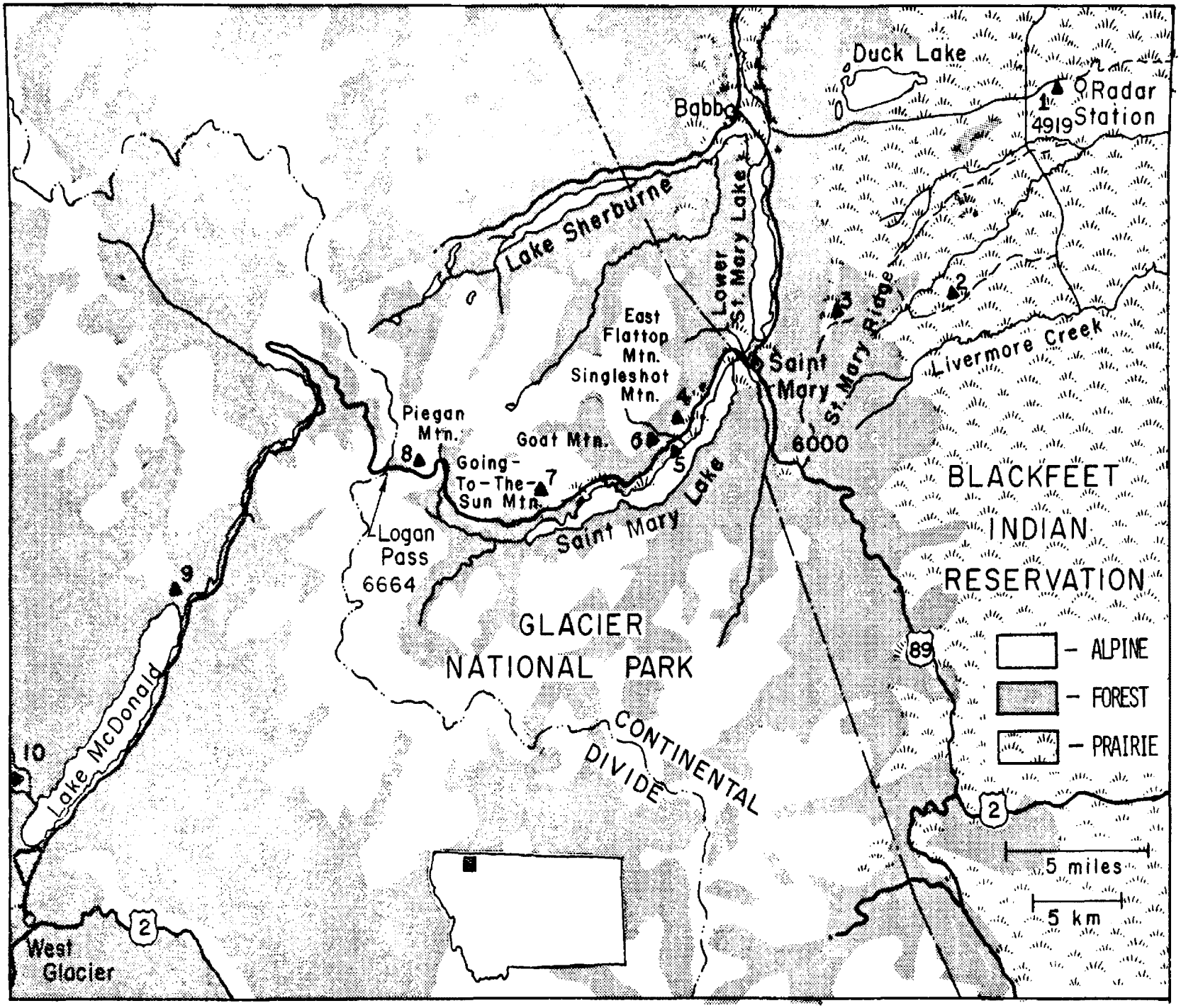
Description of the Study Area

The study area is located in the St. Mary and Lake McDonald Valleys of Glacier National Park and extends onto the prairie of the Blackfeet Indian Reservation (Fig. 3). A transect from east to west (site 1 through 10 respectively), crossing the continental divide, was established. This study encompassed a slightly larger geographic area than either Murie's or Glazier's. The boundary previously described between the two subspecies, occurs between sites 5 and 6.

Both the St. Mary and McDonald Valleys were glacially carved during the Pleistocene. These valley areas have been revegetated and invaded by animal populations during the intervening 10,000 years. Unglaciated areas both within the mountains and south and east of the glaciated terrain, may have served as refugia and recolonization sources for both plants and animals.

The habitats encountered in this study include those characterized by Glazier (1971), as well as several additional types. East of the continental divide, study sites are located primarily in grassland and coniferous forest (Douglas fir and lodgepole pine associations). Sites 1 and 2 and most of 4 are located on grassland areas. Site 3 is forested plus a clear cut area. Site 5 is located on a gravel beach adjacent to both grassland and forest. Site 6 is located primarily in a forest

Fig. 3. -- Map of the study area in northwestern Montana. Collection sites are indicated by solid triangles and numbered 1 through 10.



of Douglas fir and lodgepole pine. Sites 7 and 8 are located above the tree line and are therefore classified as alpine. West of the continental divide, sites 9 and 10, another forest association of cedar and hemlock occurs. In summary, sites 1, 2, and most of 4 are considered prairie sites; sites 3, part of 4, 5, 6, 9, and 10 are forested; and sites 7 and 8 are located in alpine areas. The distribution of these three habitat types (prairie, forest, and alpine) is shown in Figure 3.

Collection of Animals

Mice were collected between June and September of 1975 along the transect using Sherman folding live-traps (5X5X16cm) baited with peanut butter. Collection throughout the summer was unavoidable, but does assume that the genetic variation of the populations sampled is stable over time. Traps were generally placed approximately 15 meters apart, in lines. The lines were set in the early evening and were checked shortly after sunrise the following day. The number of trap-nights, per cent success and dates of collection are given in Appendix I. All animals collected from one site are referred to as a population.

Sample Preparation

The mice were returned to the laboratory at the University of Montana where they were caged until collection was completed. A mixture of chloroform, alcohol, and ether was used to anesthetize animals prior to taking morphological measurements and collecting blood and tissues (liver, heart, and kidney). The blood was centrifuged at

4000 g for 4 minutes, separating the plasma and cells. The cells were rinsed twice in saline buffer before freezing along with the tissues at -40°C. The tissues were homogenized, liver separately and heart and kidney together, using an electric tissue grinder. These homogenates were centrifuged for 15 minutes at 18,000 rpm. The supernatant was removed and again stored at -40°C. This procedure was that described by Selander et al. (1971).

Electrophoresis

The majority of the horizontal starch gel electrophoresis was performed at the Savannah River Ecology Laboratory associated with the University of Georgia, consequently the procedures employed there were adopted (Manlove et al., 1975). To make a gel, 46.3 grams of electrostarch (Lot 302) were dissolved in 400 ml of buffer and the solution was heated to boiling before being poured into plexiglass gel molds (9x180x210 cm). The gel was cooled (usually overnight) before a slit was cut into it, 6 cm from one end. Filter paper wicks, soaked with sample, were inserted into this slit. Sponges connected the gel to the buffer solution which in turn was connected to a power pack supplying DC current. The amount of current and the duration of the run depended on the buffer system employed and the particular rate of protein migration. These combinations of buffer systems, tissues, and proteins stained are listed in Table 1. Appendix II gives the relative chemical components of the buffer systems used, the voltages, and the duration of the run (Selander et al., 1971). The histochemical

TABLE 1. -- Loci examined, abbreviations assigned, alleles observed, and tissues examined (K-H, kidney and heart mixture; L, liver; Pl, plasma). Roman numerals indicate the buffer system used (for description see Appendix II).

Locus	Enzyme	Tissue	Alleles Observed	Buffer
ADA	Adenosine deaminase	K-H	100, 120	II
AGPD	Alpha-glycerophosphate dehydrogenase	L	53, 100, 127	V
GDH	Glutamate dehydrogenase	K-H	100	VI
GOT-1	Glutamate oxalate transaminase	L	72, 100, 136	V
GOT-2		L	-100, -78	V
IDH-1	Isocitrate dehydrogenase	K-H	83, 100, 115	IV
IDH-2		K-H	100, 217	V
IPO	Indophenol oxidase	K-H	100	VI
LDH-2	Lactate dehydrogenase	K-H	100, 245	IV
MDH-1	Malate dehydrogenase	K-H	100	IV
MDH-2		K-H	80, 100, 120	IV
ME	Malic enzyme	K-H	75, 100, 113 125	IV
PEP-2	Peptidase	Pl	100	I
PGI	Phosphoglucoisomerase	Pl	-150, -100, 80	I
PGM-2	Phosphoglucomutase	K-H	71, 83, 88 100, 120	III
6PGD	6-Phosphogluconate dehydrogenase	K-H	75, 88, 100 119, 138	VI
SDH	Sorbitol dehydrogenase	L	100	V
TRF	Transferrin	Pl	91, 100, 106	I

staining procedures employed are primarily those of Selander et al. (1971) and Shaw and Prasad (1970).

The selection of protein systems used in the final analysis was based on the clarity of banding and the presence of a banding pattern consistent with a genetic explanation. In addition, certain loci possessed alleles with very slight differences in mobility; since these loci could not be consistently scored, they were deleted. Consequently, several loci usually included in Peromyscus investigations are not represented.

CHAPTER III

RESULTS AND DISCUSSION

Description of Protein Systems

Fifteen biochemical systems (i.e. enzymes) representing eighteen genetic loci from 300 mice were examined using electrophoresis. Five of the eighteen loci were monomorphic in all population samples.

The majority of these protein systems have been previously reported for deer mice or closely related rodents (Rasmussen, 1968; Selander et al., 1971; Avise et al., in preparation), so that the molecular structure of the enzymes and the existence of Mendelian inheritance are already known. The nomenclature for these systems consists of abbreviations and a hyphenated numeral if multiple forms of the same enzyme occur. The loci were numbered in ascending order from the furthest anodally migrating to those cathodally migrating. The allelic variants were designated according to the relative electrophoretic mobility. One allele, generally the most common one was assigned 100; all other alleles were then assigned a numerical value representing their protein mobility relative to the 100 allele product. Any allele with a protein product which migrated cathodally was indicated by a negative sign. These allelic designations are not identical to those described by other researchers (Avise et al., in preparation; Dubach, 1975) due to differences in technique, especially variations in the duration of the run and pH of the buffer solutions. Table 1 gives the loci examined, the abbreviations used, and the alleles observed.

Genetic Variation

Allelic frequencies were calculated for each locus from the observed genotypic frequencies for each population. These gene frequencies are given in Appendix III. Thirteen of the eighteen loci examined were polymorphic in the population samples. Graphs of the gene frequencies characteristic of the population at each site with 95 percent chi-square confidence intervals illustrate the differences in allelic frequencies between the sites. (Figures 4-7 are the graphs of certain representative alleles selected to illustrate the diverse patterns in gene frequencies.) Differences in gene frequency are apparent. The complexity of the pattern of this genetic variation, however, makes immediate interpretation difficult.

In order to investigate the phenomenon of population differentiation, the distribution and pattern of genetic variation were analyzed. Nei (1973) provides a method partitioning genetic diversity into two components: within and between population variation.

$$HT = H_S + D_{ST}$$

where HT is the genetic diversity observed in the total population (i.e., all the mice sampled). H_S is the average genetic diversity or heterozygosity within the populations (i.e., variation in the mice from one site). D_{ST} is the average genetic diversity between populations sampled.

