CALIFORNIA ISLAND DEER MICE: GENETICS, MORPHOMETRICS, AND EVOLUTION

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ABSTRACT

Deer mice, *Peromyscus maniculatus*, are found on all eight California Channel Islands and are classified as separate subspecies on each island. Distinct mitochondrial DNA haplotypes, identified by restriction enzyme analysis, were found in island deer mice, and on five of the eight islands deer mice have unique haplotypes, suggesting genetic isolation and independent evolution of several island subspecies. Founder effects on mtDNA diversity in island populations relative to mainland populations are evident. The connectivity of the deer mouse populations on East, Middle, and West Anacapa Islands (*P. m. anacapae*) was assessed using sequence data from the mitochondrial cytochrome *c* oxidase subunit II gene (COII). A common haplotype was found on all three Anacapa Islets, although Middle and East Anacapa each had an additional unique haplotype. This suggests that deer mice on Anacapa are functioning as a metapopulation, with some gene flow or extinction/recolonization occurring among the islets. Discriminant function analysis of cranial and external morphological characters for three island subspecies, *P. m. anacapae*, *P. m. santacruzae*, and *P. m. elusus*, produced a high rate of correct classification, indicating strong morphological as well as genetic differentiation. The specimens used for the morphometric study were museum specimens collected at different times during the past century. A surprising result of the morphological analysis was that each subspecies had exhibited extremely rapid change in several characters over this time period.

Keywords: Morphological change, temporal variation, California Channel Islands, deer mice (*Peromyscus maniculatus*), morphometrics, discriminant function analysis, mitochondrial cytochrome *c* oxidase subunit II (COII) sequences, gene flow, evolutionarily significant units (ESU), restriction enzymes, founder effect, metapopulations.

INTRODUCTION

Deer mice, *Peromyscus maniculatus*, are an important component of the terrestrial fauna of the California Islands. Deer mice are the only nonvolant vertebrate that occurs on all eight islands and their high abundance on several of the islands necessarily dictates that they influence the ecology of those islands. Further, the modest radiation of *Peromyscus maniculatus* into multiple genetically and morphologically distinct subspecies contributes much to the biodiversity of the islands and makes them an important subject for investigations of evolutionary history and adaptive radiations on the California Islands. It is therefore not surprising that California Island deer mice have been investigated by scientists for a century (Mearns 1897), and that these investigations continue today (Pergams and Ashley 1999; Pergams et al. 2000).

The focus of this report will be recent genetic and morphological research on California Island deer mice, but we will begin with a brief review of earlier morphological and genetic investigations. Mearns (1897) identified California Island mice as *Peromyscus texanus medius*. Elliot (1903) gave the deer mice from Santa Catalina and Santa Cruz single, separate species status as *Peromyscus catalinae*. Mearns (1907) essentially renamed the remaining mice from San Clemente, San Nicolas, Santa Rosa, and San Miguel as *Peromyscus texanus clementis*. Osgood's extensive and general revision of the genus (1909) placed California Islands deer mice as the same species but two different subspecies: *Peromyscus maniculatus clementis* and *P. m. catalinae*. Later, mice from Santa Barbara, San Nicolas, and Santa Cruz were described as separate subspecies, *P. m. elusus*, *P. m. exterus*, and *P. m. santacruzae,* respectively, and mice from Santa Rosa and San Miguel together were described as *P. m. santarosae* (Nelson and Goldman 1931). von Bloeker (1940, 1941) described mice from Santa Rosa and Anacapa as separate subspecies, *P. m. santarosae* and *P. m. anacapae*, respectively.

Collins et al. (1979) commented that three of the present subspecies descriptions were made from samples which included specimens collected from other islands. Measurements for *P. m. catalinae* were taken from mice collected on Santa Catalina and Santa Cruz (Osgood 1909), measurements for *P. m. clementis* were taken from mice collected from San Clemente, San Miguel, San Nicolas, Santa

Barbara, and Santa Rosa, and measurements from *P. m. streatori* were taken from mice collected from San Miguel and Santa Rosa. Collins et al. (1979) recommended re-evaluation of these three subspecies. These authors also provide general phenotypic descriptives of California Islands deer mice, based on their own experience and other literature existing at the time. Deer mice from the California Islands have dark brownish-black tipped hairs dorsally which are subtended by a buffy band before grading into grey at the base. In general, island mice are darker than mainland mice. In terms of body size, the San Miguel and San Clemente subspecies are the smallest, and the Anacapa, San Nicolas, and Santa Barbara subspecies are the largest. All island subspecies, however, are larger than the adjacent mainland deer mice.

