Evolutionary Genetics of California Islands *Peromyscus*

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INTRODUCTION

Population genetics is at a point where its model building must be re-examined. Many of the parameters important in models are extremely hard to measure in practice, and debates on interpretations of these models have not led to clarity. It is clear that a large body of data is necessary to progress beyond the stalemate of present-day, established schools of thought.

Very little is known about the actual process of speciation, but we do know that the old axiom that evolution is merely a change in gene frequencies is not necessarily true. Some changes are homeostatic, while others are of evolutionary significance, but they can occur initially without a great deal of measurable genetic change. We must re-examine the first steps in speciation. We do not know the relative importance of morphological, biochemical, or chromosomal variation during the beginning stages of speciation. Detailed studies of specific cases are necessary to cope with the complexity of this problem, and generalizations will not be apparent until a large enough data base exists to compare the patterns in different situations.

All evolutionary change depends upon the presence of genetic variability in populations, for it is on this pool of variability that selection acts in the evolutionary process. A major concern of population genetics is the measurement of this genetic variation. We need to know what levels of variability exist in natural populations and need to gain some idea of quantitative changes in gene frequencies and genetic variability as populations evolve.

Holistic studies using a diversity of methods are important to an understanding of the variation found in natural populations (Berry and Peters 1976, Pizzimenti 1976, Shvarts 1977). If one considers only a single type of variation, the impression obtained of divergence between groups or rates of change may be quite different from that arising from a comparative analysis of different types of variation. For example, divergence between populations in electrophoretically detectable genic variation is not necessarily reflected in the levels of morphological or karyotypic variations (Lewontin 1974, Selander *et al.* 1974, Turner 1974, Nixon and Taylor 1977). Selection may operate in different ways at each of these levels.

In this evolutionary study of the Deer Mouse, *Peromyscus maniculatus*, I compare morphological and genic divergence of populations on the Channel Islands and of their closest mainland relatives. The Channel Islands are easily accessible and offer a rich opportunity for in-depth study of natural populations. Precisely because they are not divorced from the mainstream of evolution on the mainland and generally do not exhibit the strikingly aberrant forms found on oceanic islands, they may be of greater value than either mainland or oceanic species in clarifying general evolutionary processes. The time for quick and easy studies of natural populations is past, and it is time now for detailed studies on a larger scale. Continental slope islands are particularly important to general evolutionary studies because the plants and animals they support have the potential to interact with and influence the course of evolution of mainland species.

Morphological traits examined in this study include measures of overall body size, skull

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TABLE 1. Areas and distances of Channel Islands.*

Island	Area (km^2)	Distance to mainland (km)	Distance to nearest island (km)
Santa Cruz	249.0	30	7
Santa Rosa	217.0	44	5
Santa Catalina	194.0	32	34
San Clemente	145.0	79	34
San Nicolas	58.0	98	45
San Miguel	37.0	42	5
Anacapa	2.9	20	7
Santa Barbara	2.6	61	39

* Adapted from Tables 1 and 2 of the Introduction to Philbrick (1967).

anatomy, and weights of some organs. The latter traits, although far more variable than other anatomical or "hard" traits, are useful as indicators of differential adaptations at the physiological level. I have carried out factor and discriminant analyses of the subspecies of Deer Mice on the basis of these morphological characters, utilizing computer programs for multivariate analysis. In addition, I have analyzed 30 protein systems by means of horizontal starch gel electrophoresis and have calculated measures of genic variability and genetic divergence based on the electrophoretic analysis. The composite analysis of morphogenetic variability at the morphological and biochemical levels is directed to questions of (1) the comparative rates of anatomical and genic divergence among the insular populations and (2) the amounts of gene flow and the population sizes necessary to maintain the observed levels of variability. As will be seen, the analysis brings new insights into the amount of genetic variability that can be maintained in island populations and into the differing levels of heterozygosity in the various types of structural genes studied. The patterns of heterozygosity suggest different selection pressures on these various classes of structural genes.

P. maniculatus is an ideal subject for this study, for it is a highly variable and widespread species which occurs in a diversity of habitats and eats a variety of foods (Blair 1968). Furthermore, it is easily trapped and will breed in the laboratory. It is the only small mammal to have evolved endemic subspecies on all eight of the California Channel Islands (Hall and Kelson 1959, von Bloeker 1967). These islands vary in size and distance from the mainland and from their nearest island neighbors (Table 1), and, due to tectonic emergence and eustatic sea-level fluctuations, have been isolated for varying periods of time since their establishment in the late Pliocene (Thorne 1969, Vedder and Howell 1980). The subspecies of *P. maniculatus* on the eight Channel Islands, as given by Hall and Kelson (1959), are: *anacapae* von Bloeker (Anacapa), *santacruzae* Nelson and Goldman (Santa Cruz), *sanctaerosae* von Bloeker (Santa Rosa), *streatori* Nelson and Goldman (San Miguel), *exterus* Nelson and Goldman (San Nicolas), *elusus* Nelson and Goldman (Santa Barbara), *catalinae* Elliott (Santa Catalina), and *clementis* Mearns (San Clemente). The closest mainland relative is *P. maniculatus gambelii* Baird, which ranges from Baja California to Washington.

MATERIALS AND METHODS

Deer Mice belonging to different nominal subspecies of *Peromyscus maniculatus* were live-trapped from populations on the eight Channel Islands and from California mainland sites

in Ventura, Riverside, San Bernardino, and Inyo Counties. All the island mice and those from Ventura County were transported live to the laboratory, where they were weighed and measured. They were maintained for at least a week under standard laboratory conditions and were subsequently bred or processed for electrophoresis. The other mainland mice were live-trapped by other researchers for their own studies and later made available to me. For these samples, data on body measurements at the time of capture are lacking. A list of the samples taken at each site detailing the site, subspecies, abbreviations for subspecies, dates of capture, and number captured follows:

Santa Cruz Island, *santacruzae* (CRU), July 1975, 123.
Middle Anacapa Island, *anacapae* (ANA), July 1976, 25.
Santa Catalina Island, *catalinae* (CAT), Nov. 1975 and March 1976, 17.
Santa Barbara Island, *elusus* (ELU), Nov. 1976, 34.
San Miguel Island, *streatori* (STR), May 1975 and Sep. 1976, 140.
Santa Rosa Island, *sanctaerosae* (ROS), Feb., Apr., and May 1976 and May 1977, 70.
San Nicolas Island, *exterus* (EXT), Apr. 1975 and May 1976, 25.
San Clemente Island, *clementis* (CLE), Jan. and Apr. 1975 and Apr. 1976, 57.
Pt. Mugu and Camarillo, Ventura County, *gambelii* (GAM, MUG), Mar., Apr., and May 1976 and Apr. 1977, 19.
Riverside, Riverside County, *gambelii* (GAM, RIV), received July 1977 and Feb. 1978, 35.
Black Mountain, Riverside County, *gambelii* (GAM, BKM), received Feb. 1978, 10.
Heart Bar Campground, San Bernardino County, *gambelii* (GAM, HTB), received Feb. 1978, 16.
Owens Valley, Inyo County, *sonoriensis* (OWV), received Feb. 1977, 10.

Many of the females live-trapped in the field were pregnant and their offspring (approximately 300 individuals) were born in the laboratory. Crosses were set up within and between subspecies in the laboratory, as well. The data thus obtained from known relatives were used to establish the genetic basis of all electrophoretic variants reported in this study; only those variants that represent allozymes at a genetic locus are included in the analysis. Litters were reared in the laboratory until they reached maturity and only adults were used in the morphological and electrophoretic analyses. Pelage type was used to determine age classes in field-caught Deer Mice.

There are two developmental molts in *Peromyscus maniculatus*: (1) the post-juvenal molt from the gray juvenal pelage to the browner, subadult pelage, which occurs within an age range of 4 to 12 weeks (Layne 1968), and (2) the post-subadult molt to the adult pelage, which begins at an average age of 16 weeks and is usually completed by 21 weeks (based on data from *P. m. gambelii*, McCabe and Blanchard 1950). Only animals that had completed the post-subadult molt to the adult pelage were used in this study. Growth rate in body dimensions becomes essentially zero by eight weeks of age in *P. maniculatus* (Layne 1968), so that the field-caught animals compared in this study can be assumed to have reached their full adult growth. Both eruption of molars and sexual maturity occur at an early age in *P. maniculatus* and are not suitable characters for determining adult status in this study. We have observed in our laboratory-reared animals that all three molars have already erupted in mice with juvenal pelage; incisors erupt at a mean age of 5.7 days (Layne 1968) and become yellowish while the animals are still juveniles. Sexual maturity has been reported to be as early as seven weeks in females and nine in males (McCabe and Blanchard 1950). This finding is consistent with results in my laboratory where 2½-month-old sibs have produced litters. Since the gestation period is 23 to 24 days (Layne 1968), this is evidence of sexual maturity at seven to eight weeks of age in both sexes.