These two components of genetic variation are discussed in the following two sections.

Fig. 4. -- Graph illustrating the frequency of ADA allele 100 in populations from sites 1 through 10 and 95 per cent chi-square confidence intervals. The allelic frequency in each population is indicated by an (*) and the confidence interval by a series of dashed lines.

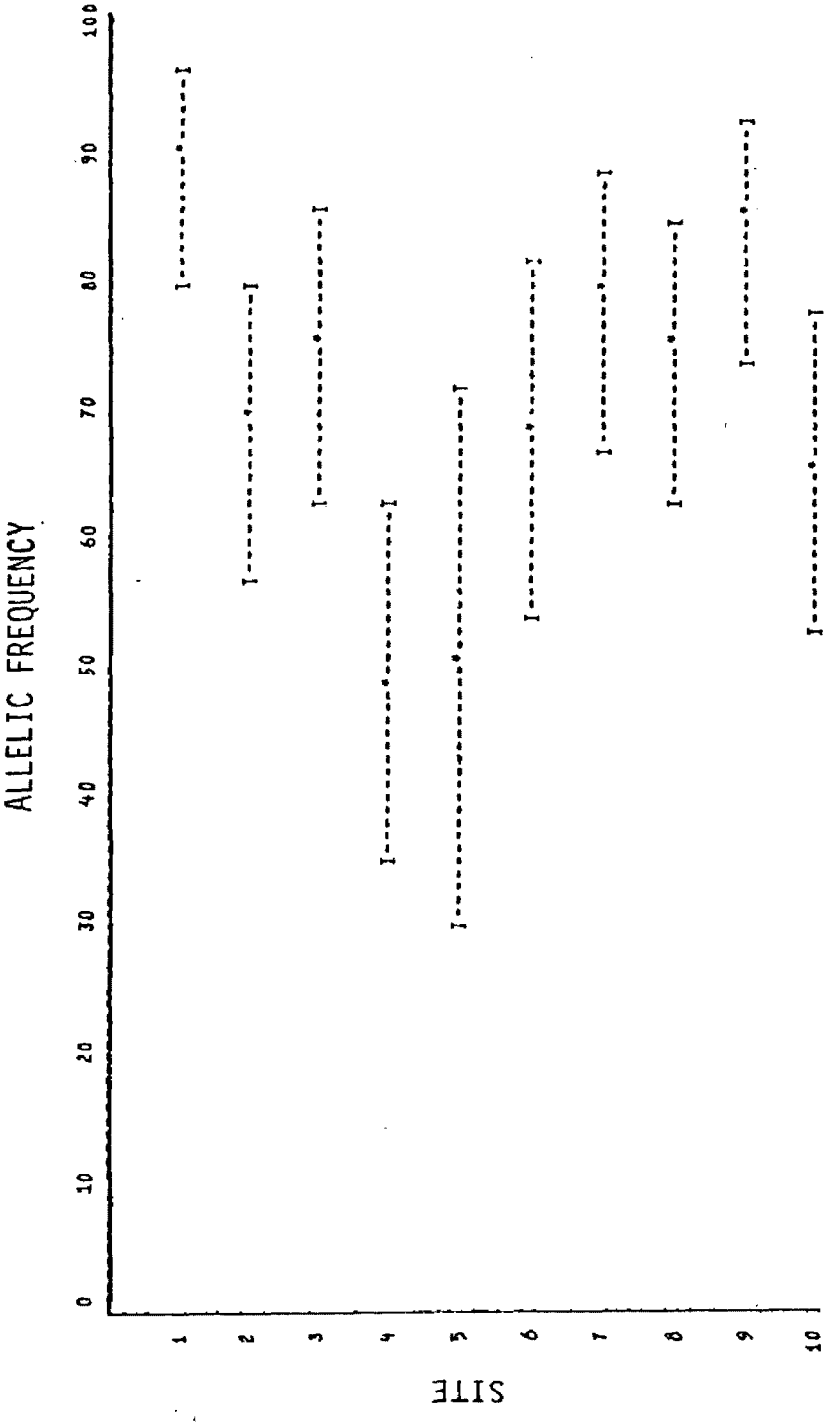


Fig. 5. -- Graph illustrating the frequency of GOT-1 allele 100 in populations from sites 1 through 10 and 95 per cent chi-square confidence intervals. The allelic frequency in each population is indicated by an (*) and the confidence interval by a series of dashed lines.

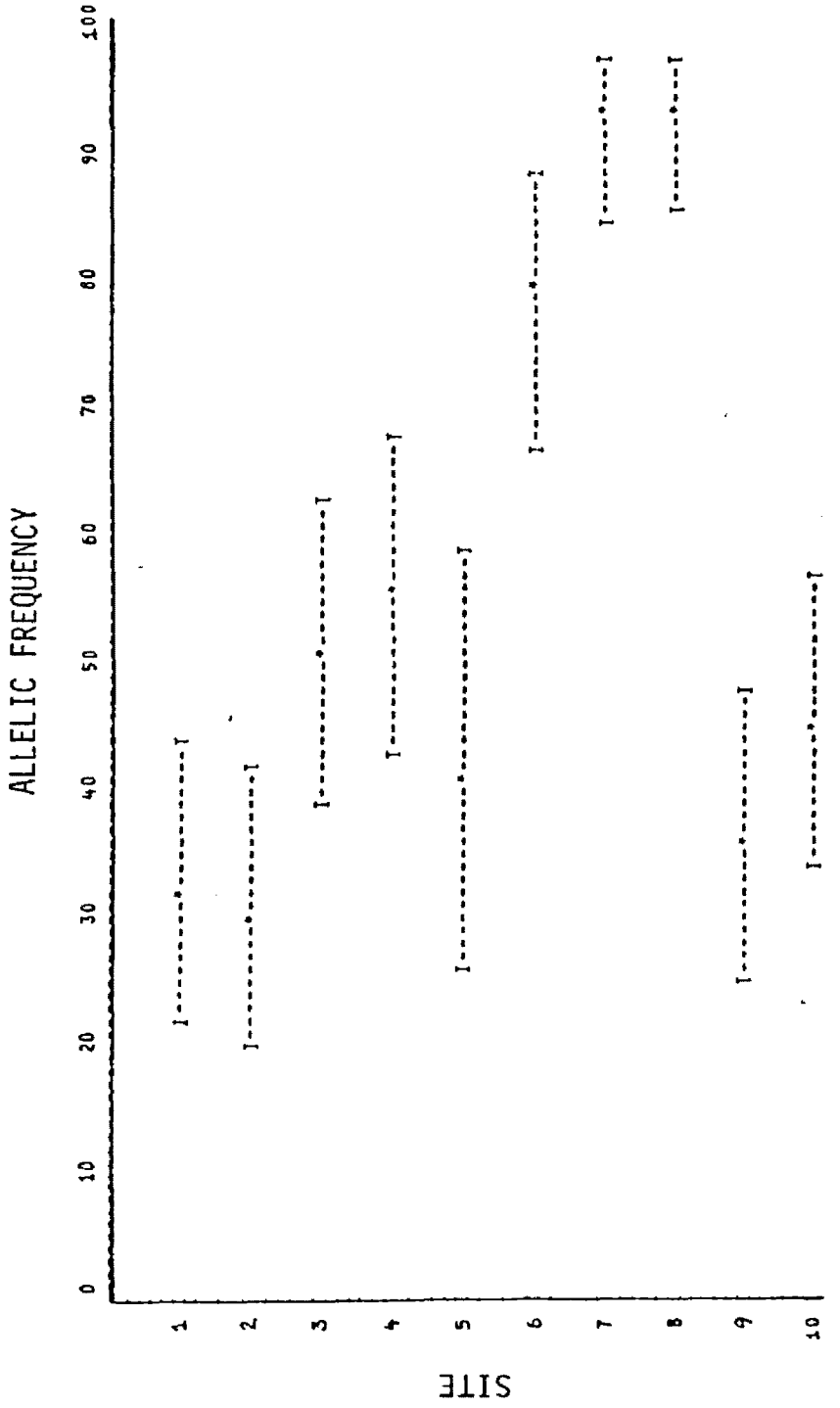


Fig. 6. -- Graph illustrating the frequency of 6PGD allele 100 in populations from sites 1 through 10 and 95 per cent chi-square confidence intervals. The allelic frequency in each population is indicated by an (*) and the confidence interval by a series of dashed lines.