The first formal morphometric treatment of California Island deer mice was conducted by Gill (1980). She applied stepwise discriminant function analysis to 219 specimens from all eight islands, as well as the mainland subspecies *P. m. gambelli*. An 85% classification rate was achieved from 17 external and cranial measurements. The majority of misclassifications involved mice from San Miguel, Santa Rosa, and San Nicolas. In a second analysis, mice from these three islands were removed, and a 98% classification rate was then achieved using 12 external and cranial measurements. Gill (1980) concluded that California Island deer mice have undergone significant morphological divergence.

Gill (1980) also conducted the first genetic study of California Island deer mice, an examination of variation at 30 protein-coding loci among island and adjacent mainland deer mice. She reported that levels of genetic variation were relatively high on the islands (average mean heterozygosity, H=0.066), but reduced from that found among California mainland deer mice (H=0.083). Genetic distances (Nei's *D* 1972) were small among all comparisons, less than 0.10.

We have conducted additional genetic studies, employing rapidly evolving mitochondrial DNA (mtDNA), and have performed discriminant function analysis of morphological characters of California Island deer mice. The work we present here has three separate components that examine different aspects of the evolution of island deer mice. First, we summarize results of previous mtDNA restriction fragment analysis of all the subspecies of *P. maniculatus* that occur on the California Islands as well as the adjacent mainland. The mitochondrial genome is maternally inherited and evolves rapidly relative to nuclear genes, and thus provides greater resolution of patterns of differentiation, isolation, and colonization than do nuclear-coded markers such as allozymes. The mtDNA results are compared to previous genetic and morphometric studies. Second, sequences of a mitochondrial gene, cytochrome *c* oxidase subunit II (COII), were used to look at an even finer evolutionary scale, that of *P. m. anacapae* populations on the three small islets that comprise Anacapa Island. We were interested in determining whether East, Middle, and West Anacapa Island deer mouse populations were genetically isolated from each, or alternatively, whether they functioned as a metapopulation connected by gene flow. Finally, we draw on the extensive collection of California Island deer mice specimens in museums taken over nearly a century to document rapid morphological evolution in three of the island subspecies, *P. m. elusus*, *P. m. anacapae*, and *P. m. santarosae*. Taken together, these results provide a relatively detailed picture of California Island deer mice evolution at different spatial and temporal scales and for both morphological and genetic characters.

MATERIALS AND METHODS

Mitochondrial DNA RFLP Analysis

Table 1 provides information regarding specimens used for various aspects of this study. Purified mtDNA samples from 131 island and mainland mice collected from 1983 to 1985 (Table 1) were analyzed for restriction fragment length polymorphisms (RFLPs). Fresh tissue samples (liver, heart, kidney and spleen) were used for mtDNA purification following differential centrifugation (Lansman et al. 1981; Ashley and Wills 1987). All samples were digested with nine restriction enzymes, *Eco*RI, *Hin*d III, *Bst*EII, *Pst* I, *Bgl* II, *Ava* I, *Ava* II, *Mbo* I and *Hin*f I. Approximately 15 nanograms of purified mtDNA were used for each digestion and fragments were end-labeled with ^{32}P using the large fragment of *E. coli* polymerase I. Fragments were electrophoresed on 1% horizontal agarose slab gels or in 3.5% vertical polyacrylamide gels along with a molecular weight size standard. After electrophoresis, gels were dried and autoradiographed using Kodak XAR-5 film (Ashley and Wills 1987).

Sequence divergence (*p*) was estimated from the proportion of shared restriction fragments using the formula of Upholt (1977) and weighting the estimation relative to the total number of base pairs recognized by each type of restriction enzyme. Restriction pattern diversity, *h*, was estimated using Nei and Tajima (1981).

The relationships between geographic distance, genetic differentiation and morphological divergence of the California island subspecies were examined (Ashley and Wills 1989). Four distances matrices were compared using Kendall's tau statistic, K_c. Significance levels of observed K_c values were estimated by 2,000 random permutations of the elements of matrices (Dietz 1983). The elements of the four matrices were: 1) genetic distances (Nei's *D*, 1972) based on allozymes (Gill 1980); 2) morphological distance measured as distance in canonical-variable units between subspecies (Gill 1980); 3) sequence divergence (*p*) based on mtDNA RFLP comparisons of the predominant mtDNA genotype in each subspecies; and 4) geographic distance between islands.