I used starch gel electrophoresis to detect protein variation in blood, liver, and kidney

TABLE 2. External measurements of body size in nine subspecies of *Peromyscus maniculatus*.*

Subspecies†	*n*	Body weight (g) Mean	S.D.	Lengths (mm) Body Mean	S.D.	Tail Mean	S.D.	Foot Mean	S.D.	Ear Mean	S.D.
CRU	134	26.0	4.0	87.8	6.2	80.1	5.1	19.5	1.1	17.0	1.3
ANA	25	25.8	2.8	85.2	3.7	81.2	3.0	19.7	0.6	16.7	1.0
CAT	15	21.8	2.5	88.7	4.8	77.5	4.7	20.1	1.2	20.0	0.9
ELU	24	24.6	3.2	89.7	3.3	68.7	3.2	16.8	0.8	19.5	0.6
STR	132	23.9	4.6	88.5	4.7	70.4	4.9	18.2	1.5	19.0	1.5
ROS	65	23.8	4.0	88.1	7.5	70.8	4.5	19.4	0.7	17.2	1.1
EXT	20	21.5	4.2	84.0	5.6	72.5	2.6	18.9	0.7	17.8	0.9
CLE	54	19.7	2.6	83.6	4.9	67.8	4.6	19.0	0.9	17.8	1.2
Island total	469	23.9	4.4	87.4	5.8	73.8	6.8	18.9	1.3	17.9	1.6
GAM	14	19.5	3.3	79.4	7.6	65.3	3.8	18.1	0.8	15.9	1.1
Grand total	483	23.8	4.4	87.2	6.0	73.5	6.9	18.9	1.3	17.9	1.6

* Samples include pregnant females.

† Samples are from these islands: CRU = Santa Cruz, ANA = Anacapa, CAT = Santa Catalina, ELU = Santa Barbara, STR = San Miguel, ROS = Santa Rosa, EXT = San Nicolas, and CLE = San Clemente; and these mainland sites: GAM = Pt. Mugu and Camarillo.

samples. The experimental procedures employed were essentially those described by Yang in the appendix to the paper by Selander *et al.* (1971). Animals were weighed just before they were sacrificed. The blood samples were prepared, and plasma and hemolysate extracts run immediately. The kidneys and liver were then dissected, homogenized, and centrifuged in preparation for immediate electrophoretic runs, or the whole animal was kept frozen at −76°C and the tissues prepared later. The organs were weighed before extracts were prepared.

After tissues had been dissected for electrophoresis, the skull and flat skin of each animal were prepared as museum specimens. These specimens are housed at the University of Nevada at Reno. Specimens of mice born both in the field and in the laboratory were prepared. The following 12 measurements were taken on each skull with dial calipers accurate to 0.1 mm: (1) greatest skull length, (2) nasal length, (3) nasal breadth (greatest distance across nasal bone), (4) zygomatic breadth (greatest distance across zygomatic arches), (5) interorbital constriction, (6) palatal length, (7) mastoidal breadth (greatest distance across mastoidal bones), (8) maxillary breadth, (9) length of upper toothrow, (10) length of lower toothrow, (11) rostral depth (least vertical distance from top of skull to anterior border of toothrow), and (12) mandible length. All the raw data from skull measurements were transformed into logarithms in the computer analyses. These transformations improve comparisons of the subspecies samples, since growth-related characters generally vary proportionally rather than arithmetically.

MORPHOLOGICAL VARIATION

Three different sets of morphological characters are analyzed here: (1) the external characters of body size measured for all animals caught in the field, (2) organ and body weights for both field-born and laboratory-born mice, and (3) skull indices for field and laboratory mice. The

means and standard deviations of the size characters—body weight, body plus head length, and lengths of tail, hind foot, and ear—are given in Table 2, with data arranged in order of generally decreasing body size. As is typical of size relationships in small mammals, the mainland subspecies, *P. m. gambelii*, is smaller than any of the island subspecies. Among the island mice, San Clemente Island mice are smallest. There is great variation in size among the mice on the different islands and this inter-island variation is highly significant ($P < 0.001$ by analysis of variance) for each of the five measurements. Analyses of variance show the differences between average island measurements of body size and average mainland measurements to be highly significant ($P < 0.001$) for every trait except foot length, for which the difference is not as great, but is still significant ($P < 0.05$). When males and females were analyzed separately, it was found that males of different subspecies differ in foot length, but females do not. There was no sexual dimorphism in body size except for foot length ($P < 0.01$, analysis of variance), males having larger feet. The lack of sexual differences in body weight usually found in this species was no doubt due to the fact that many females caught in the field were pregnant.

San Clemente Island mice are the smallest insular mice not only in general body size but in relative organ weights, as well (Table 3). Their characteristically small size persists in their laboratory-born progeny (Table 4). Santa Barbara Island mice (ELU, Table 3) also have small kidneys and livers. The body and relative organ weights are significantly different among the island populations ($P < 0.001$, analyses of variance) for all four variables, suggesting differences in physiological adaptations among the insular populations. Morpho-physiological indices are very responsive to environmental changes and their high variability between populations reflects development under different environmental conditions and not random fluctuations (Shvarts 1975). This comparison of organ weights, although approximate because it does not deal with seasonal variation, clearly shows that, even when the island mice are maintained in a uniform environment and their progeny develop in a uniform environment, differences between San Clemente Island mice and others remain. This suggests that genetic changes have occurred (Tables 3 and 4), distinguishing San Clemente Island mice from the other island forms in organ size. Mainland mice have relatively heavier kidneys and hearts than island mice ($P < 0.01$, analyses of variance), but there is no significant difference in relative liver weights. Sexual dimorphism in kidney weights was observed in field-caught animals, but the differences were relaxed in laboratory-born animals. The animals on which the data in Tables 3 and 4 were based did not include any pregnant females, and a highly significant sexual dimorphism in body weight was found ($P < 0.001$, analyses of variance) in both field- and laboratory-born animals (the mean weight ± standard deviation for field-caught males is 24.7 ± 6.6 g, and for females 22.8 ± 7.8 g; laboratory males weigh 22.7 ± 3.6 g, females weigh 19.5 ± 3.1 g).

The relative liver weights of laboratory-born Deer Mice—which live in a uniform environment, unlike their parents born on the islands or mainland—were much lower (42.0 ± 6.4 mg/g) than those of island (49.7 ± 11.8 mg/g) or mainland mice (46.6 ± 6.8 mg/g). The liver is important in regulation and metabolism (*e.g.*, in controlling blood sugar levels, the interconversion of various nutrients, and detoxification of injurious chemical compounds). The larger liver weights of field-born mice may be due to such factors as greater storage of glycogen or a heavier workload on this organ in natural habitats, which have a variety both in type of food (including, probably, some toxic compounds in the plants they eat) and in its availability. Not only did liver weights decline in the laboratory-born progeny but the coefficients of variation were also greatly decreased for progeny of all insular mice (calculated from S.D./mean, Tables 3 and 4). The greatest change in relative liver weights occurred between Santa Rosa and Anacapa Island mice and their progeny. Despite the fact that relative organ weights reflect greater sensitivity to environmental factors than the other morphological measures used in this

TABLE 3. Body and organ weights of field-caught *Peromyscus maniculatus*.*

		Body weight (g)		Relative weights (mg/g)					
				Liver		Kidneys		Heart	
Subspecies†	*n*	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CRU	82	25.5	4.7	50.0	8.8	10.8	2.1	4.6	1.1
ANA	18	23.4	3.2	57.2	10.0	11.8	1.0	4.5	0.9
CAT	12	22.2	3.9	48.2	16.1	11.1	1.7	4.9	0.8
ELU	24	26.8	3.5	41.9	5.8	9.6	1.3	4.2	0.7
STR	111	23.6	5.0	53.2	9.8	10.6	1.7	4.1	0.7
ROS	65	23.6	3.8	51.6	16.2	10.7	1.5	4.9	1.1
EXT	20	23.3	3.1	50.1	11.4	10.0	1.4	4.6	1.0
CLE	43	22.8	4.1	38.7	5.4	9.2	1.6	3.8	0.7
Island total	375	24.1	3.7	49.7	11.8	10.5	1.8	4.4	1.0
GAM(M)	15	19.7	2.9	47.0	7.6	11.5	2.0	5.2	1.0
GAM(R)	5	20.4	3.1	45.3	4.2	12.0	2.1	4.6	0.4
Mainland total	20	19.9	2.9	46.6	6.8	11.7	1.9	5.0	0.9
Grand total	395	23.8	4.5	49.5	11.6	10.5	1.8	4.4	1.0

* Samples do not include any pregnant females.