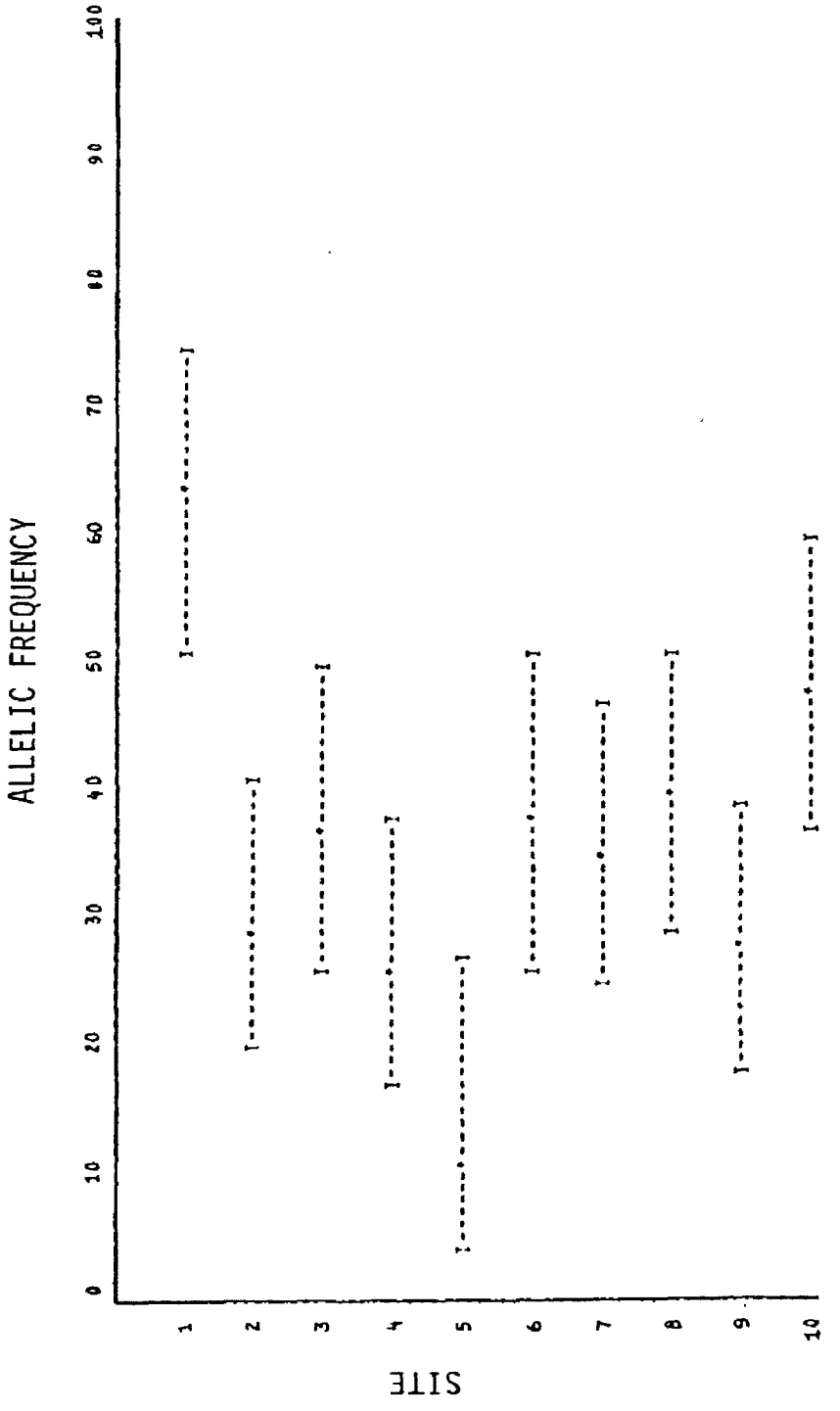
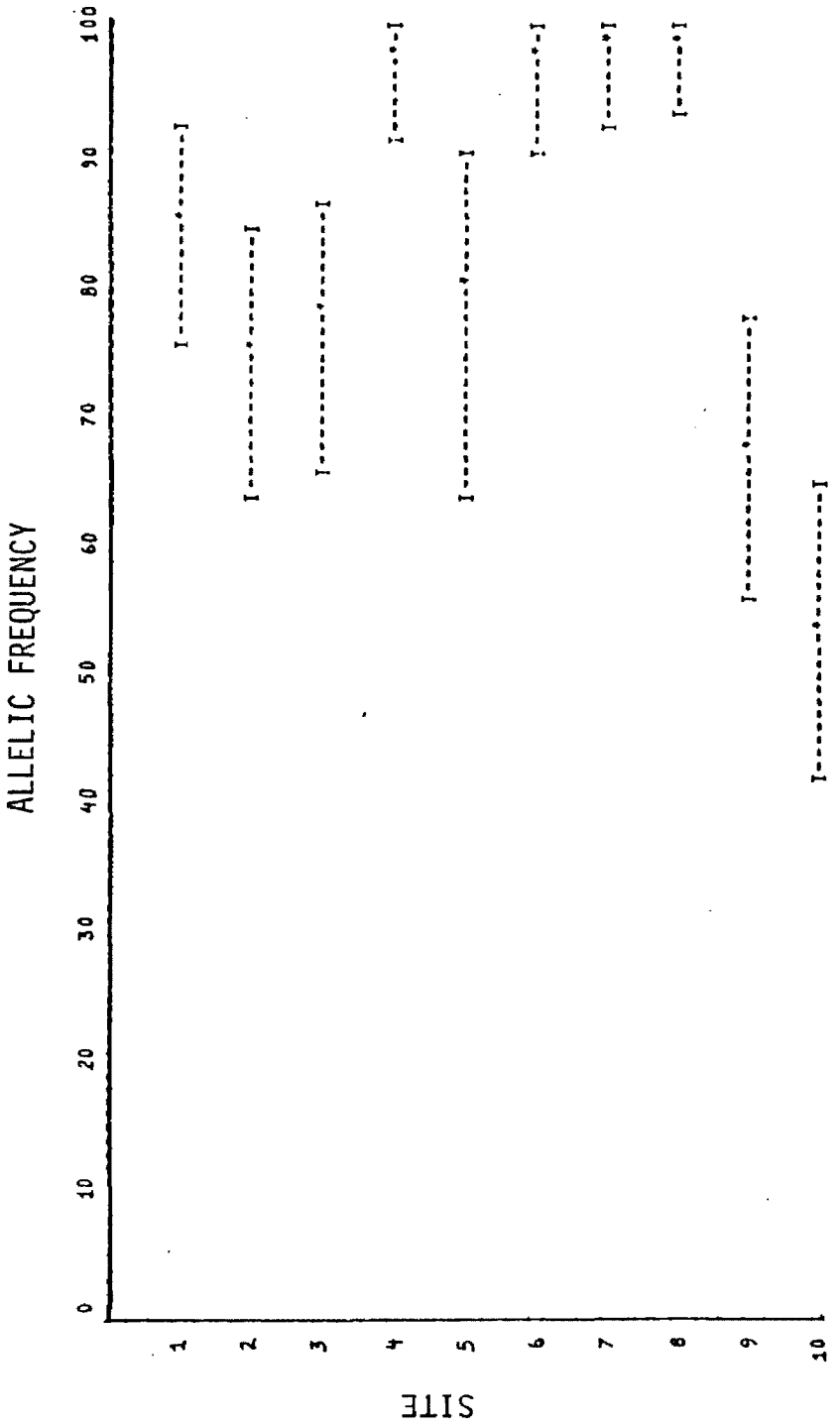


Fig. 7. -- Graph illustrating the frequency of TRF allele 100 in populations from sites 1 through 10 and 95 per cent chi-square confidence intervals. The allelic frequency in each population is indicated by an (*) and the confidence interval by a series of dashed lines.



Within Population Genetic Variation (H_S)

The genetic variability within a population can be estimated by calculating the probability of nonidentity for two genes, randomly chosen from individuals within a population (Nei, 1973; Nei and Roychoudhury, 1974). A measure of genetic variation, average heterozygosity, $\overline{H}$, was obtained for each population sampled by the following formula which encompasses all alleles and loci simultaneously:

$$\overline{H} = \sum_{k=1}^{r} h_k / r$$

where

$$h_k = 1 - \sum_{i=1}^{n} x_i^2$$

where x_i is the gene frequency of the i^{th} allele, n is the number of alleles at a particular locus and r is the number of loci. The standard error of average heterozygosity is estimated by: $\sqrt{V(\overline{H})}$ where

$$V(\overline{H}) = \left[\sum_{k=1}^{r} (h_k - \overline{H})^2 / (r-1) \right] / r$$

The relative validity of this estimate of genetic variation rests on the assumption that the isozyme loci sampled by electrophoresis reflect the relative amount of genetic variation found at other loci in the genome (Allendorf, 1975).

The average heterozygosity values for each population are given in Table 2. No significant differences between sites occurs. The range for these values is 7.8 to 14.5 per cent with an average value of 11.6 per cent.

TABLE 2. -- Sample size (N), per cent of loci polymorphic ($P_{.05}$ and $P_{.01}$), and average heterozygosity ($\bar{H}$) with standard error (S.E.) for each of the ten populations. A locus is considered polymorphic if the most common allele has a frequency less than .95 ($P_{.05}$) or .99 ($P_{.01}$).

Site	N	$P_{.05}$	$P_{.01}$	$\bar{H}$	S.E.
1	31	22	39	.096	.044
2	31	28	39	.131	.051
3	29	33	44	.130	.049
4	30	22	39	.125	.055
5	15	28	33	.136	.053
6	26	17	50	.103	.045
7	34	28	39	.078	.035
8	36	28	39	.080	.038
9	33	28	56	.137	.052
10	34	28	39	.145	.053

$\bar{H}$ Average .116

This value indicates that an average individual is heterozygous at approximately 12 per cent of the loci in its genome. The average value for vertebrate species is lower than this, between 5 and 6 percent (Powell, 1975; Selander and Johnson, 1973). Other species of Peromyscus also show generally lower average heterozygosity values than those found in the present study (Avise et al., 1974a; Avise et al., 1974b; Selander et al., 1971; Smith et al., 1973). This is noteworthy since no esterase loci, which are characteristically variable, are included in the present study. Recent studies of P. maniculatus (Avise et al., in preparation; Dubach, 1975) also give high values for average heterozygosity, reporting means of 9.1 and 13.3 per cent, respectively. These average heterozygosities are the highest yet reported for any species of Peromyscus. There are a number of theories postulating a relationship between genetic and environmental variability, some with conflicting predictions (Bryant, 1974; Valentine, 1976). There are studies on P. maniculatus currently underway to investigate this relationship in this species (Joule, personal communication).

Heterozygosity employs the concept of probability of nonidentity between genes from individuals within one population as a measure of genetic variation in this population. This term, therefore is only appropriate for a randomly mating population (Nei, 1973). In order to determine if the mice for each sample site represent one randomly mating population, chi-square tests were performed to indicate deviations from Hardy-Weinberg proportions.

The Hardy-Weinberg principle describes a state of genetic equilibrium for large random-mating populations in the absence of forces (i.e., migration, mutation, selection, or genetic drift) instrumental in changing gene frequencies. At equilibrium, allelic frequencies do not vary from one generation to another. The genotypic frequencies also remain constant and are predictable from the gene frequencies. A goodness-of-fit chi-square test is used to determine if the genotypic frequencies observed represent those from a population in Hardy-Weinberg equilibrium. A significant chi-square value indicates a deviation from the expected genotypic proportions.

In actuality, the goodness-of-fit chi-square test is not very sensitive to deviations from Hardy-Weinberg proportions, and consequently detection of deviations may be difficult (Ward and Sing, 1970). An illustration of this potential difficulty is an analysis of a case of population substructuring. A computer simulation generated the sample sizes necessary to detect significant deviations at a locus with two alleles due to sampling two individual demes (assuming equal sample size of the two demes). The probability of detection of significant deviations (i.e., significant chi-square values) depends on the magnitude of the allelic differences between the subpopulations and/or the sample size. These relationships are illustrated in Table 3. It can be seen that the smaller the allelic frequency differences, the larger the sample size must be in order to demonstrate a significant chi-square value. In particular, unless the gene frequency differences between the subpopulations are approximately 0.5, the sample size must be

TABLE 3. -- Sample sizes required to demonstrate a significant chi-square value at the .05 level as a result of sampling two subpopulations with gene frequencies, P1 and P2.

P2 \ P1	0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0										
.1	1386									
.2	311	9988								
.3	123	983	21600							
.4	61	267	1693	31799						
.5	35	106	393	2212	37636					
.6	21	51	138	465	2400	37636				
.7	13	27	60	150	465	2212	31799			
.8	9	16	30	60	138	393	1693	21600		
.9	6	9	16	27	51	106	267	983	9988	
1.0	4	6	9	13	21	35	61	123	311	1386

large (greater than 50) for a significant chi-square value. Consequently only those loci which meet these criteria will demonstrate significant chi-square values.

Chi-square tests were performed on each polymorphic locus in every population sampled. All categories less than one were combined for the analysis. These chi-square values and degrees of freedom are presented in Appendix III. A total of 77 tests were performed and four of these are significant at the .05 level. These data are given in Table 4. With a total of 77 tests, approximately four would be expected to demonstrate statistical significance at the 5 per cent level due to chance alone. This suggests that the significant deviations observed are due to chance.

Three of these cases, are observed in one locus, GOT-1, and all are the result of a deficiency of heterozygotes, suggesting something other than chance operating at this particular locus. The simplest explanation is the existence of a null allele. An operational definition of a null allele is an allele which does not stain on a starch gel. The reason for this inactivity may occur at several levels. The allele may not be transcribed, no enzyme may be produced, or the enzyme which is produced may be inactive. The presence of a null allele would result in an observed deficiency of heterozygotes, since heterozygotes for the null allele are difficult to detect electrophoretically. A deficiency of heterozygotes is precisely what is observed in the case of GOT-1. In addition, this deficiency is of similar magnitude in all three populations suggesting the frequency of the null allele

TABLE 4. -- Loci demonstrating significant deviations from Hardy-Weinberg proportions.