Mitochondrial COII Sequence Analysis

Sequences of the mitochondrial COII gene were obtained for a total of 35 samples (Table 1), representing *P. m. anacapae* from each of the Anacapa islets (East, Middle

Table 1. Samples used in this study.

Species/ Subspecies	Collection Location	Collection Year	N	mt RFLP	mt COII	Morpho- metrics
P. m. anacapae	West Anacapa Island	1940-1996	40		11	29
	Middle Anacapa Island	1940-1996	49	8	9	35
	East Anacapa Island	1917-1997	26		7	24
P. m. catalinae	Santa Catalina Island	1983	7	7		
P. m. clementis	San Clemente Island	1985	10	10		
P. m. elusus	Santa Barbara Island	1897-1983	56	14	1	42
P. m. santacruzae	Santa Cruz Island	1983	48	10	1	38
P. m. santarosae	Santa Rosa Island	1985	10	10	1	
P. m streatori	San Miguel Island	1983	13	13		
P. m. exterus	San Nicolas Island	1983-1984	14	14		
P. m. gambelii	Los Angeles Co.	1903	13			13
	Los Padres National Forest, Santa Barbara Co.	1983	1	1		
	La Jolla, San Diego Co.	1983-1985	28	28	1	
	Cleveland National Forest, Riverside Co.	1985	5	5		
	Idyllwild, Riverside Co.	1985	3	3		
	Jalama, Santa Barbara Co.	1985	1	1		
	Las Flores ranch	1985	3	3	1	
	Los Alamos	1985	4	4	1	

and West) as well as two additional island subspecies (*P. m. elusus* and *P. m. santacruzae*) and one mainland subspecies (*P. m. gambelii*). Samples consisted of either purified mtDNA isolated by differential centrifugation (those collected from 1983 to 1985), or total genomic DNA (those collected from 1996 to 1997) isolated from frozen heart or liver tissue by standard phenol-chloroform extractions. The COII gene (684 bp) was amplified via the polymerase chain reaction (PCR) with primers designed from $tRNA^{Asp}$ and $tRNA^{Lys}$ genes that flank COII in the mammalian mitochondrial genome (Adkins and Honeycutt 1994). Two external and two internal primers were also used for amplification and/or sequencing (Pergams et al. 2000). PCR amplifications were performed in 50μl reaction volumes which included 0.2μM of each primer, 0.2μM dNTPs, 1U Taq polymerase, 1X reaction buffer, and approximately 2ng of DNA. Amplification proceeded for 32 to 40 cycles of 94^{o}C for 1 min, 50-51^{o}C for 1 min, and 72^{o}C for 2 min. Excess primers and dNTPs were removed from successful PCR reactions and these were sequenced either manually or on an automated sequencer (ABI 373A; Pergams et al. 2000).

Levels of mitochondrial variation were examined using indices of nucleotide diversity, π (Nei 1987). Gene flow, *Nm*, an estimate of migration averaged over evolutionary time, was estimated for the Anacapa islet populations from COII sequence data using equation 2 of Nei (1982). Calculations were performed using the computer program DNAsp 2.9 (Rozas and Rozas 1998).

Morphometric Analysis

Skulls and skins of total of 151 adult mice were measured for a study of temporal variation in three subspecies of island deer mice, *P. m. elusus, P. m. anacapae,* and *P. m. santacruzae* (Table 1). The collection years of the specimens are as follows. *P. m. elusus*: 1897 (N = 2), 1919 (3), 1939 (13), 1940 (3), 1955 (1), 1972 (2), 1974 (1), 1978

(14), and 1979 (3). *P. m. anacapae*: 1940 (38), 1978 (35). *P. m. santacruzae*: 1917 (5), 1938 (3), 1939 (9), 1941 (2), 1967 (13), 1983 (2), 1986 (1), and 1988 (1).