† Subspecies abbreviations for the island mice are identified in Table 2. GAM(M) are from Pt. Mugu and Camarillo, GAM(R) from Riverside.

TABLE 4. Body and organ weights of laboratory-born *Peromyscus maniculatus*.*

		Body weight (g)		Relative weights (mg/g)					
				Liver		Kidneys		Heart	
Subspecies†	*n*	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CRU	19	21.4	4.3	43.4	5.0	13.3	2.8	4.8	0.9
ANA	20	22.5	4.1	43.5	4.4	9.4	1.3	3.7	1.1
CAT	3	23.5	4.4	49.6	4.3	11.7	2.2	4.8	1.1
STR	85	21.6	3.3	43.1	6.4	10.7	2.3	4.1	0.9
ROS	24	20.5	4.4	38.1	5.9	9.9	2.0	4.4	1.0
EXT	16	20.5	2.4	44.1	7.3	9.9	1.5	4.1	0.9
CLE	21	18.7	2.9	35.9	3.3	8.8	1.1	3.0	0.6
GAM(M)	5	19.5	2.7	39.4	6.8	10.7	2.4	5.1	0.4
GAM(R)	12	20.1	4.3	44.1	7.5	12.7	2.2	5.3	0.7
Total	214	21.1	3.7	42.0	6.4	10.6	2.4	4.1	1.0

* No laboratory ELU were available.

† Subspecies identification given in Tables 2 and 3.

TABLE 5. Representative skull indices of field-caught *Peromyscus maniculatus*.

		Total length (mm)		Zygomatic breadth (mm)		Rostral depth (mm)	
Subspecies*	*n*	Mean	S.D.	Mean	S.D.	Mean	S.D.
CRU	60	26.22	.82	13.56	.48	6.04	.30
ANA	17	25.86	.61	13.65	.34	5.96	.18
CAT	12	25.40	.69	13.47	.31	5.67	.30
ELU	23	25.42	.41	13.38	.18	5.87	.18
STR	65	25.27	.66	13.14	.35	5.86	.30
ROS	43	25.51	.70	13.39	.36	5.96	.24
EXT	18	25.66	.81	13.15	.50	5.71	.32
CLE	34	24.74	.57	13.01	.46	5.78	.23
GAM(M)	14	24.09	.71	12.89	.31	5.67	.20
GAM(R)	6	24.55	.62	12.91	.32	5.85	.25
Total	292	25.44	.88	13.29	.45	5.88	.29

* Subspecies identification given in Tables 2 and 3.

study, significant differences among the progeny of island and mainland mice still remained.

Skull measurements are particularly useful for comparative purposes, both among living groups and for fossil specimens. Analyses of skull indices revealed significant differences among the island populations and between island and mainland populations in all 12 variables measured. As with the other morphological measurements, the island mice are larger than the mainland mice in skull indices. Representative data on three of the skull indices measured on field-caught mice, which give an idea of the volume of the skull, are presented in Table 5. Variation among island populations is highly significant for all three variables ($P < 0.001$, analyses of variance), San Clemente mice having the smallest skulls and Santa Cruz Island mice the largest. The island mice have significantly longer ($P < 0.001$), wider ($P < 0.001$), and deeper skulls ($P < 0.009$) than the mainland mice. A sample of 122 laboratory progeny of these mice were also measured; they were found to differ significantly, depending on island of origin, displaying differences similar to those found between island and mainland progenitors. There was no sexual dimorphism found in these skull indices.

In terms of morphological, morpho-physiological, and anatomical variability as measured by body size, organ weights, and skull indices, respectively, the insular populations of Deer Mice are distinct from one another and from the mainland populations. They are larger than the mainland populations, but vary greatly in size among themselves.

Multivariate Analysis of Morphological Data

Morphological data were analyzed by factor analysis, using BMDP4M (Dixon and Brown 1977) to determine correlations of the variables and common factors. Analysis of the skull measurements of 409 field-caught and laboratory-born Deer Mice revealed that the total length of the skull was the variable that correlated most highly with all other skull indices, its squared multiple correlation being 0.805. Skull length had the highest unrotated factor loading (0.911) for the largest principal component. Two factors of notably different character accounted for 61 per cent of the variance in skull indices, the other factors being minor. The first factor alone, for which the variables of zygomatic breadth and mandible length had the highest loadings after

rotation of the factors, accounted for 50.2 per cent of the variance. Rostral depth and palatal and skull lengths also had relatively high loadings for this factor. Only two variables, the lengths of the lower and upper toothrows, had high loadings on the second factor, which accounted for an additional 10.6 per cent of the variance in skull variables. It is, of course, obvious that selection would lead to correlation of the lengths of the toothrows, although they are no doubt under separate genetic control.

Variance in body measurements of 316 field-caught mice was also assessed using factor analysis, and three factors were found to account for 73 per cent of the variance. Body and organ weights had high loadings for factor 1, which accounted for 49 per cent of the variance. Only tail and foot lengths were important in factor 2, and ear length in factor 3, the latter two factors each contributing about 10 per cent of the variance.

The measurements that contribute most to the factors accounting for the largest part of the variance are not necessarily the measurements that are most important in discriminating between groups. A stepwise discriminant analysis (BMDP7M, Dixon and Brown 1977) was used to determine if the *P. maniculatus* subspecies were separable on the basis of the morphological variables described. Using a subset of 219 animals for which the entire battery of 22 measurements was available showed a high percentage of correct subspecific classification (85 per cent) based on 17 of these variables. The remaining five variables did not improve the discrimination. Tail and ear lengths were the variables first in importance to discrimination, followed by three skull indices of minor importance in the factor analyses. The finding that almost all the variables entered into the discrimination shows that the differentiation of subspecies is not due to change in one or a few major variables but to distinct changes in many variables, each contributing in its own way to the discrimination of the groups. A large number (eight) of canonical variables were needed to discriminate between the subspecies; none of these variables had particularly large eigenvalues.

In discriminating between two groups, a canonical variable is that combination of the original variables that maximizes the distance between groups. For a larger number of groups, discrimination between them can be visualized in the following way for three variables: each group is represented by a point in three-dimensional space which corresponds to the values of the three measurements (*e.g.*, mean skull length, rostral depth, and palatal length). As an example, if these points form a football-shaped cluster, the first canonical variable would lie along the long axis and would be some combination of the original variables. Its eigenvalue would be proportional to its length through the cluster of points. The second canonical variable, a different combination of the measurements, would be perpendicular to the first, and its eigenvalue would be proportional to its length. In this football-shaped example, the second and third canonical variables would have equal eigenvalues which would be smaller than the eigenvalue of the first canonical variable. In the discrimination of the nine subspecies in the present study, the eigenvalues of the first three canonical variables, which accounted for 74 per cent of the total dispersion, were 2.977, 1.934, and 1.063, respectively. The canonical variables consisted largely of contrasts between skull length and other skull indices: canonical variable 1 consisted of a contrast of skull length and mastoidal breadth with palatal length, and canonical variable 2 contrasted skull length and zygomatic breadth with mandible length and maxillary breadth. Thus, in Figure 1, where the subspecies are graphed in units of the first two canonical variables, it can be seen that the mice from Anacapa and Santa Cruz Islands have relatively longer skulls and greater mastoidal breadth than palatal length, the reverse holding true for the mice from San Clemente Island and the mainland *gambelii* subspecies. Discrimination between the other insular populations occurs along canonical variable 2. In this case, skull length and zygomatic breadth are relatively greater than mandible length and maxillary breadth