Locus	Site	N	Genotypic Frequencies			Chi-square Value	d.f.
			100/100	100/72	72/72		
GOT-1	6	26	.69	.19	.12	4.660*	1
GOT-1	7	34	.88	.09	.03	4.220*	1
GOT-1	8	36	.89	.08	.03	4.606*	1

Locus	Site	N	Genotypic Frequencies			Chi-square Value	d.f.
			100/100	100/120	120/120		
ADA	9	27	.78	.15	.07	4.606*	1

* Indicates significance at the .05 level.

is also similar in the three populations. Null allele frequencies were estimated from the expected Hardy-Weinberg proportions (Cavalli-Sforza and Bodmer, 1971). These values are given in Appendix IV along with the non-significant chi-square values generated when a null allele is assumed to be present. This occurrence in sites 6, 7, and 8 lends support to the null allele hypothesis since these sites are distinct in frequency at other loci. These null allele frequencies were not included in any of the calculations (such as average heterozygosity and similarity coefficients) performed on the other allelic frequencies.

The data were also examined for allelic differences between females and males within a population using contingency chi-square tests. No significant deviations were observed.

In summary, this analysis suggests panmixia in all the populations sampled. Three of the four instances of significant deviation from Hardy-Weinberg proportions can be explained by chance or by a locus-specific phenomenon. The within population component of genetic variation, H_S, is 11. 6 per cent, a comparatively high value.

Genetic Variation Between Populations

The between population component of genetic variation, D_{ST}, is also estimated in terms of probability of nonidentity of two genes. In this case, the genes are randomly chosen from individuals in two different populations. This calculation can also be performed over all alleles and loci simultaneously for each pair of populations sampled, yielding a genetic dissimilarity coefficient.

Several methods of computation have been used, two are employed here; one developed by Nei (1972) and one by Rogers (1972). Nei's D-value, a measure of genetic diversity, and standard error were computed for all pair-wise comparisons between the sites.

$$D = - \log_e \ (J_{XY} / \sqrt{J_X \ J_Y})$$

where J_X, J_Y and J_{XY} are the arithmetic means of j_x, j_y and j_{xy} over all loci (including monomorphic ones).

$$j_X = \sum_{i=1}^{n} x_i^2 \qquad j_y = \sum_{i=1}^{n} y_i^2 \qquad j_{xy} = \sum_{i=1}^{n} x_i y_i$$

where x_i and y_i are the sample gene frequencies of the i^{th} allele in populations X and Y respectively, and n is the number of alleles at a particular locus. From Nei's D-value, genetic similarity (I) was calculated.

$$-\log_e I = D$$

$$\log_e I = -D$$

$$I = e^{-D}$$

In a similar manner, Rogers' coefficient measures the average geometric distance between allele frequency vectors on a scale from 0 to 1 as defined by:

$$D = \frac{1}{L} \sum_{i=1}^{L} \left(\frac{1}{2} \sum_{j=1}^{A_i} (P_{ijx} - P_{ijy})^2 \right) \frac{1}{2}$$

where L is the number of loci, A_i is the number of alleles at the i^{th} locus and P_{ijx} and P_{ijy} are the frequencies of the j^{th} allele at the i^{th} locus in populations x and y, respectively. This value, D, is a

genetic dissimilarity measure. A genetic similarity index, S, is defined by 1 - D, and ranged from 0 - 1, with an S-value of 1 indicating genetic identity.

Table 5 gives both Nei's I-values and Rogers' S-values, the former are generally higher numbers but show similar patterns (Chakraborty and Tateno, 1976). Nei's genetic similarity values range from .964 to .999 with an average value of .982. Rogers' values range from .901 to .983 with an average value of .937. Computing both similarity coefficients facilitates comparison with other data since these are the two indices most commonly employed.

Similarity coefficients have been calculated for a number of organisms at various taxonomic levels. A summary of this work, primarily using Rogers' coefficient, was compiled by Avise (1975).

In general, the average S-values for the Peromyscus species that have been investigated are in agreement with the other taxa studied at the species level, particularly other mammals. There is a tendency for high S-values between species; the range for congenerics is .34 to .99 with an average value of .66 (Avise, 1975). This somewhat obscures the definitive boundary of S-values between congenerics and conspecifics. The populations of mice in this present study have S-values above .90 and are considered conspecific.

The within species similarity values are generally high; this seems to be the rule and not the exception in Peromyscus species (Table 6). In P. leucopus (Smith et al., in preparation) and P. maniculatus (Avise et al., in preparation), the two most widespread species in this genus, each have within species S-values above .87. The S-values for

TABLE 5. -- Nei's (above diagonal) and Rogers' (below diagonal) genetic similarity values between populations from sites 1 through 10.

	1	2	3	4	5	6	7	8	9	10
1		.987	.991	.975	.970	.981	.973	.974	.986	.982
2	.939		.996	.987	.993	.978	.966	.966	.995	.991
3	.950	.968		.989	.990	.991	.984	.984	.992	.992
4	.920	.941	.948		.993	.989	.976	.978	.982	.980
5	.918	.956	.949	.962		.977	.965	.965	.986	.986
6	.934	.935	.949	.946	.923		.996	.997	.975	.975
7	.930	.912	.938	.918	.901	.962		.999	.964	.965
8	.930	.915	.939	.923	.903	.967	.983		.964	.966
9	.941	.963	.952	.939	.944	.917	.906	.905		.992
10	.931	.954	.953	.937	.946	.924	.905	.911	.958	

TABLE 6. -- Rogers' coefficients of genetic similarity between conspecific populations for several species of Peromyscus.

Organism	#Loci	Conspecifics Average	Conspecifics Range	Reference
Peromyscus (16 spp.)	21	.95	.71 - 1.00	Avise, 1975
P. attwateri	17	.97	.94 - 1.00	Kilpatrick and Zimmerman, 1975
P. boylei	17	.92	.84 - .98	Kilpatrick and Zimmerman, 1975
	21	.95	.89 - .99	Avise et al., 1974a
P. difficilis	22	.79	.77 - .87	Avise et al., in preparation
P. leucopus			>.90	Smith et al., in preparation
P. floridanus	39		.96 - .98	Smith et al., 1973
P. maniculatus	21	.93	.87 - .97	Avise et al., in preparation
	18	.94	.90 - .98	present study
P. melanotis	21	.92	.88 - .98	Avise et al., in preparation
P. pectoralis	21	.79	.75 - .84	Avise et al., 1974a
	17	.82	.69 - .98	Kilpatrick and Zimmerman, 1975
P. polionotus	32	.95	.81 - .93	Smith et al., 1973
P. truei	22	.75	.58 - .95	Avise et al., in preparation

P. maniculatus were based on comparisons between samples from northern Canada to Mexico, and from California to Virginia. In some cases, data were pooled, but this fact itself does not explain the overall high similarity among the populations.

The genetic similarity values for the populations in this study are above .90. This value, calculated for sites along a 75-kilometer transect, is of the same magnitude as that observed for the entire species over continental North America (Avise et al., in preparation). Genetic differentiation in this present study, although not numerically great, is similar in amount to that observed in the species, P. maniculatus, as a whole. Avise et al. (in preparation) explain this macrogeographic conservatism in levels of genetic divergence by the relatively recent separation of populations, coupled with genetic inertia resulting from a selected cohesion of the genome.

The observed microgeographic heterogeneity is further investigated by examining individual loci. Contingency chi-square tests were performed to test for differences between the frequencies at a given level of significance. The loci for which significant differences in allelic frequency were observed between the populations are listed in Appendix IV. This compilation of differences allows comparison between the populations and yet retains the identity of the individual loci involved. In pair-wise comparisons between the populations, nine loci show significant differences: IDH-2, GOT-1, GOT-2, TRF, 6PGD, ADA, LDH, MDH, and PGM-2. A matrix of the number of significant differences observed between two sites was constructed (Table 7). This illustration confirms that

TABLE 7. -- Number of loci which are significantly different ($p < .05$) in pairwise comparisons between populations from sites 1 through 10.

SITE	1	2	3	4	5	6	7	8	9
1									
2	5								
3	4	1							
4	5	4	3						
5	4	1	2	2					
6	5	4	5	4	4				
7	4	6	4	5	5	3			
8	5	5	5	5	5	3	1		
9	4	2	2	3	2	5	5	5	
10	4	2	2	2	2	5	5	4	2

these samples differ significantly at many genetic loci. In particular, the populations from sites 6, 7, and 8 and site 1 differ from other populations at four or more loci.

Considerable microgeographic heterogeneity is demonstrated among populations from sites less than 20 kilometers apart and in some cases less than two kilometers apart. In particular sites 4, 5, and 6 are in very close proximity to each other and et sites 4 and 5 remain significantly different from site 6 at a number of loci. Avise et al. (1974b) studying Peromyscus species of the subgenus Haplomylomys, also found heterogeneity in allelic frequencies among collection localities, although the sample points are further apart. Similar geographic heterogeneity has been reported in other Peromyscus species (Selander et al., 1971; Smith et al., 1973). Avise et al. (1974b) suggest that allelic heterogeneity may be "the rule in vertebrate populations." The origin and maintenance of this differentiation remain a matter for speculation.

In summary, genetic similarity values were calculated in pairwise comparisons of all the populations, and were found to be within a range common for conspecifics. The similarity values for the populations are greater than .90, a value comparable in magnitude to the S-values between populations sampled over the entire species range. This indicates a level of microgeographic genetic differentiation comparable to the macrogeographic variation of the species. Further investigation of this microgeographic variation reveals significant differences between populations at nine different loci.