Twelve cranial measurements were taken (Pergams and Ashley 1999): intermeatus width (IW), length of nasals (LN), length of palate plus incisor (LPI, measured as the greatest distance from the anterior edge of the alveoli of the incisors to the mesopterygoid fossa), breadth of rostrum (BR), alimentary toothrow (AL), length of incisive foramen (LIF), rostral width (RW, the narrowest width of the rostrum and premaxilla dorsally and directly anterior to the infraorbital foramen), zygomatic breadth (ZB), interorbital breadth (IB), depth of braincase (DBC), breadth of braincase (BB), and breadth of zygomatic plate (BZP). All cranial measurements were taken to the nearest 0.01 mm. The four standard external measurements were originally made by 18 different museum preparers and recorded from museum tags: total length (TOT), tail length (TAIL), hind foot length (HIND), and ear length (EAR). EAR was not available for six *P. m. elusus* and five *P. m. santacruzae*.

SYSTAT v. 7.0 (SPSS, Inc. 1997) was used for statistical analysis. Normality of data was determined by visual inspection of normal probability plots, following the method of Afifi and Clark (1996). We plotted 85% ellipses of concentration as group scatterplot matrices. To determine if temporal change had occurred, three data sets were created: data from all years 1897 to 1988 (N = 140), data from only 1897 to 1941 (68), and data from only 1955 to 1988 (72). Since no mice had been collected between 1941 and 1955, these years served as an obvious, albeit arbitrary, cut-off between "early" and "late" collections. One-way ANOVAs and complete discriminant analyses were performed on each data set. The year the specimen was collected was used as the time variable. Results from all ANOVAs were considered significant at the 95% confidence level.

To compare differentiation between subspecies to relative morphological change over time, we performed a two-way ANOVA with subspecies and time as factors. Time was included by comparing time classes 1897 to 1941 and 1955 to 1988. Mean squared errors (MSE, or the sum of squares over degrees of freedom) of the two factors were compared (Sokal and Rohlf 1997). The variable with the larger MSE was considered to have the greater variance.

We used discriminant function analysis to characterize the direction, degree, and nature of the morphological changes over time. Our discriminant analysis used the "Weight" function in SYSTAT's data handling component to assign a weighting of "0" or "1" to individuals and thereby apportion them to time class. Individuals collected from 1897 to 1941 were fully weighted, and individuals collected from 1955 to 1988 were given zero weight. A complete discriminant function was derived only from individuals from 1897 to 1941, but applied to individuals from 1955 to 1988 as well. Centroid coordinates of the late groups were calculated by hand as the means of individual canonical scores. Mahalanobis distances between the centroids of early and late groups were calculated by hand using the distance formula.

RESULTS

RFLP analysis of the mtDNA genome revealed a total of 26 different haplotypes (Table 2). Of these, 10 haplotypes were identified among the 87 island mice surveyed, and the remaining 16 were found among southern California mainland *P. maniculatus gambelii*. No haplotypes occurred in both mainland and island deer mice, and six of the island subspecies (*P. m. anacapae*, *P. m. exterus*, *P. m. santacruzae*, *P. m. clementis*, *P. m. catalinae*, and *P. m. elusus*) had one or two unique haplotypes. In the cases of *P. m. anacapae*, *P. m. clementis*, *P.m. catalinae*, and *P. m. elusus*, only haplotypes unique to each subspecies were found. A common haplotype (#3) was found on four of the islands, Santa Rosa, San Nicolas, San Miguel and Santa Cruz. At most, two haplotypes were found on any island, and a single haplotype was found on San Miguel, Santa Rosa, and Santa Barbara Islands. Heterogeneity, h, ranged from 0 to 0.44 (mean 0.20) within island samples and from 0.67 to 1.00 (mean 0.79) within mainland samples from a single location. Estimated percent sequence divergence, p, ranged from 0.32% to 0.95% for haplotype comparisons (Ashley and Wills 1987).

Tests of association among the various distance matrices indicated that only two pairs of matrices are significantly correlated: mtDNA divergence versus allozyme divergence (P=0.002) and geographic distance versus allozyme divergence (P=0.0005; Ashley and Wills 1989).

For COII sequence analysis, at least 606 bp of sequence were obtained for three island subspecies (*P.m. elusus*, *P.m. santarosae*, and *P. m. anacapae)* and one mainland subspecies (*P. m. gambelii).* A total of 18 sites were polymorphic within *P. maniculatus*, 14 of which represented silent substitutions. Among the *P. maniculatus* subspecies, nucleotide diversity π was less than 1%, reflecting levels of differentiation similar to those found using RFLP analysis. The distribution of haplotypes identified by sequences also confirmed the RFLP results, with each haplotype identified by restriction enzyme analysis of the entire mtDNA genome also exhibiting nucleotide differences within the COII gene. The RFLP analysis for Anacapa only included specimens from Middle Anacapa Island and had identified two unique haplotypes. All specimens from West Anacapa Island had the most common of these two haplotypes. Specimens from East Anacapa also shared this haplotype, but a third haplotype was also identified (Figure 1).