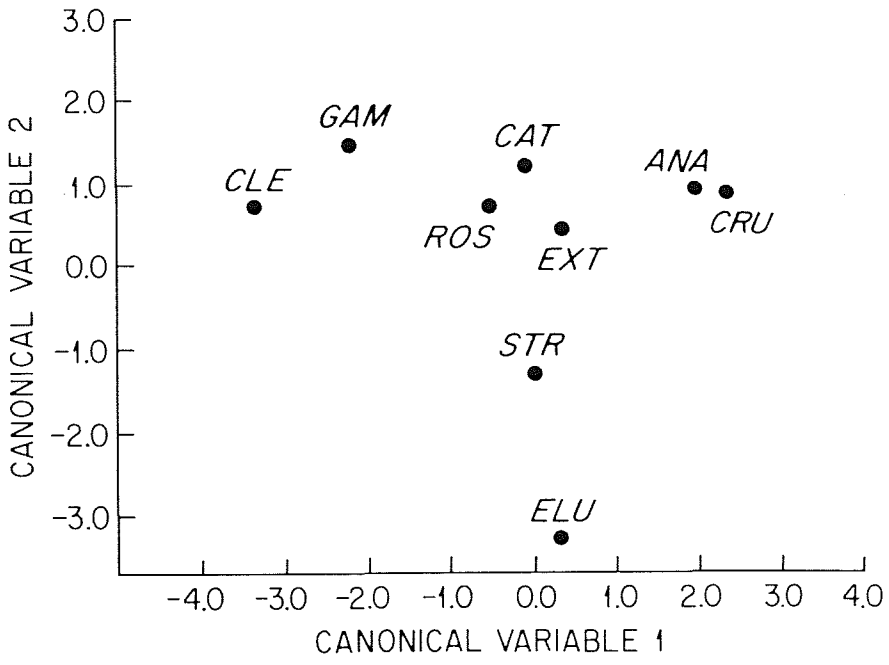

FIGURE 1. *Discrimination between nine subspecies of* Peromyscus maniculatus, *based on the first two of eight canonical variables. The means of the subspecies are shown. The coefficients of the original variables that contributed most heavily to the canonical variables are: for canonical variable 1, 31.6 skull length, 37.7 mastoidal breadth, and* −40.2 palatal length; and, for canonical variable 2, 45.5 skull length, 45.5 zygomatic breadth, −31.5 mandible length, and −26.1 maxillary breadth.

for mice from Santa Catalina, Santa Rosa, and San Nicolas Islands, compared with those from San Miguel and Santa Barbara Islands.

The majority of misclassifications between the nine subspecies in the stepwise discrimination involved mice from San Miguel (subspecies STR), Santa Rosa (ROS), and San Nicolas (EXT) Islands. I therefore removed those three subspecies from the analysis and compared the remaining five island and one mainland subspecies (reducing the sample size to 116). Using 12 of the 22 variables, the discrimination improved to 98 per cent correct classification. One of the *P. m. clementis* mice was statistically misclassified as *gambelii*, and one of the *santacruzae* mice as *catalinae;* the other 114 mice were correctly classified to subspecies. Not only was the discrimination almost completely accurate, but the "jackknifed" classification was also high—97 per cent. The P7M program uses the "jackknife" (Mosteller and Tukey 1977) to cross-validate classifications. This procedure gives a good indication of the reliability of classification for additional data—so it can be seen from the highly accurate jackknifed classification that morphological variables provide a reliable basis for classification of these subspecies. The relationships between morphological variables that are important in the discrimination of subspecies can be gauged from the canonical variables generated in the

analysis. Five canonical variables were generated to discriminate between these six subspecies, the first variable consisting of a contrast of tail length and mastoidal breadth with palatal length, and the second canonical variable a contrast of foot and palatal lengths with lengths of mandible and ear. It is clear that six of the subspecies are now so divergent in skull and body measurements that they can be accurately distinguished on this morphological basis. Discrimination is not as sharp between the populations of Deer Mice on San Nicolas, San Miguel, and Santa Rosa Islands, but even for this subset (sample size 103) a separate discriminant analysis showed 82 per cent correct classification. The canonical variables, again, involved contrasts of skull indices.

GENIC VARIATION

Horizontal starch gel electrophoresis was used to obtain measures of genic variation among the *P. maniculatus* subspecies. This method allows the detection of proteins of different net charge and mobility in an electric field (Hubby and Lewontin 1966, Harris 1966). Since the proteins are the initial gene products, we can infer that there is variation in the alleles controlling proteins of different mobility. Comparisons of known relatives were used to establish the electrophoretic variants as allozymes of genetic loci. The method provides a lower limit estimate of genetic variability because it can only distinguish proteins of different net charge.

Protein Systems

Thirty loci were analyzed; 23 of these were found to be polymorphic within or between subspecies. Ten subspecies were compared: the eight insular subspecies (labeled as ANA, CRU, ROS, STR, EXT, ELU, CAT, and CLE) and two California mainland subspecies (*P. m. gambelii*, represented by four mainland populations [MUG, RIV, BKM, and HTB; see Materials and Methods section for identification], and *P. m. sonoriensis*, represented by one mainland population [OWV]). *P. m. gambelii* is considered the closest mainland relative of the island mice (Hall and Kelson 1959) and *sonoriensis* is a neighboring subspecies included for comparison. The thirty loci analyzed are a good representation of the three groups of proteins: (I) glucose-metabolizing enzymes, (II) other enzymes, and (III) nonenzymatic proteins.

Glucose-metabolizing Enzymes

The polymorphic systems include two enzymes found in the kidneys, 6-phosphogluconate dehydrogenase (6-Pgd) and a lactic dehydrogenase (Ldh-1), which is polymorphic only in *P. m. sonoriensis*. Polymorphic systems found in the liver are α-glycerophosphate dehydrogenase (αGpd), a malate hydrogenase that is Nadp-dependent (Mdh-3), and three phosphoglucomutases (Pgm-1, Pgm-3, and Pgm-4). Another phosphoglucomutase (Pgm-2) is variable, but cannot be clearly interpreted. The monomorphic glucose-metabolizing enzymes are two malate dehydrogenases (Mdh-1 anodal and Mdh-2 cathodal) in liver and a lactic dehydrogenase (Ldh-1) in kidney.

Other Enzymes

Ten polymorphic nonglucose-metabolizing enzymes are found in liver. These are sorbitol dehydrogenase (Sdh), two peptidases (Pept-1 and Pept-2), an indophenol oxidase (Ipo-1) that is polymorphic only in the Santa Barbara Island mice, two glutamic oxaloacetic transaminases (Got-1 and Got-2) which are polymorphic only in the Santa Rosa Island population, and four esterases in liver and hemolysate that are not included because their separation and genetic basis are not yet clarified. The monomorphic Group II enzymes are leucine amino peptidase (Lap) in plasma, alcohol dehydrogenase (Adh) in liver, and an indophenol oxidase (Ipo-2) in liver.

Nonenzymatic Proteins

Hemoglobin (Hb) from hemolysate is polymorphic only in *P. m. sonoriensis* and serves to differentiate *sonoriensis* from *gambelii* (L. Snyder, pers. comm.) and the insular subspecies.

Genetic control of the hemoglobin loci in mainland *P. maniculatus* is described by Snyder (1978a and 1978b). There are two variable general proteins (Gp-1 and Gp-2) also found in hemolysate. Three polymorphic proteins are found in plasma, transferrin (Trf) and two general proteins (Gp-3 and Gp-4). There is only one monomorphic protein, albumin (Alb), found in plasma.

Subspecific Variation in Allozymes

Allelic frequencies for the 23 variable loci found in *P. maniculatus* are given in Table 6. Frequencies are given for eight insular populations, each representing a different subspecies, and for five mainland populations, four of *gambelii* subspecies (MUG, RIV, BKM, and HTB) and one of *sonoriensis* (OWV). The number of alleles sampled for each population was not constant because animals were trapped over an extended period and all the systems were not perfected at the same time. For the majority of loci analyzed, the number of alleles sampled (two per individual) was as follows: ANA 48, CRU 164, ROS 120, STR 190, EXT 46, ELU 50, CAT 30, CLE 102, MUG 32, RIV 66, OWV 20, BKM 20, and HTB 32. The smallest sample sizes were obtained for the esterases in most cases, for which the minimum numbers of alleles sampled in the given population were: ANA 14, ROS 34, STR 86, EXT 22, CAT 14, MUG 10, RIV 24, BKM 18, and HTB 24. The minimum numbers of alleles sampled in the remaining populations were: CRU 60 for Mdh-3, ELU 16 for Pgm, CLE 58 for Mdh-3, and OWV 14 for Pept.