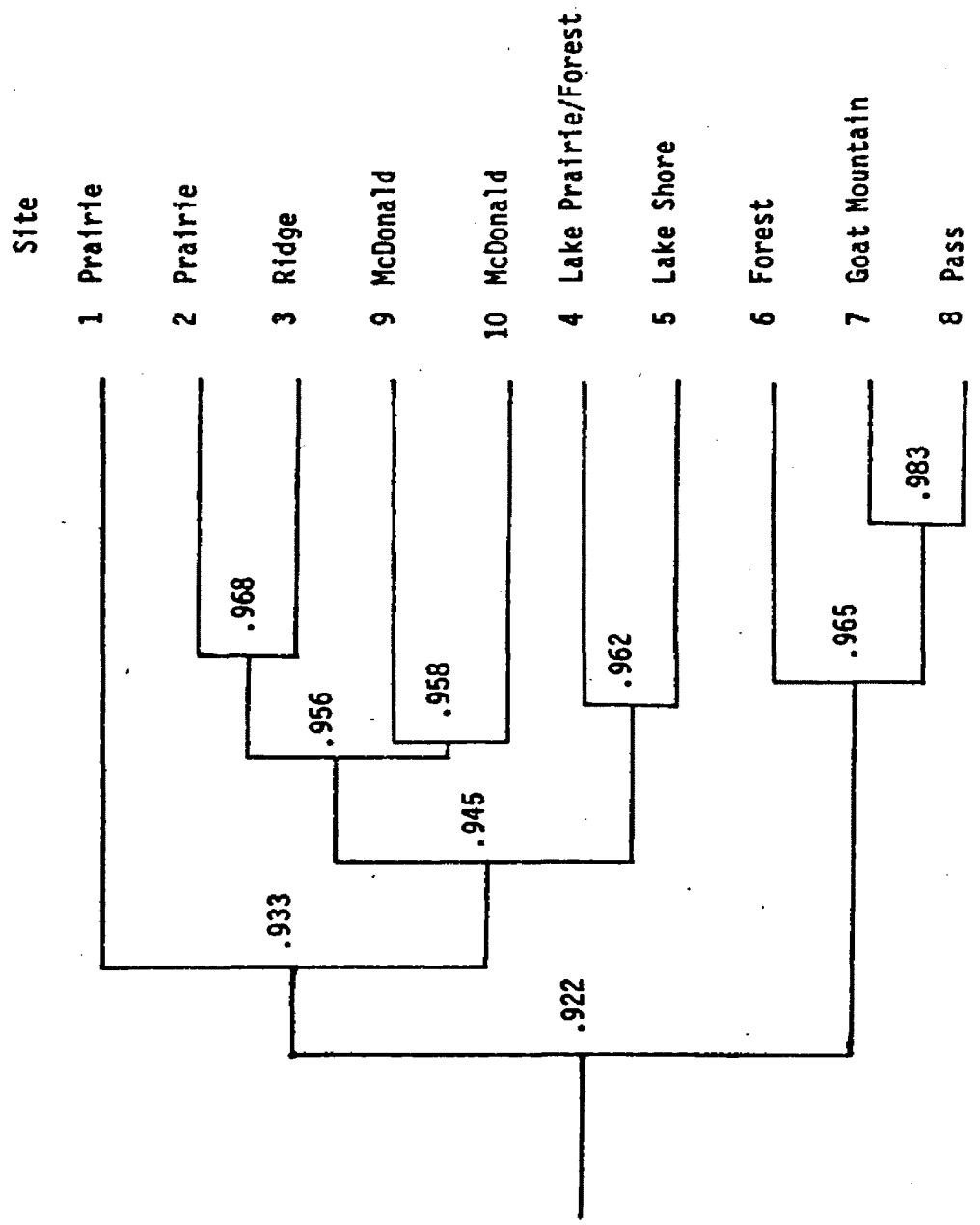
Patterns of Genetic Variation Among Populations

The two components of total genetic diversity indicate the amount of variation and the distribution of this within and between populations. The genetic similarity value, calculated from the between population component, is used to elucidate the pattern of genetic variation. The magnitudes of the S-values are a measure of the relative similarities among populations. Simply surveying the similarity matrix (Table 5), does not immediately reveal the overall patterns.

Further clarification and illustration of the patterns of similarities among the sites are achieved by construction of a dendrogram employing the unweighted average linkage method (Sneath and Sokal, 1973) (using a computer program obtained from Joseph Felsenstein, University of Washington). Figure 8 shows the dendrogram describing the relationships among the populations using Rogers' genetic similarity as a measure of divergence. There are two main groups; sites 6, 7, and 8 form one, and sites 1, 2, 3, 4, 5, 9, and 10 form the other. Site 1 represents a possible third group; unfortunately, there is no way to readily assess this relative dissimilarity. Therefore, all subsequent discussion considers only two main groupings.

The previous morphological and ecological work on the mice in this area indicated one point of discontinuity; and subspecific terminology was adopted to indicate this place of differentiation. This site of delineation on Figure 3 occurs between sites 5 and 6. *P. m. osgoodi* is found to the east and *P. m. artemisiae* to the west.

Fig. 8. -- Biochemical similarity dendrogram summarizing the relationships among populations from sites 1 through 10, based on average unweighted linkage cluster analysis of Rogers' genetic similarity coefficients.



The genetic analysis of the population differentiation in this area also indicates two main groups, but these are not identical with the subspecific groupings. On the east side of the mountains the genetic change observed coincides with the subspecific boundary exactly (between sites 5 and 6). At this point there is the previously described morphological change from the eastern short-tailed P. m. osgoodi to the western long-tailed P. m. artemisiae. Since this represents the previously described contact zone, sampling was concentrated in this area. Thus the 3 sample sites are located less than two kilometers apart. Also at this point there is a distinct habitat change from prairie to forest. However, habitats to the east of this point are not all grassland, since St. Mary Ridge is forested and there are numerous stands of aspen along both Upper and Lower St. Mary Lake (St. Mary Ridge was sampled, site 3, and is genetically similar to the surrounding prairie sites.)

Westward along the transect another habitat change occurs between sites 6 (which is forested) and 7 (above treeline). Here there are no associated morphological (Glazier, 1971) or genetic changes.

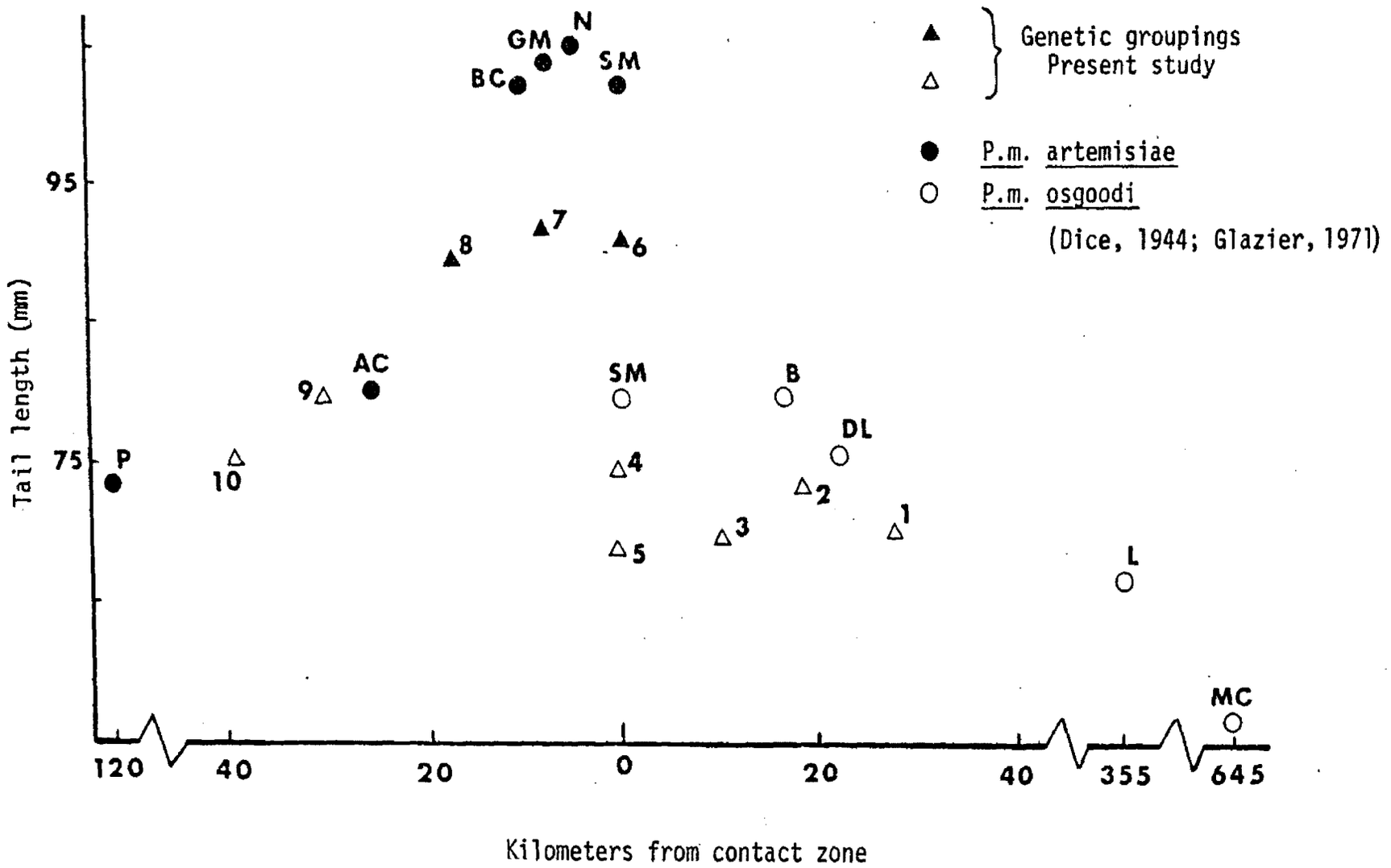
Sites 9 and 10 are located in forest, which indicates another change in habitat from sites 7 and 8 above treeline. Morphological data are poor for the western side (since Glazier did not sample there). However, Murie (1933) refers to the fact that P. m. artemisiae at St. Mary and vicinities has a much longer tail than in the rest of its range, suggesting differences within P. m. artemisiae. Glazier (1971) also refers to morphological data from the Lake McDonald drainage (Dice, 1944),

indicating shorter tail length on the west than in the mountains. A corresponding genetic change does occur between sites 8 and 9. Unfortunately, since this point of differentiation was not anticipated, the two sites are quite far apart (approximately 15 kilometers). Since this point of differentiation was not noted previously, there is no taxonomic designation. The nature of this genetic change on the western side is such that sites 9 and 10 are similar genetically to the eastern-most sites (sites 1, 2, 3, 4, and 5) (Fig. 8).

There is a central group, sites 6, 7, and 8, which is different from the genetically similar extremes. There is as much microgeographic differentiation within a subspecies (P. m. artemisiae) as between the two designated subspecies. The linear nature of the transect may have introduced a biased indication of the distribution of the two groups, even so, it appears the existing taxonomic description is not adequate for the complex genetic situation.

The genetic pattern, however, does not disagree with the pattern for the morphological data. Tail length data (Dice, 1944; Glazier, 1971; present study) are presented in Figure 9. The pattern of variation in tail length is similar to the pattern observed for the genetic variation (i.e., sites 6, 7, and 8 appear as a group distinct from the rest of the sites).

Fig. 9. -- Geographic variation in means of tail lengths of Peromyscus maniculatus across Montana (modified from Glazier, 1971). P, Polson, Montana; AC, Avalanche Creek, Glacier National Park; L, Lewiston, Montana; MC, Miles City, Montana; from Dice (1944). BC, Baring Creek; GM, Goat Mountain; DL, Duck Lake; from Glazier (1971). Numbers 1 through 10 represent sites from the present study.



CHAPTER IV

ORIGIN AND MAINTENANCE OF DIFFERENTIATION

Despite the absence of an obvious interpretation of the geographic distribution of these two genetically distinct groups, the most parsimonious explanation may be derived from the glacial history of the area and the patterns of recolonization.