Gene flow, *Nm*, or the number of individuals per generation migrating, was estimated for the three Anacapa islets using the COII sequence data. Approximately seven individuals are estimated to migrate between West and Middle Anacapa per generation, approximately the same number are estimated to migrate between Middle and East Anacapa, and approximately two individuals are estimated to migrate between West and East Anacapa per generation.

Table 2. mtDNA RFLP haplotypes observed among the samples of *P. maniculatus*. Letters describing mtDNAs, from left to right, refer to restriction fragment patterns for the restriction enzymes *Eco*R l, *Hin*d lll, *Bst*E ll, *Pst* l, *Bgl* ll, *Ava* l, *Ava* ll, *Mbo* l, and *Hin*f l, respectively. Each composite haplotype is numbered. The last column shows the number of mice sampled that show a particular mtDNA composite haplotype.

	mtDNA Genotype	Collection Site	Number of Mice
	California Channel Islands:		
1)	AACAAAAAG	Anacapa	7
2)	BBAAAAAAM	Anacapa	1
3)	AAAAAAAAF	Santa Rosa	10
		San Nicolas	12
		San Miguel	13
		Santa Cruz	8
4)	AAAAAAACF	San Nicolas	2
5)	AAAAAAAGK	Santa Cruz	2
6)	AABAAAAAE	Santa Barbara	14
7)	BBAAAAACH	Santa Catalina	6
8)	AAAAAAAHA	Santa Catalina	1
9)	ABAAAAAJD	San Clemente	3
10)	ABAAAAAAD	San Clemente	8
	Southern California Mainland:		
11)	ABABAAAAA	La Jolla	1
12)	ABAAAABAB	La Jolla	1
		Los Padres National Forest	1
13)	ABCAAAAAA	La Jolla	1
14)	AAAAAAJAA	La Jolla	1
15)	BBAAAAACA	La Jolla	1
16)	ABAAAACAA	La Jolla	15
		Cleveland National Forest	1
		Idyllwild	1
		Los Alamos	2
17)	ABAAAAAAA	La Jolla	3
		Idyllwild	2
		Cleveland National Forest	2
		Las Flores ranch	1
18)	ABAAAADAC	La Jolla	1
19)	ABABAAAAB	La Jolla	2
20)	FBAAAAAAD	La Jolla	1
21)	ABAAAAAAE	Cleveland National Forest	1
22)	ABAAAAIEA	Cleveland National Forest	1
23)	ABAAADAAA	Los Alamos	2
24)	ABAAAAABA	Las Flores ranch	1
25)	ABAAAAADA	Las Flores ranch	1
26)	ABAAAAALA	Jalama	1

Morphometric Analysis

Measurements of all characters were determined to be approximately normal in distributions so no transformations were made to the raw data, thereby avoiding distancing ourselves from the data (Reyment 1972). Results of analyses for identifying morphological change over time are given in Table 3. For *P. m. elusus*, six measurements (LN, AL, TOT, TAIL, HIND, & EAR) were significantly correlated with time. For *P. m. anacapae*, ten measurements (IW, BR, LIF, RW, DBC, BZP, TOT, TAIL,