For all but two loci, the populations differ only in the frequencies of alleles, and, if an allele has reached fixation, it is the same allele in all populations (Table 6). The two exceptions to this are the Got-1 and Trf loci, for which different alleles have reached fixation in the San Clemente Island population than in other populations. The Got-1 locus is highly variable, only the Anacapa and San Clemente populations being monomorphic for it. The Got-1 allele found in the Anacapa Island mice is also common in neighboring Santa Cruz Island mice, in the Santa Barbara and San Miguel Island populations, and in the mainland *sonoriensis* population (OWV). The Got-1 allele that is fixed in the San Clemente Island population is common on Santa Catalina Island. All four *P. m. gambelii* populations and the populations on Santa Rosa and San Nicolas Islands have extremely similar frequencies at this locus, suggesting selection may be maintaining the alleles close to a ratio of 0.57 to 0.43. The analysis of the Got-1 system reported here differs from that given for an earlier sample from four of the islands (Gill 1976) because a different buffer system is used here. I found that I obtained far better results using lithium hydroxide buffers—LiOH AB gel buffer and LiOH A, pH 8.1 tray buffer (buffer system 2 *in* Selander *et al.* 1971)—than the previously employed continuous triscitrate II, TC pH 8.0 gel and tray buffer system (system 5 *in* Selander *et al.* 1971). The dimeric nature of the Got-1 system is clear in LiOH, the three bands of the heterozygote being sharp and distinct. The TC pH 8.0 buffer is still used for the cathodal system, Got-2, since better results are obtained.

Only three populations are monomorphic for Trf. The same allele has reached fixation in the Anacapa and San Nicolas mice and a different allele is fixed in the San Clemente Island population. Again, the San Clemente Island allele is common in the Santa Catalina Island population. The allele fixed in the Anacapa and San Nicolas Island populations is common in the remaining Northern Channel Island populations.

There are five glucose-metabolizing enzymes for which the same allele is common in all populations; it has reached fixation in most. These are Ldh-1 (for which only *P. m. sonoriensis* has a rare alternate allele), α-Gpd, Mdh-3, Pgm-1, and Pgm-3. In contrast, both 6-Pgd and Pgm-4 are highly variable, showing no particular pattern of variation.

For most of the enzymes not involved with glucose metabolism (Group II), the same pattern of common alleles is found in the populations. The island populations are monomorphic for Sdh and Pept-2, with the same allele common on the mainland. All populations but one are

TABLE 6. Allele frequencies at 23 variable loci in *Peromyscus maniculatus* subspecies.

Loci and alleles		Island								Mainland				
		ANA*	CRU	ROS	STR	EXT	ELU	CAT	CLE	MUG	RIV	OWV	BKM	HTB
Group I. Glucose-metabolizing enzymes.														
Ldh-1	a†											0.10		
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00
α-Gpd	a	1.00	1.00	1.00	0.61	1.00	1.00	1.00	1.00	0.89	1.00	0.75	1.00	1.00
	b											0.15		
	c				0.39					0.11		0.10		
Mdh-3	a			0.01								0.10		
	b	0.94	0.98	0.96	1.00	1.00	1.00	0.90	0.97	0.90	1.00	0.90	1.00	0.81
	c			0.03					0.03	0.10				0.19
	d	0.06	0.02					0.10						
6-Pgd	a	0.47					0.10	0.10		0.22	0.19		0.20	0.50
	b	0.53	0.74	0.67	0.36	0.39	0.80	0.15	0.60	0.56	0.56	0.80	0.80	0.50
	c		0.26	0.33	0.64	0.61	0.10	0.75	0.40	0.22	0.08	0.10		
	d										0.17	0.10		
Pgm-1	a		0.30		0.05						0.07			
	b	1.00	0.70	0.98	0.95	1.00	1.00	1.00	1.00	1.00	0.89	1.00	0.95	0.97
	c			0.02							0.04		0.05	0.03
Pgm-3	a	1.00	1.00	1.00	1.00	1.00	1.00	0.77	1.00	0.93	1.00	1.00	1.00	0.93
	b							0.23		0.07				0.07
Pgm-4	a	0.39	0.09	0.27	0.11	0.33	0.50	0.13	0.40	0.31	0.54	0.50	0.15	0.37
	b	0.22	0.89	0.35	0.59	0.13		0.80	0.11	0.31	0.09	0.20	0.25	0.25
	c	0.39	0.02	0.38	0.30	0.54	0.50	0.07	0.49	0.38	0.37	0.30	0.60	0.38
Group II. Other enzymes.														
Sdh	a											0.10		
	b											0.30	0.20	0.06
	c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.60	0.80	0.94
Pept-1	a			0.06							0.05			
	b	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	0.94	0.95	0.71	0.90	1.00
	c									0.06		0.29	0.10	
Pept-2	a									0.11	0.05	0.15	0.10	
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.95	0.85	0.90	1.00
Ipo-1	a	1.00	1.00	1.00	1.00	1.00	0.79	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b						0.21							
Got-1	a		0.19	0.54	0.37	0.57	0.08	0.87	1.00	0.54	0.59	0.20	0.55	0.59
	b	1.00	0.81	0.46	0.63	0.43	0.92	0.13		0.46	0.41	0.80	0.45	0.41
Got-2	a	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b			0.02										
Es-2	a	0.07	0.01			0.15			0.03					
	b	0.93	0.99	1.00	1.00	0.85	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00
Es-3	a								0.07			0.17		
	b	0.86	1.00	1.00	1.00	1.00	1.00	0.92	0.85	1.00	0.75	0.83	1.00	1.00
	c	0.14						0.08	0.08		0.25			
Es-5	a		0.03							0.25	0.19	0.23	0.18	0.37
	b	0.50	0.59	0.73	0.84			0.64	0.05	0.50	0.16	0.23		0.17
	c			0.06		0.27								
	d	0.50	0.38	0.21	0.16	0.73	1.00	0.36	0.92	0.25	0.65	0.54	0.82	0.46
	e								0.03					
Es-6	a								0.35	0.56	0.17		0.15	0.12
	b			0.55		0.08	0.39			0.11		0.23		
	c	0.30	0.82	0.45	0.91	0.46	0.35	0.57	0.55	0.22	0.10	0.09	0.30	0.46
	d	0.50	0.18		0.09	0.29	0.26	0.43	0.10	0.11	0.50	0.64	0.30	0.29
	e	0.20				0.17					0.23	0.04	0.25	0.13
Group III. Nonenzymatic proteins.														
Hb	a											0.25		
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00

TABLE 6. (Cont.).

Loci and alleles		Island								Mainland				
		ANA*	CRU	ROS	STR	EXT	ELU	CAT	CLE	MUG	RIV	OWV	BKM	HTB
Trf	a												0.05	0.03
	b	1.00	0.99	0.88	0.80	1.00	0.24	0.05		0.38	0.68	0.25	0.40	0.59
	c		0.01	0.12	0.20		0.76	0.95	1.00	0.62	0.32	0.75	0.55	0.38
GP-1	a				0.05				0.01					
	b	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
GP-2	a	0.13			0.03	0.03								
	b	0.87	0.90	1.00	0.93	0.97	1.00	1.00	1.00	0.86	0.89	1.00	0.95	1.00
	c		0.10		0.04					0.14	0.11		0.05	
GP-3	a									0.03	0.08			
	b	1.00	1.00	1.00	0.98	1.00	1.00	0.95	1.00	0.90	0.80	1.00	1.00	1.00
	c				0.02			0.05		0.07	0.12			
GP-4	a										0.05	0.15	0.56	
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	0.85	0.33	0.93
	c										0.05		0.11	0.07

* Samples are from these islands: ANA = Anacapa, CRU = Santa Cruz, ROS = Santa Rosa, STR = San Miguel, EXT = San Nicolas, ELU = Santa Barbara, CAT = Santa Catalina, CLE = San Clemente. Mainland *gambelii* are from MUG = Pt. Mugu and Camarillo, RIV = Riverside, BKM = Black Mountain, HTB = Heart Bar Campground, and *sonoriensis* is from OWV = Owens Valley.