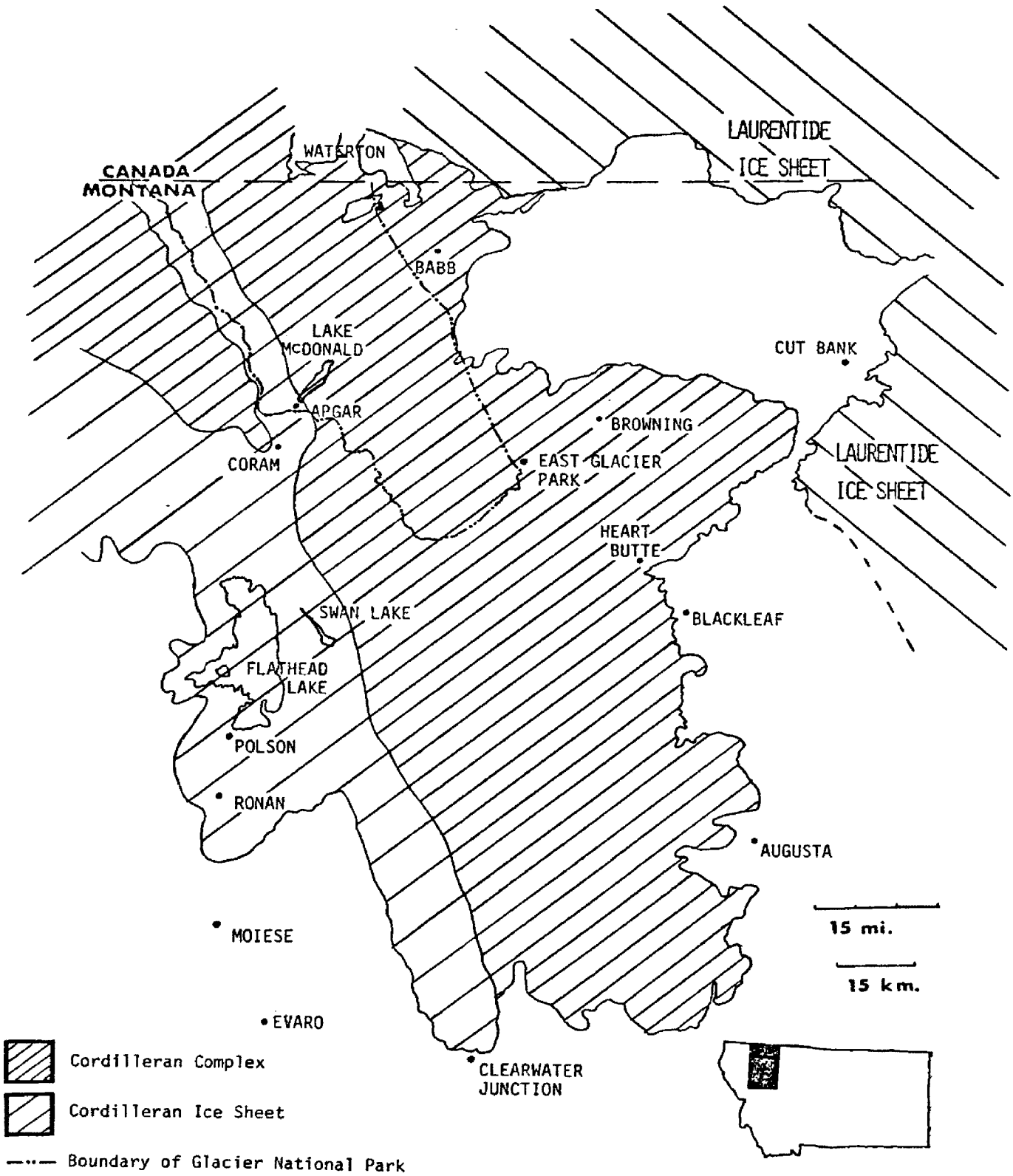
The earliest fossils assigned to the genus Peromyscus were found in Early Pliocene deposits in the western U.S. (Hibbard, 1968). In general, the fossil record is poorly known, apparently due to the small size of the animal and its probable scarcity until the Pliocene or early Pleistocene. Even so, there is a paucity of Peromyscus fossils from several of the glacial and interglacial deposits of the Pleistocene. The fossil record is quite depauperate until the Illinoian and Sangamon, at which time examination of finds demonstrated increased differentiation and complexity of form. Fossils from the Wisconsin deposits (formed during the last glacial event) are referable to living species. This indicates the recent origin (approximately 10,000 B.P.) of such groups as P. maniculatus and P. leucopus; which are considered products of late Pleistocene adaptive radiation (Hibbard, 1968). This recent origin may explain the high genetic similarity values found between and within many Peromyscus species.

Most of the knowledge of the extent and effect of glaciation comes from the Wisconsin, since this is the most recent. Undoubtedly, the zoogeography of the region was profoundly affected by all the glacia-

tions, however, their effects can only be conjectured. During the Wisconsin, a period lasting approximately 60,000 years, the study area was situated primarily within the vicinity affected by the Cordilleran glacial complex (Alt, personal communication; Flint, 1971). In general, this complex was characterized by local origins of ice. Most of this glacial ice remained associated with mountains and valleys, but some flowed downward forming piedmont glaciers, and eventually an extensive ice sheet. This cordilleran ice sheet centered in British Columbia and extended southward between the coast ranges and the Rocky Mountains (Flint, 1971; Richmond et al., 1965). Thus, the area of Glacier National Park was predominated by local mountain glaciers (Alden, 1953). This local nature of glacial ice particularly in the east stems partly from the effect of a strong rain shadow produced by the Rockies (Flint, 1971).

The occurrence of refugia within and around glaciated areas has been postulated by other workers (Kilpatrick and Zimmerman, 1976 for Peromyscus; Uzzell, 1964 for Ambystoma). The existence of a large refuge in central Alaska is well documented and smaller refuges further south are postulated. Evidence for nonglaciated areas in the vicinity of Glacier National Park is given by Alden (1932, p. 116). For example, the Kennedy Ridge area north of St. Mary Lake apparently remained unglaciated, surrounded by the St. Mary Glacier on the south and east, Belly Glacier on the west and the Laurentide ice sheet on the north (Fig. 10). There is evidence for survival of various taxa in high altitudes through the glacial ages by "overwintering" in refuges not covered by ice (Flint, 1971). For example, pollen from certain species

Fig. 10. -- Distribution of the glacial ice in northwestern Montana during the maximum extent of the Wisconsin glaciation (modified from Alden, 1932 and 1953; Alt, personal communication).



of plant which today occupy nunataks in Greenland, has been found in the nonglaciated areas on the coast of Alaska and British Columbia (Heusser, 1955). These hardy plants are believed to have survived the last glaciation on isolated ice-free areas. The survival capacity of such "hardy" organisms as P. maniculatus can be inferred from present distributions which include both arctic and alpine habitats (Baker, 1968, p. 112).

During the Wisconsin, the two western sites, 9 and 10, were covered with one of the largest of the Pleistocene glaciers found within the limits of Glacier National Park (Alden, 1953). The origin and path of the McDonald Creek Glacier can be identified today by the topography of the McDonald Valley which it carved. Lake McDonald now occupies part of this glacial trough. The southern extent is more difficult to delineate but appears to be just north of Coram, Montana (Alden, 1953). Habitation of this area during most of the Wisconsin was consequently impossible.

The eastern edge of Glacier National Park and the Blackfeet Indian Reservation were influenced primarily by the piedmont glaciers which spread from the eastern edge of the Rocky Mountains (Fig. 10). One of these, the St. Mary Glacier, 36 or more miles in length, occupied the St. Mary Valley during the Wisconsin (Alden, 1932, p. 116). Consequently, much of this area at the eastern base of the mountains was also uninhabitable (including sites 4, 5, and 6). To the east of these piedmont glaciers (in particular, south of 49° latitude) was unglaciated terrain. This ice-free area was bordered on the east and north by the eastern continental (Laurentide) ice sheet. An unglaciated corridor led southward where it eventually merged with the area south of the glacial border (Fig. 10).

Overall reconstruction of the glacial history of this area suggests the existence of remnant populations in the unglaciated sections of the mountains in and around Glacier National Park. These relic populations from a once widespread species may have existed through all of the glaciations or perhaps only the Wisconsin, following invasion during the Sangamon interglacial. With a gradual thinning of the ice sheet, the ridges were exposed (Richmond et al., 1965), creating potential mountain routes for recolonization from these isolated refugia. Both the eastern and western edges of Glacier National Park were uninhabitable during this time; the previous populations moved south before the advancing ice, establishing the geographic isolation of the mountain and southern populations.

The genetic evidence suggests that, after the glaciers receded, the eastern and western regions of Glacier National Park were re-colonized from a genetically homogeneous source, probably the southern population(s). This is entirely possible, since reconstruction of the biogeography of this region following glacial retreat suggests that the entire area was predominantly lodgepole pine forest (Flint, 1971; Hansen, 1948).

The relevance of this historical construct to the genetic relationships among the populations sampled is readily apparent. In this model, the central group (sites 6, 7, and 8) represent animals whose ancestors resided in mountain refugia during the glaciation, whereas the eastern and western groups are descendants of more recent colonists from populations to the south. The average heterozygosity values for the

populations from sites 6, 7, and 8 are generally lower than those from the other populations. Lower heterozygosity, produced by genetic drift, would be predicted in these presumably small, isolated refugia populations (Kilpatrick and Zimmerman, 1976).

Although the sampling in this study was done in a rather limited geographic area, the situation of other post-glacial populations should be similar enough to allow certain generalizations and predictions. Other species, in particular, other populations of P. maniculatus found in the area covered or affected by glaciers probably demonstrate similar patterns. The influence of glaciers may extend south of the actual borders due to habitat alterations from the climate change associated with periods of glaciation (Blair, 1965). Lowland areas would support large continuous populations during glaciation and would maintain high genetic similarities. Whereas high altitude refugia or isolated ice-free areas within the glaciated region would produce populations which are differentiated from the lowland populations and possibly from each other.

This model predicts that areas which were isolated during glaciation e.g., mountainous areas, are essentially "terrestrial islands," supporting populations which are genetically dissimilar to surrounding populations. Many lowland sites which were inhabited by or recolonized from a large continuous population, possess high genetic similarity over large distances. For example, it seems reasonable that deer mouse populations in the Rocky Mountains and in the ranges to the west, would show a pattern similar to that observed here; high similarity between lowland populations and low similarity between highland and lowland populations. This prediction

of the genetic similarity based on the probable origin of the populations, could be tested by sampling in other areas.

The relationship of the pattern of morphological change to that of genetic change is potentially useful in detecting areas of population differentiation since many zones of morphological change have been documented. During glacial events, isolated populations in different habitats very probably are placed under differential selection regimes and change accordingly, perhaps morphologically. Simultaneously, neutral allelic frequency variation in these populations undergo drift. After the glaciers retreat, contact may be resumed. During subsequent years, these previously isolated populations may remain differentiated morphologically and if examined genetically, may demonstrate corresponding genetic differences.

The probable origin of the differentiation observed has been discussed in terms of the glacial history of the area. Isolation during the time of glaciation allowed differentiation of the populations inhabiting the two areas. The resulting differences under these circumstances could be the result of either stochastic processes or natural selection. The observation of any differences today represent products of the action of these two factors, in both the past and present.

Location in different habitats should result in differences in those populations due to differential selection regimes. This argument appears to be true for certain morphological characteristics in deer mice. There is a general trend noted over the entire United States for long-tailed, dark forms to be associated with forested areas and

short-tailed, light-colored forms with grassland (Blair, 1950; Hooper, 1968). However, this does not appear to be true for the biochemical loci studied. Avise, et al. (in preparation) found no consistent allelic frequencies associated with the two morphological types. These researchers identified morphological types according to subspecies, which potentially is a problem as noted in this study. Even so, there does not appear to be a relationship between habitat and morphology with biochemical loci commonly studied. Within this present study, neighboring collection sites located in different habitats show few or no significant changes in gene frequency. Certain of the eastern-most sites are located on the prairie and yet are similar biochemically to the most western forested sites, 9 and 10.

If certain of the genes that control morphology are under selection, then the selection intensity must be known in order to evaluate the relative contributions of gene flow and selection to the similarity between two populations for these particular morphological traits. The earlier work on deer mice postulated knowledge about gene flow from the pattern of change of morphological characters. This was done in spite of the studies on such characters as pelage color (Baker, 1968; Blair, 1947; and Kaufman, 1974), demonstrating the importance of selection in maintaining regional differentiation even with gene flow between regions. Recently, there has been a re-emphasis on the importance of selection maintaining similarities or resulting in differences and de-emphasizing the role of gene flow as a predominant evolutionary phenomenon (Ehrlich and Raven, 1969).

The same reasoning applies to the patterns of allelic frequencies; unless the variants of the enzymes sampled are neutral, no insight can be gained into the patterns of gene flow. Assumption of the selective neutrality of the biochemical alleles is in accord with the available evidence and allows postulations about the current pattern of gene flow.

The genetic similarities between the eastern and western groups, presumably established by common origin, have remained through the intervening 10,000 years, in spite of the differences in habitat today. The relatively recent separation alone may explain the degree of similarity. However, it is feasible that its maintenance may be assisted by current, even if intermittent, gene exchange.

The discontinuities at the points of contact between the mountain group and the eastern and western populations, established presumably during the period of isolation during glaciation, have also been maintained. This observation suggests very limited gene exchange at both contact points.

The differences between the two groups may have persisted through time due to random processes without regard to the selective value of any individual traits in different areas. The amount of migration perhaps is not sufficient to obliterate the differences which existed. This lack of migration may be due to the local nature and generally limited dispersal range of P. maniculatus (Howard, 1949). In addition, individual mice may remain within a particular habitat. Wecker's (1963) study indicates the important role of early experience on habitat preferences. Wecker (1963) and Harris (1952) give evidence for a

genetic component in habitat selection in studies of subspecies of P. maniculatus in Michigan. In a situation with limited dispersal tendencies and/or habitat preferences, contact between groups in different habitats is minimal and no or very limited gene exchange occurs. Consequently, those differences established during allopatry persist.

Individuals of the two major forms possibly do make contact but do not interbreed. This, according to the earlier investigators (Murie, 1933; Glazier, 1971), is the case between P. m. osgoodi and P. m. artemisiae, which are not entirely restricted to one habitat especially in disturbed areas. This contact with no gene flow suggests the existence of selection against such an exchange.

If selection is occurring, it may do so on several different levels. The selection, presumably occurring at loci other than those sampled electrophoretically, may be against alleles at particular loci or against hybrids. Selection against hybrids may represent selection against mixing two coadapted genomes (Mayr, 1970; Avise et al., in preparation). Hunt and Selander (1973) studying Mus, concluded that "the internal genetic environment plays an important role in determining the selective values of alleles... since the degree of ...introgression is not equal over all loci."

If selection is sufficiently intense, development of isolating mechanisms may take place, further contributing to the maintenance of genetic differences. Types of isolating mechanisms found in natural populations are well-known (Mayr, 1963), but it is difficult to determine which of these are operative in the Peromyscus populations. The

evidence currently available suggests that both habitat isolation (Wecker, 1963) and ethological isolation, in particular mate selection (Harris, 1954; Moore, 1965; Smith, 1965), are likely mechanisms.

CHAPTER V

SUMMARY

The genetic relationshps among populations of two subspecies of the deer mouse, Peromyscus maniculatus artemisiae, a long-tailed form, and P. m. osgoodi, a short-tailed form, were investigated. Most of the previous taxonomic work on these mice has been based on morphology. This previous work concluded no, or limited, interbreeding between adjacent populations at the subspecific contact zone which occurs on the east side of the Rocky Mountains in northwestern Montana.

In order to investigate this systematic relationship genetically, starch gel electrophoresis was performed on 300 mice, which were live-trapped at each of ten sites along a transect located primarily in Glacier National Park. Examination of 18 biochemical loci revealed high levels of protein variability (average heterozygosity equal to 11.6 per cent).

Genetic similarity values were calculated in pair-wise comparisons of all the sample populations and were found to be within a range common for conspecifics. Rogers' genetic similarity values for the populations are greater than .90, a value comparable in magnitude to the average similarity value for the entire species. Investigation of the micro-geographic variation revealed significant differences between populations at nine different loci.

Analysis of the pattern of genetic variation revealed two groups, a central one which differed from the rest. This analysis reveals as much population differentiation within a subspecies, P. m. artemisiae,

as between the two designated ones. The genetic pattern, however, is similar to the pattern of change in tail length.

The origin of the pattern of differentiation among the populations is presumed to be glacial isolation. Populations with highest genetic similarity probably share a common origin during glacial times. These populations have remained similar in the intervening years and presumably continue to exchange genes. Those populations observed which are different, apparently differentiated during the time of isolation. These genetic differences persist today because of no or very limited gene flow between the populations.

The genetic pattern for this species is an overall high degree of similarity yet there are indications of local heterogeneity. Analysis of the patterns of this local genetic heterogeneity allows interpretation of the current patterns of gene flow. In addition, predictions of similar patterns of gene exchange for other areas have been generated. Contact areas of the long- and short-tailed forms. of P. maniculatus may show similar oversimplified taxonomy.

There may be consistent patterns of gene flow throughout the entire species. By investigation of other contact areas of the two morphological types this consistency could be determined. Insight into the validity of the assumption of the neutral nature of the protein variants would also be gained. Additional data on the deer mouse, P. maniculatus, have the potential to reveal the nature of evolutionary change in this species.

Appendix I.- Collection data for each site 1 through 10.

Site	Collection Dates	# Trap Nights	# Mice Captured	% Trap Success
1	3 June	200	31	17
2	5 June	200	41	20.5
3	28 July, 4-5 Aug.	290	42	14.5
4	9-10 June, 4-9 July	810	34	4.5
5	28-31 July	84	20	23.8
6	8-10 June	200	32	16
7	10-31 July	360	41	11.4
8	15-27 July, 5 Aug.	262	50	19
9	20-27 Aug., 3 Sept.	535	36	6.7
10	15-27 Aug.	418	45	10.8

Appendix II. - Description of the buffer systems used.

I Lithium hydroxide LIOH

Gel: 1:9 mixture of 0.03 M lithium hydroxide - 0.19 M boric acid, pH 8.1
0.05 M tris - 0.008 M citric acid, pH 8.4
Electrode: 0.03 M lithium hydroxide - 0.19 M boric acid, pH 8.1
350 volts for 5 hours

II Potassium Phosphate PGI

Gel: 1:9 dilution of electrode buffer
Electrode: 0.138 M potassium phosphate - 0.062 M sodium hydroxide, pH 6.7
130 volts for 9 hours

III Discontinuous tris-citrate (Poulik) P+/B+*

Gel: 0.076 M tris - 0.005 M citric acid, pH 8.7
Electrode: 0.30 M borate, pH 8.2
250 volts for 3.5 hours

IV Continuous tris citrate I TC 6.7

Gel: 0.008 M tris - 0.003 M citric acid, pH 6.7
Electrode: 0.223 M tris - 0.086 M citric acid, pH 6.3
150 volts for 3 hours

V Continuous tris citrate II TC 8.0

Gel: 22.89 mM tris - 5.22 mM citric acid, pH 8.0
Electrode: 0.687 M tris - 0.157 M citric acid, pH 8.0
130 volts for 4 hours

VI Tris-maleate TM+

Gel: 1:9 dilution of electrode buffer
Electrode: 0.10 M tris - 0.10 M maleic acid -
0.01 M EDTA - 0.01 M magnesium chloride, pH 7.4
100 volts for 5 to 7 hours

*(+) indicates NADP (nicotine adenine dinucleotide phosphate) was added to buffer solution

Appendix III.- Frequencies of alleles and chi-square values, x^2, from goodness-of-fit tests to Hardy-Weinberg proportions for populations from sites 1 through 10.

ADA

Site	N	100	120	x^2	d.f.
1	25	.900	.100	.309	1
2	29	.690	.310	3.666	1
3	26	.750	.250	.154	1
4	22	.478	.523	2.954	1
5	9	.500	.500	.111	1
6	19	.684	.315	.903	1
7	26	.789	.212	1.872	1
8	28	.750	.250	.571	1
9	27	.852	.148	4.606*	1
10	26	.654	.346	2.667	1

AGPD

Site	N	53	100	127	x^2	d.f.
1	31		1.000			
2	31	.032	.952	.016	1.286	3
3	29		1.000			
4	30		1.000			
5	15		1.000			
6	26		1.000			
7	34		1.000			
8	36		1.000			
9	33		.970	.030	.032	1
10	34		.956	.044	.072	1

GOT-1*

Site	N	72	100	136	ϕ	x^2	d.f.
1	31	.532	.307	.161		4.967	3
2	31	.710	.290			.285	1
3	29	.500	.500			.310	1
4	30	.450	.550			.466	1
5	15	.600	.400			.185	1
6	26	.212	.789			4.660*	1
6'	26	.169	.654		.177	1.167	1
7	34	.074	.927			4.220*	1
7'	34	.062	.827		.111	3.096	1
8	36	.069	.931			4.540*	1
8'	36	.057	.827		.116	2.887	1
9	33	.652	.349			.000	1
10	34	.559	.441			3.316	1

sites for which null allele frequencies were dicated with a (').

Appendix III. - Continued.

GOT-2

Site	N	-100	-78	x^2	d.f.
1	31	1.000			
2	31	1.000			
3	29	1.000			
4	30	1.000			
5	15	1.000			
6	26	1.000			
7	34	1.000			
8	36	.917	.083	2.678	1
9	33	1.000			
10	34	1.000			

IDH-1

Site	N	83	100	115	x^2	d.f.
1	31		.984	.016	.008	1
2	31		1.000			
3	29	.017	.983		.009	1
4	30		1.000			
5	15		1.000			
6	26		1.000			
7	34		.985	.015	.008	1
8	36		1.000			
9	33		1.000			
10	34		1.000			

IDH-2

Site	N	100	217	x^2	d.f.
1	31	.984	.016	.008	1
2	32	.828	.172	1.716	1
3	29	.845	.155	3.400	1
4	30	.750	.250	.015	1
5	15	.667	.333	.600	1
6	26	.962	.039	.042	1
7	34	1.000			
8	36	1.000			
9	33	.758	.242	.793	1
10	34	.735	.265	1.484	1

Appendix III.- Frequencies of alleles and chi-square values, x^2, from goodness-of-fit tests to Hardy-Weinberg proportions for populations from sites 1 through 10.

ADA

Site	N	100	120	x^2	d.f.
1	25	.900	.100	.309	1
2	29	.690	.310	3.666	1
3	26	.750	.250	.154	1
4	22	.478	.523	2.954	1
5	9	.500	.500	.111	1
6	19	.684	.315	.903	1
7	26	.789	.212	1.872	1
8	28	.750	.250	.571	1
9	27	.852	.148	4.606*	1
10	26	.654	.346	2.667	1

AGPD

Site	N	53	100	127	x^2	d.f.
1	31		1.000			
2	31	.032	.952	.016	1.286	3
3	29		1.000			
4	30		1.000			
5	15		1.000			
6	26		1.000			
7	34		1.000			
8	36		1.000			
9	33		.970	.030	.032	1
10	34		.956	.044	.072	1

GOT-1*

Site	N	72	100	136	ϕ	x^2	d.f.
1	31	.532	.307	.161		4.967	3
2	31	.710	.290			.285	1
3	29	.500	.500			.310	1
4	30	.450	.550			.466	1
5	15	.600	.400			.185	1
6	26	.212	.789			4.660*	1
6'	26	.169	.654		.177	1.167	1
7	34	.074	.927			4.220*	1
7'	34	.062	.827		.111	3.096	1
8	36	.069	.931			4.540*	1
8'	36	.057	.827		.116	2.887	1
9	33	.652	.349			.000	1
10	34	.559	.441			3.316	1

sites for which null allele frequencies were dicated with a (').