† Alleles are listed in order of increasing mobility; *a* is the slowest.

monomorphic for Ipo-1 and Got-2, and in the polymorphic populations the second allele is rare. Mainland populations are monomorphic for Es-3, with the same allele common throughout the island populations; for both Pept-1 and Es-3, one allele is common throughout all populations and fixed in most of them. Only Got-1 (already described), Es-5, and Es-6 show more variability. Both esterase loci have a larger number of detected alleles; no single allele is common throughout the populations sampled.

With the exception of Trf, there is little variability in the nonenzymatic proteins. The inheritance of Trf is consistent with a one-locus, codominant allelic system, as described by Rasmussen and Koehn (1966) for *P. maniculatus*. In this study, in addition to the two common alleles, a rarer, slow allele was found paired with the intermediate allele in a heterozygote from Black Mountain, and with the fast allele in a heterozygote from Heart Bar Campground, both mice of the *gambelii* subspecies. Hemoglobin is variant only in *P. m. sonoriensis*, as mentioned earlier. In all the insular subspecies and *gambelii*, it exhibits the usual two-banded electrophoretic pattern. For each of the general proteins, one allele is common in all populations and has reached fixation in most. The only exception to this is the variation for Gp-4 in the Black Mountain population (BKM) of *gambelii*, where a usually rare allele has reached high frequency. Among the insular populations, that on San Miguel Island (STR) has the most variable general proteins.

The likelihood ratio (Wilks' λ) was used to test for equilibrium at all loci. All of the populations are in Hardy-Weinberg equilibrium for transferrin and Got-1, except the San Miguel Island population, which has an excess of heterozygotes for Got-1. The only other loci for which there is an excess of heterozygotes in some insular populations are Pgm-4 and Es-6. At the other esterase loci there is an excess of homozygotes in some of the populations, both island and mainland. All the populations are in equilibrium for the remaining loci, with the following exceptions at which there is an excess of homozygotes: 6-Pgd in some island and mainland populations, GP-3 and Pept-2 in RIV, Sdh in MUG and BKM, α-Gpd in STR, and Pept-1 in ROS. This deficiency of heterozygotes, occurring mostly in the mainland populations, suggests that those populations are subdivided.

Measures of Genic Variability

Measures of genic variability based on the electrophoretic data for the 30 protein systems analyzed are given in Table 7. Three measures of variability are calculated for each of the different kinds of loci and for the total of all loci, with island and mainland populations considered separately. The three measures are: the mean number of alleles per locus ($\bar{A}$), the fraction of loci polymorphic ($\bar{P}$), and the mean heterozygosity per locus per individual ($\bar{H}$). The criterion for polymorphism is that the most common allele does not exceed a frequency of 0.99. Heterozygosity per individual was determined for each locus by dividing the number of heterozygotes by the sample size; the unweighted mean over all loci, $\bar{H}$, was then calculated. The standard error of $\bar{H}$ over all loci is also given in Table 7.

What is immediately impressive about the data in Table 7 are the high levels of polymorphism apparent in the mainland populations. Forty-three per cent of the loci in mainland populations are polymorphic, as compared, for example, with 23 per cent for *Peromyscus polionotus*, 29 per cent for *Mus musculus musculus*, and 29 per cent for *Homo sapiens* (Lewontin 1974). As Selander (1975) notes, it was evident early in electrophoretic work that some proteins are more likely to be polymorphic than others. The particular protein systems included in a study will therefore influence the levels of variability. For example, the addition of four new systems in this study and the exclusion of two used in a previous study (Gill 1976) resulted in a change of some measures of variability, although the overall pattern remained the same. In this study, all three groups of loci in mainland populations have the same level of

TABLE 7. Genic variation in island and mainland populations of *Peromyscus maniculatus:* mean number of alleles per locus ($\bar{A}$), fraction of loci polymorphic ($\bar{P}$), and mean heterozygosity per locus per individual ($\bar{H}$).

	Group I loci			Group II loci			Group III loci			All loci			
Population	$\bar{A}$	$\bar{P}$*	$\bar{H}$	$\bar{A}$	$\bar{P}$	$\bar{H}$	$\bar{A}$	$\bar{P}$	$\bar{H}$	$\bar{A}$	$\bar{P}$	$\bar{H}$	± S.E.
Island populations													
ANA†	1.40	.30	.065	1.38	.31	.083	1.14	.14	.007	1.33	.27	.059	.028
CRU	1.50	.40	.070	1.38	.31	.073	1.29	.29	.030	1.40	.33	.062	.022
ROS	1.60	.40	.073	1.46	.38	.106	1.14	.14	.035	1.43	.33	.079	.039
STR	1.50	.40	.138	1.23	.23	.081	1.71	.57	.082	1.43	.37	.100	.032
EXT	1.30	.20	.056	1.46	.31	.070	1.14	.14	.008	1.33	.23	.051	.025
ELU	1.30	.20	.100	1.31	.23	.079	1.14	.14	.052	1.27	.20	.080	.041
CAT	1.60	.40	.046	1.31	.31	.066	1.29	.29	.015	1.40	.33	.048	.016
CLE	1.40	.40	.072	1.46	.31	.051	1.14	.14	.004	1.37	.27	.047	.030
Island averages	1.45	.34	.078	1.37	.30	.076	1.25	.23	.029	1.37	.29	.066	
Mainland populations													
MUG	1.70	.50	.100	1.62	.38	.081	1.57	.43	.076	1.63	.43	.086	.031
RIV	1.70	.30	.079	1.69	.46	.049	1.88	.57	.093	1.73	.43	.069	.030
OWV	1.80	.50	.077	1.85	.54	.167	1.43	.43	.086	1.73	.50	.118	.040
BKM	1.40	.30	.040	1.62	.46	.086	1.71	.43	.102	1.57	.40	.074	.027
HTB	1.60	.50	.046	1.54	.31	.080	1.43	.29	.071	1.53	.37	.067	.028
Mainland averages	1.64	.42	.068	1.66	.43	.093	1.60	.43	.086	1.64	.43	.083	

* Criterion for polymorphism is 0.01.
† Abbreviations for populations are explained in Table 6.

polymorphism, whereas in many studies a greater variability has been found in Group II compared with Group I enzymes (for example in *Drosophila*, Gillespie and Kojima 1968). The mean number of alleles per locus is also similar for all three groups of loci in mainland populations—about 1.64 alleles per locus.

Heterozygosities are relatively high for all loci in mainland populations: 0.068 for Group I, 0.093 for Group II, and 0.086 for Group III loci, with an overall mean of $\bar{H} = 0.083$. These measures can be compared with an average heterozygosity of 0.039 for mammals (25 taxa), calculated from the literature by Powell (1975), or 0.054 for rodents (26 taxa), calculated by Selander (1975).

Not only is variability high on the mainland, but a great deal of variation is maintained in the California insular populations, which have unusually high levels of variability compared with other insular populations surveyed (Gill 1976). However, the reduction in variability that is found in the insular populations is not uniform. With the exception of *P. m. streatori*, in all island populations the reduction of variability in nonenzymatic proteins of Group III is far greater than the reduction in variability of enzymes, especially in terms of mean individual heterozygosity, $\bar{H}$. This dramatic decrease in variability of the nonenzymatic proteins, compared with enzymes, strongly suggests different selection pressures on these different groups of loci. Mean heterozygosity for glucose-metabolizing enzymes is actually higher in the island populations than on the mainland (0.078 compared with 0.068) but there is a wide range of Group I heterozygosities among the island populations (0.046 to 0.138) and among the mainland populations (0.040 to 0.100). The *P. m. streatori* population on San Miguel Island again exhibits a pattern of variation different from the other insular populations. It has the exceptionally high heterozygosity of 0.138 for glucose-metabolizing loci. For most of the island populations the highest heterozygosities are found for Group II enzymes, followed by Group I glucose-metabolizing enzymes, with much reduced heterozygosities in Group III nonenzymatic proteins. An explanation of the higher variability of Group II loci was offered by Kojima *et al.* (1970), who reasoned that enzymes with a variety of substrates that may vary in concentration are likely to be more variable than enzymes (such as Group I enzymes) with a single, fairly constant substrate. Only *P. m. elusus* on Santa Barbara Island and *clementis* on San Clemente Island, like *streatori*, are more highly heterozygous for Group I than Group II enzymes. *P. m. streatori* is also exceptional both in its pattern of variability and in being the most variable of the island populations.