Appendix III. - Continued.

GOT-2

Site	N	-100	-78	x^2	d.f.
1	31	1.000			
2	31	1.000			
3	29	1.000			
4	30	1.000			
5	15	1.000			
6	26	1.000			
7	34	1.000			
8	36	.917	.083	2.678	1
9	33	1.000			
10	34	1.000			

IDH-1

Site	N	83	100	115	x^2	d.f.
1	31		.984	.016	.008	1
2	31		1.000			
3	29	.017	.983		.009	1
4	30		1.000			
5	15		1.000			
6	26		1.000			
7	34		.985	.015	.008	1
8	36		1.000			
9	33		1.000			
10	34		1.000			

IDH-2

Site	N	100	217	x^2	d.f.
1	31	.984	.016	.008	1
2	32	.828	.172	1.716	1
3	29	.845	.155	3.400	1
4	30	.750	.250	.015	1
5	15	.667	.333	.600	1
6	26	.962	.039	.042	1
7	34	1.000			
8	36	1.000			
9	33	.758	.242	.793	1
10	34	.735	.265	1.484	1

Appendix III. - Continued

LDH-2

Site	N	100	245	x^2	d.f.
1	31	1.000			
2	32	1.000			
3	29	1.000			
4	30	.967	.033	.036	1
5	15	.967	.033	.018	1
6	26	.904	.096	.294	1
7	34	1.000			
8	36	1.000			
9	33	1.000			
10	34	1.000			

MDH-2

Site	N	80	100	120	x^2	d.f.
1	31		1.000			
2	32		1.000			
3	29		1.000			
4	30		1.000			
5	15		1.000			
6	26		.981	.019	.010	1
7	34		.927	.074	.214	1
8	36		.972	.028	.029	1
9	33	.015	.985		.008	1
10	34		1.000			

ME

Site	N	75	100	113	125	x^2	d.f.
1	31		1.000				
2	32		1.000				
3	29		.983	.017		.009	1
4	30	.017	.983			.009	1
5	15		1.000				
6	26		1.000				
7	34		1.000				
8	36		1.000				
9	33		.985		.015	.008	1
10	34		1.000				

Appendix III. - Continued

PGI

Site	N	-150	-100	80	x^2	d.f.
1	31		1.000			
2	32		.969	.031	.033	1
3	29	.035	.948	.017	.086	3
4	30		1.000			
5	15		1.000			
6	26		.981	.019	.010	1
7	34	.029	.971		.031	1
8	36		1.000			
9	33		.985	.015	.008	1
10	34		1.000			

PGM-2

Site	N	71	83	88	100	120	x^2	d.f.
1	31				.952	.048	.080	1
2	32				1.000			
3	29				1.000			
4	30				1.000			
5	15				1.000			
6	26	.019			.981		.010	1
7	34	.015		.044	.941		.133	3
8	36	.028		.028	.944		.125	3
9	33		.015		.970	.015	.032	3
10	34		.029		.956	.015	.072	3

6PGD

Site	N	75	88	100	119	138	x^2	d.f.
1	31	.016	.242	.629	.097	.016	11.332	10
2	32	.047	.344	.281	.297	.031	5.822	10
3	29		.414	.362	.190	.035	2.494	6
4	30	.250	.267	.250	.233		4.120	6
5	15	.100	.500	.100	.300		10.333	6
6	26	.077	.385	.365	.173		6.339	6
7	34		.559	.338	.103		2.466	3
8	36	.014	.486	.389	.111		4.719	6
9	32	.281	.281	.266	.156	.016	10.472	10
10	34	.074	.397	.471	.059		3.974	6

Appendix III. - Continued

TRF

Site	N	91	100	106	x^2	d.f.
1	31	.113	.855	.032	4.397	3
2	32	.250	.750		.889	1
3	29	.172	.776	.052	1.647	3
4	30	.017	.983		.009	1
5	15	.200	.800		.417	1
6	26	.019	.981		.010	1
7	34		.985	.015	.008	1
8	36	.014	.986		.007	1
9	33	.333	.667		1.091	1
10	34	.471	.529		.105	1

Appendix IV. - Names of loci and the chi-square values which demonstrate a significant difference in pairwise comparisons between the populations at each site. Specific allele is indicated in parentheses.

Site:Site	Allele	Chi-square
1 : 2	IDH-2 (100)	8.872**
	GOT-1 (136)	10.878***
	TRF (91)	3.967*
	6PGD (100)	15.368***
	ADA (100)	7.086**
1 : 3	IDH-2 (100)	7.591**
	GOT-1 (136)	10.206**
	6PGD (100)	8.539**
	ADA (100)	3.946*
1 : 4	IDH-2 (100)	14.641***
	GOT-1 (136)	10.542**
	6PGD (100)	17.753***
	TRF (100)	6.688**
	ADA (100)	22.021***
1 : 5	IDH-2 (100)	19.328***
	GOT-1 (136)	5.429*
	6PGD (100)	22.803***
	ADA (100)	16.000***
1 : 6	GOT-1 (100)	26.354***
	TRF (100)	5.610*
	LDH-2 (245)	6.238*
	6PGD (100)	7.863**
	ADA (100)	6.451*
1 : 7	GOT-1 (100)	53.529***
	TRF (100)	7.777**
	MDH-2 (100)	4.739*
	6PGD (100)	10.993***
1 : 8	GOT-1 (100)	56.445***
	GOT-2 (100)	5.407*
	TRF (100)	8.314**
	6PGD (100)	7.682**
	ADA (100)	4.040*
1 : 9	IDH-2 (100)	14.217***
	GOT-1 (136)	11.548***
	TRF (100)	6.163*
	6PGD (100)	16.843***
1 : 10	IDH-2 (100)	16.062***
	GOT-1 (136)	11.882***
	TRF (100)	15.903***
	ADA (100)	8.846**

Appendix IV. - Continued.

Site:Site	Allele	Chi-square
2 : 3	GOT-1 (100)	5.531*
2 : 4	GOT-1 (100)	8.453**
	TRF (100)	14.246***
	6PGD (75)	10.293**
	ADA (100)	5.468*
2 : 5	6PGD (100)	3.869*
2 : 6	IDH-2 (100)	5.129*
	GOT-1 (100)	28.112***
	TRF (100)	12.220***
	LDH-2 (100)	6.434*
2 : 7	IDH-2 (100)	12.752***
	GOT-1 (100)	55.892***
	TRF (100)	16.269***
	MDH-2 (100)	4.889*
	PGM-2 (100)	3.881*
	6PGD (119)	7.845**
2 : 8	IDH-2 (100)	13.466***
	GOT-1 (100)	58.883***
	GOT-2 (100)	5.407*
	TRF (100)	17.268***
	6PGD (119)	7.351**
2 : 9	6PGD (75)	12.817***
	ADA (100)	4.128*
2 : 10	TRF (100)	6.933**
	6PGD (119)	12.991***

Appendix IV. - Continued.

Site:Site	Allele	Chi-square
3 : 4	TRF (100)	12.132***
	6PGD (75)	16.612***
	ADA (100)	8.660**
3 : 5	6PGD (100)	6.848**
	ADA (100)	5.288*
3 : 6	IDH-2 (100)	4.148*
	GOT-1 (100)	9.862**
	TRF (100)	10.366**
	LDH-2 (100)	5.845*
	6PGD (75)	4.628*
3 : 7	IDH-2 (100)	11.366***
	GOT-1 (100)	28.900***
	TRF (100)	13.900***
	MDH-2 (120)	4.440*
3 : 8	IDH-2 (100)	12.006***
	GOT-1 (100)	30.841***
	GOT-2 (100)	5.065*
	TRF (100)	14.771***
	PGI (100)	3.810*
3 : 9	TRF (91)	4.174*
	6PGD (75)	19.140***
3 : 10	TRF (91)	12.525***
	6PGD (119)	5.114*

Appendix IV. - Continued.

Site:Site	Allele	Chi-square
4 : 5	TRF (100)	9.366**
	6PGD (88)	4.821*
4 : 6	IDH-2 (100)	9.678**
	GOT-1 (100)	7.069**
	6PGD (75)	5.926*
	ADA (100)	4.037*
4 : 7	IDH-2 (100)	19.257***
	GOT-1 (100)	24.099***
	6PGD (119)	3.593*
	MDH-2 (100)	4.589*
	ADA (100)	11.472***
4 : 8	IDH-2 (100)	20.308***
	GOT-1 (100)	25.815***
	GOT-2 (100)	5.236*
	6PGD (75)	17.126***
	ADA (100)	9.046**
4 : 9	GOT-1 (100)	5.168*
	TRF (100)	21.112***
	ADA (100)	17.643***
4 : 10	TRF (100)	34.319***
	6PGD (75)	7.533**

Appendix IV. - Continued.

Site:Site	Allele	Chi-square
5 : 6	IDH-2 (100)	13.234***
	GOT-1 (100)	12.562***
	TRF (100)	7.966**
	6PGD (100)	6.826**
5 : 7	IDH-2 (100)	25.240***
	GOT-1 (100)	32.128***
	TRF (100)	10.777**
	6PGD (75)	7.015**
	ADA (100)	7.313**
5 : 8	IDH-2 (100)	26.606***
	GOT-1 (100)	34.141***
	TRF (100)	11.473***
	6PGD (100)	8.355**
	ADA (100)	5.463*
5 : 9	6PGD (88)	4.288*
	ADA (100)	12.012***
5 : 10	TRF (100)	6.421*
	6PGD (100)	12.452***
6 : 7	GOT-1 (100)	4.857*
	6PGD (75)	5.410*
	LDH-2 (100)	6.826**
6 : 8	GOT-1 (100)	5.427*
	GOT-2 (100)	4.552*
	LDH-2 (100)	7.218**
6 : 9	IDH-2 (100)	9.354**
	GOT-1 (100)	22.686***
	TRF (100)	18.288***
	6PGD (75)	7.799**
	LDH-2 (100)	6.630*
6 : 10	IDH-2 (100)	10:855***
	GOT-1 (100)	14.712***
	TRF (100)	30.115***
	6PGD (119)	3.985*
	LDH-2 (100)	6.826**

Appendix IV. - Continued.

Site:Site	Allele	Chi-square
7 : 8	GOT-2 (-100)	5.918*
7 : 9	IDH-2 (100)	18.718***
	GOT-1 (100)	48.673***
	TRF (100)	23.913***
	6PGD (88)	10.304**
	MDH-2 (120)	5.039*
7 : 10	IDH-2 (100)	20.745***
	GOT-1 (100)	37.038***
	TRF (100)	37.038***
	6PGD (75)	5.189*
	MDH-2 (100)	5.189*
8 : 9	IDH-2 (100)	19.741***
	GOT-1 (100)	51.438***
	GOT-2 (-100)	5.748*
	TRF (100)	25.294***
	6PGD (88)	5.975*
8 : 10	IDH-2 (100)	21.870***
	GOT-1 (100)	39.363***
	GOT-2 (-100)	5.918*
	TRF (100)	40.487***
9 : 10	6PGD (75)	9.984**
	ADA (100)	5.616*

* Significance at .05 level.
** Significance at .01 level.
*** Significance at .001 level.

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