Gene flow between the islands would, of course, affect the patterns of genic variability. To gain some insight into present possible levels of gene flow, we have set up crosses in the laboratory to study the interfertility of the subspecies. Although this investigation is not complete, certain trends are noticeable in the data from 103 crosses already analyzed. All subspecies have bred successfully in the laboratory; however, no offspring have been produced by *P. m. streatori* females, although 25 crosses were set up with males from other islands and 9 with *streatori* males. *P. m. streatori* males, on the other hand, have mated successfully with females from many of the other subspecies. These results suggest that gene flow between the mice on San Miguel Island and the other islands is possible through migrant *streatori* males entering other populations, but the results are not informative as to the possibility of gene flow due to the matings of female *streatori*. San Clemente Island females have bred successfully only with *catalinae* males and their own subspecies in the laboratory, although 24 crosses were set up with other subspecies. *P. m. streatori* and *clementis* females differ in this respect from females of the other subspecies that have been adequately tested in the laboratory (*anacapae*, *catalinae*, *exterus*, and *gambelii*), all of which have mated with males from several other subspecies and produced offspring.

TABLE 8. Ecological parameters for the eight Channel Islands.*

Island	Elevation (ft)	Number† of native plants	Number‡ of native mammals	Number of endemic plants	Trapping success (per cent)
Anacapa	930	70	1	0	32
Santa Cruz	2,470	420	10	7	36
Santa Rosa	1,560	340	4	3	41
San Miguel	830	190	2	0	51
San Nicolas	910	120	4	2	8
Santa Barbara	635	40	1	1	23
Santa Catalina	2,125	392	9	3	8
San Clemente	1,965	235	7	11	18

* Island areas and distances to mainland and nearest neighbor are given in Table 1. Trapping success is from this study. All other data, except where specified, are adapted from Johnson *et al.* (1968).

† Includes species, subspecies, and varieties. Anacapa data from Raven (1967).

‡ Includes recent (living) native land mammals. Data from von Bloeker (1967).

The islands differ in a number of ecological factors and it would be of value to know if the pattern of genetic variability observed was related in some discernible manner to ecological variants. To investigate this question, a canonical correlation analysis was done to relate the following two data sets compiled for each of the eight islands: (1) measures of genetic variability—the mean individual heterozygosities, $\bar{H}_{\text{I}}$, $\bar{H}_{\text{II}}$, and $\bar{H}_{\text{III}}$, for the three types of loci calculated for each population (Table 7); and (2) ecological measures—the area, distance to the mainland, and distance to the nearest island (Table 1), and elevation, number of native plant species, number of recent native land mammal species, number of endemic plants, and per cent trapping success (Table 8). Trapping success is based on field work done in this study and is a rough measure of population density. The other data in Table 8 are adapted from papers given at an earlier symposium on the California Islands (von Bloeker 1967, Raven 1967) and from a paper by Johnson *et al.* (1968). The canonical analysis was done with logarithms of distances and areas and square roots of frequencies. The program (BMDP6M, Dixon and Brown 1977) also calculates the correlations between variables; the results showed that many of the ecological parameters are highly correlated. Correlations between island area and elevation and the numbers of native plants and mammals ranged from 0.83 to 0.93. The number of endemic plant species is also well correlated with elevation (0.77). There is a strong correlation between trapping success and distance to the nearest island (0.90). As far as the genetic parameters are concerned, heterozygosities of Group I and Group III loci are highly correlated (0.9); their correlations with Group II heterozygosities are low (0.23 with $\bar{H}_{\text{I}}$ and 0.42 with $\bar{H}_{\text{III}}$). Because of the high correlations between some of the ecological parameters, only a few of these parameters are needed to check for dependency between the genetic and ecological sets of variables. Island area, distance to the mainland, and distance to the nearest island were chosen since these parameters are of basic significance and are not highly correlated with each other; all the other ecological parameters correlated with one of these three parameters. No significant relationship was found between the heterozygosities and the ecological parameters.

Each of the heterozygosities was regressed on the three ecological parameters (BMDP2R, Dixon and Brown 1977). The analysis suggested that distance to the nearest island may be a

TABLE 9. Genetic distance (above diagonal) and standard error (below diagonal) between populations of *Peromyscus maniculatus*.*

	Island								Mainland				
	ANA	CRU	ROS	STR	EXT	ELU	CAT	CLE	MUG	RIV	HTB	BKM	OWV
Island													
ANA		.033	.035	.046	.034	.045	.096	.100	.045	.028	.029	.057	.052
CRU	.033		.027	.021	.045	.071	.071	.099	.050	.056	.045	.072	.075
ROS	.034	.030		.023	.028	.054	.058	.066	.027	.037	.030	.058	.060
STR	.040	.027	.028		.042	.079	.049	.083	.039	.062	.047	.080	.079
EXT	.034	.043	.031	.038		.047	.070	.053	.044	.024	.028	.046	.065
ELU	.039	.049	.043	.052	.040		.084	.045	.042	.036	.037	.038	.029
CAT	.058	.049	.045	.041	.049	.054		.040	.040	.064	.051	.077	.077
CLE	.059	.059	.048	.054	.043	.039	.037		.035	.041	.039	.043	.063
Mainland													
MUG	.039	.041	.030	.037	.039	.038	.037	.034		.025	.017	.040	.040
RIV	.031	.044	.036	.046	.029	.035	.047	.037	.029		.015	.029	.038
HTB	.031	.039	.032	.040	.031	.036	.042	.037	.024	.022		.028	.042
BKM	.044	.050	.045	.053	.040	.036	.052	.038	.037	.032	.031		.038
OWV	.042	.051	.045	.052	.047	.031	.052	.046	.037	.036	.038	.036	

* Abbreviations for populations are explained in Table 6.

helpful variable in predicting heterozygosities for Group II loci. Distance to the nearest island is the best predictor of $\bar{H}_{\text{II}}$. The regression coefficients of the island distance parameters are remarkably similar for each of the three dependent variables: 0.027 in the case of $\bar{H}_{\text{I}}$, 0.024 for $\bar{H}_{\text{II}}$, and 0.026 for $\bar{H}_{\text{III}}$. Even if not a significant effect, there seems to be a consistent relationship between the heterozygosities and the distance to the nearest island. As mentioned before, distance to the nearest island is highly correlated with trapping success, which is a rough measure of population density, and it may well be that heterozygosity actually depends on population size.

Genetic Distance Between Populations

Genetic distance, D, which is a measure of the accumulated number of gene differences per locus between populations (Nei 1971, 1972), was used as an overall measure of genetic divergence between the populations sampled in this study. $D = -\log_e I$, where I is the normalized identity of genes between two populations, X and Y, with respect to all loci. $I = J_{XY}/\sqrt{J_X J_Y}$ where J_{XY} is the arithmetic mean of the probability of identity of a gene from X and a gene from Y, the probability being $\Sigma x_i y_i$ for a locus i, calculated for all loci including monomorphic loci; and J_X and J_Y are the arithmetic means of the probabilities of identity for randomly chosen genes within $X(\Sigma x_i^2)$ and within Y (Σy_i^2), respectively. Genetic distances between pairs of all island and mainland populations are given in Table 9, with the standard errors of D. The standard error of genetic distance, S_D, is calculated according to Nei's (1971) formula: $S_D = [(I - I)/In_s]_{1/2}$ where I is the normalized identity of genes and n_s is the number of proteins. The standard error depends only on I, and for closely related populations, for which I is large, standard errors are also large, as can be seen in Table 9. An inordinately large number of loci would have to be included to reduce the standard errors noticeably.

Genetic distances between subspecies of *P. maniculatus* and mainland populations of *P. m.*

gambelii are small, as is expected of such closely related groups. Nei (1976), using published electrophoretic data on gene frequencies, estimated D for a variety of organisms at various levels of differentiation: for local races of rodents, D lies between zero and 0.058; for subspecies of rodents, D lies between 0.004 and 0.262. In Table 9, all pairwise comparisons are between subspecies, except for comparisons among MUG, RIV, HTB, and BKM, which are all populations of *P. m. gambelii*. The smallest genetic distances occur between the pairs MUG-HTB and RIV-HTB (0.017 and 0.015, respectively). Distances between subspecies range from 0.021 to 0.100.

The striking feature of the genetic distances in this study is their consistency with the distribution and known history of the islands. The smallest genetic distances between island subspecies are found between the populations on Santa Cruz, Santa Rosa, and San Miguel Islands—three neighboring islands in the Northern Channel Island group which are thought to have been temporarily interconnected about 20,000 years ago. The populations on Anacapa, the fourth island in the Northern Channel Island group, and San Nicolas Island are genetically closer to these three islands than they are to any of the other islands. The remaining three Southern Channel Island populations are more distinct genetically from the other island populations, just as the islands themselves have been isolated for a longer period of time than have the northern islands. The San Clemente Island population is at the greatest genetic distance from other populations; Santa Catalina Island mice are similarly distinct.

The genetic distance of island subspecies from the closest mainland subspecies, *P. m. gambelii*, or even from *sonoriensis*, is relatively small—smaller than some of the genetic distances found between certain of the Southern and Northern Channel Island populations. As was stated earlier, the populations differ for the most part in the frequencies of alleles, rather than in the presence or absence of alleles. Furthermore, all populations, insular as well as mainland, have a high degree of variability (Table 7) within the populations, which tends to produce smaller genetic distances.

Sarich (1977) has suggested that there are two sets of proteins, one changing ten times as rapidly as the other, and that the fast group contributes the bulk of the measured genetic distance during the first five to six million years of divergence. The rapidly evolving proteins include the plasma proteins, nonspecific esterases, and certain enzymes not involved in complex metabolic pathways (*i.e.*, many Group II and III loci). His suggestion is supported by the evidence in this study, for the loci contributing most to genetic distances are Trf, Got-1, Es-5, Es-6, and Pgm-4. Sarich also proposes a test for neutral alleles, arguing that if the alleles sampled are neutral, higher heterozygosity values would be expected for more rapidly evolving loci. The exceptionally low heterozygosity values found for Group III loci in all insular populations except *P. m. streatori* clearly do not fit the neutral allele hypothesis. There seem to be selective forces on these islands whose actions result specifically in reduced variability of nonenzymatic proteins.

DISCUSSION AND CONCLUSIONS

The populations of Deer Mice inhabiting the Channel Islands have undergone significant morphological divergence from mainland populations and among themselves. This morphological divergence has not been accompanied by a comparable rate of genic divergence—genetic distances between insular and mainland subspecies remain relatively small. The factor that underlies this disparity is no doubt the high level of genetic variability maintained in insular populations. In the island populations, selection can act on a large available store of genetic variability, as measured by levels of polymorphism and individual heterozygosity.

To allow a direct comparison of morphological divergence between islands with genic divergence, as measured by genetic distance (Table 9), I have compiled a table of inter-island distances based on skull measurements (Table 10). These are the distances in canonical units

TABLE 10. Distance between island subspecies of *Peromyscus maniculatus* based on skull measurements.*

	CRU	ROS	STR	EXT	ELU	CAT	CLE
ANA†	0.44	2.58	3.02	1.68	4.57	2.05	5.35
CRU		3.02	3.30	2.09	4.72	2.49	5.78
ROS			2.13	1.03	4.08	0.83	2.77
STR				1.78	1.97	2.66	3.61
EXT					3.69	1.02	3.77
ELU						4.62	5.40
CAT							3.38

* Distances are given in units of the canonical variables depicted in Figure 1.
† Abbreviations for populations are explained in Table 6.

between the subspecies' means depicted in Figure 1, in which the axes are the first two canonical variables discriminating the subspecies. Only the island populations are included here because comparable data were not available for most of the mainland populations. A significant positive correlation, $r = 0.380$ ($P = 0.05$), was found between elements of the genetic data (genetic distance, Table 9) and elements of the morphological data (skull distance, Table 10) for all pairwise comparisons between islands ($n = 28$). None of the correlations for individual islands (which involve only the seven possible comparisons with each of the other islands) was significant, but the correlations were relatively high for Santa Cruz, Anacapa, and San Clemente Islands (0.661, 0.483, and 0.454, respectively). The relationship between Santa Barbara Island and the other islands is surprising in that it has the only population for which there is a negative correlation between genetic and morphological distances (−0.382). These correlations for individual islands give an idea of trends, but there are too few comparisons possible (seven) to establish significance. In general, there is a significant correlation between genetic and morphological distances between islands, although the rate of morphological divergence has been much greater than the rate of genic divergence.

Highly significant changes have occurred in body size, the island mice all being larger than the mainland mice, but size varies significantly between islands. San Clemente Island mice are the most distinct in all morphological traits. Significant differences in organ weights—differences which are maintained among the progeny of island mice reared in a uniform environment—suggest the existence of differential physiological adaptations in the island subspecies. Comparison of skull indices clearly shows the distinctness of the island populations and the increase in their average size over mainland populations.

The *P. maniculatus* subspecies can be distinguished on the basis of skull indices and body size variables and it is important to note that this discrimination is based on distinct changes in many different variables, rather than a few major ones. It is likely that many different genes are involved in the traits that distinguish the subspecies. *P. maniculatus* is known for marked geographic variation in morphological characters, as documented especially in the work of Dice (*e.g.*, 1940, 1941). Blair (1950) has suggested that the wide distribution and variation in morphological traits and in ecological preferences shown by *P. maniculatus* leads to an increase in its genetic variability and may favor the survival of the species and increase its opportunities for speciation.

Genetic variability is unusually high for both mainland and island subspecies in this study, compared with other mammals, but it is not maintained at the same level in the insular

populations for all types of genetic loci. There is evidence for selection acting to reduce variability in nonenzymatic proteins in the insular habitat, while variability remains high in loci coding for glucose-metabolizing and other enzymes. There are also differences in the pattern of variability among insular populations, *P. m. streatori* on San Miguel Island being exceptional for its high levels of heterozygosity for nonenzymatic proteins and glucose-metabolizing enzymes. No correlation could be found between any of the major ecological parameters characterizing the islands and the levels of genic heterozygosity found. There is, however, a consistent relationship between the heterozygosities of the three groups of loci (I, glucose-metabolizing enzymes; II, other enzymes; and III, nonenzymatic proteins) and the distance to the nearest island, a parameter that has a high correlation with trapping success, which is, in turn, a rough measure of population density.

The genetic distances (based on Nei's measure, 1971, 1972) calculated from the electrophoretic data are relatively small because, for most of the variable loci, the populations differ in the frequencies of alleles rather than in the kinds of alleles. The pattern of variation in the magnitude of genetic distance is entirely consistent with the spatial distribution and known history of the islands. Furthermore, it agrees extremely well with the morphological differentiation of the subspecies. Just as *P. m. clementis* is morphologically the most distinct subspecies, it is also genetically the most distant from other insular subspecies and displays relative behavioral isolation in mating. Although most of the subspecies are well differentiated morphologically, with almost 100 per cent correct classification possible, there is some overlap between samples from San Miguel, Santa Rosa, and San Nicolas Islands. Genetic distances between these insular populations are also relatively small; this is especially true for the two Northern Channel Island populations on San Miguel and Santa Rosa.

The morphological and genic data indicate possible routes of gene flow among the islands in the past. The greatest amount of gene flow apparently occurred between the three westernmost Northern Channel Islands, probably during their period of interconnection. The mice on Anacapa Island most closely resemble their neighbors on Santa Cruz Island, apparently having less gene exchange with the other two Northern Channel Islands. It is likely that rafting or transportation by humans contributed both to the exchange of genes of Santa Rosa and San Miguel Deer Mice with San Nicolas mice and to gene flow between San Clemente and Santa Catalina Island mice. This pattern of gene flow seems consistent with the fact that there is a cold ocean current flowing south past San Miguel and Santa Rosa Islands toward San Nicolas Island and a warm current that goes north past San Clemente and Santa Catalina Islands. The distinct morphological differences that have evolved in the insular populations, distinguishing them, indicate a fair amount of isolation and argue against much gene flow at the present time. Even if there is some individual movement of Deer Mice between islands, these individuals would have to participate in breeding to contribute to the gene pool. There is some evidence from laboratory breeding studies of differential breeding behavior among females of different subspecies to support the idea of behavioral blocks to gene exchange.

The high levels of genic variability maintained in the insular populations have not undergone periods of serious diminution. The founders apparently carried fairly large stores of genetic variability and the populations seem not to have suffered severe reductions in size. Based on the data summarized in Table 9, there is no evidence for large random fluctuations in gene frequencies from one island to the next, as might occur with small population sizes and genetic bottlenecks. On the contrary, the patterns of gene frequencies are consistent with the distribution and history of the islands. That the high levels of variability are maintained by a sufficiently large population, rather than by gene flow between islands, is indicated by the highly significant anatomical and external morphological differences that have developed between insular populations.

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