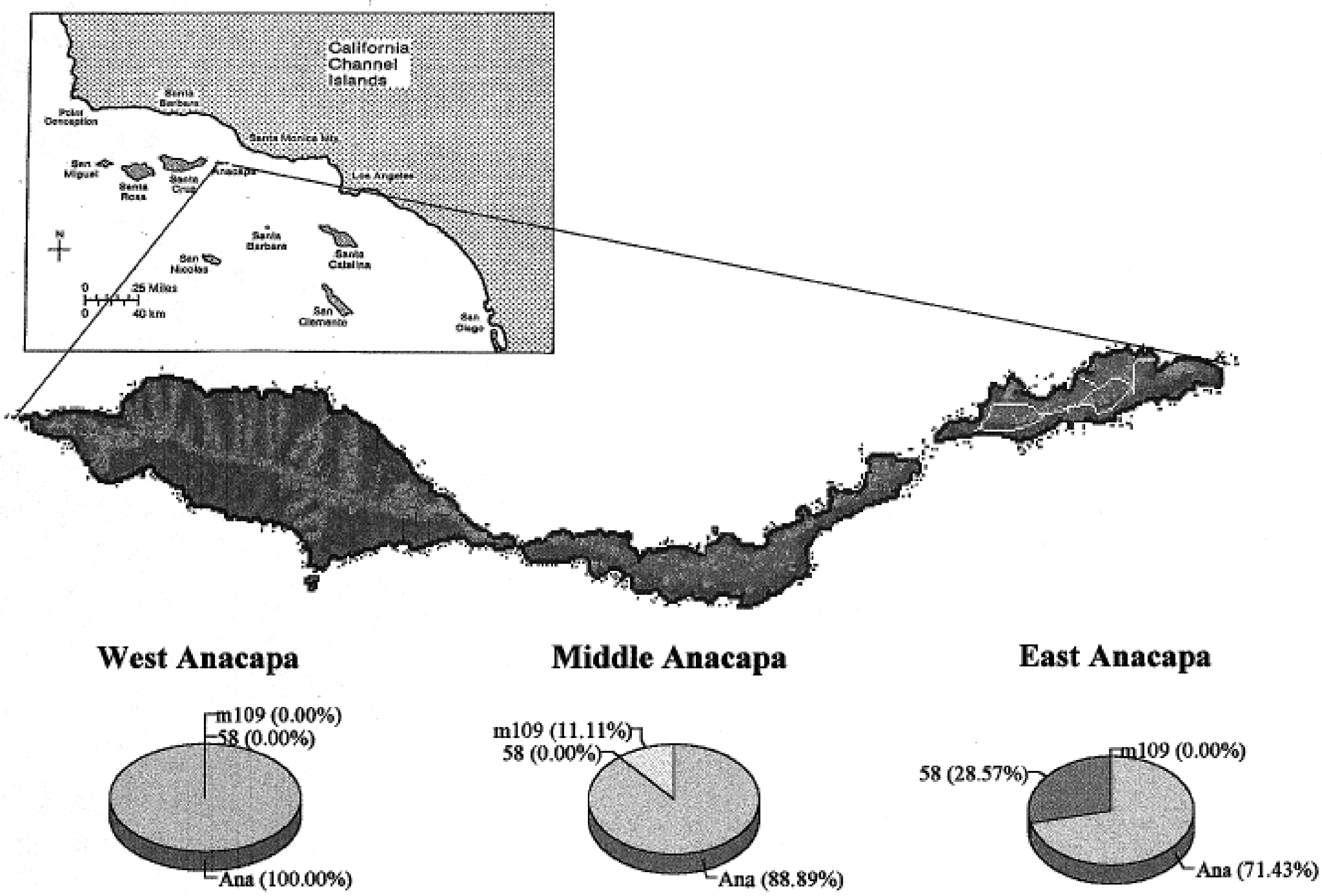

Figure 1. Map of Anacapa islets with distribution of haplotypes. *Ana* is the common haplotype and corresponds to RFLP haplotype 1, Table 2. *m109* corresponds to RFLP haplotype 2, Table 2. *58* is a new haplotype.

HIND, & EAR) were significantly correlated with time. For *P. m. santacruzae*, six measurements (IW, BR, TOT, TAIL, HIND, & EAR) were significantly correlated with time. On all three islands TOT, TAIL, HIND, & EAR showed significant change over time. Direction of change was the same for TOT, TAIL, and HIND (becoming smaller) but differed for EAR. Using our method of nested two-way ANOVA and comparing MSEs, we found that the temporal change in five characters (ZB, TOT, TAIL, HIND, & EAR) exceeded differentiation between subspecies. Complete discriminant analysis of data from all years (Figure 2a) correctly classified 85% (119/140) of individuals, whereas 1897 to 1941 data alone (Figure 2b) correctly classified 99% (67/68) and 1955 to 1988 data alone (Figure 2c) correctly classified 96% (69/72). Altogether, 97% (136/140) of individuals were correctly classified by correcting for temporal change. Had we not tested for temporal variation in this data, we would have lost the power to classify 12% (17/140) of our sample.

Given the extent of temporal change, we expected the discriminant function giving full weight to individuals from 1897 to 1941 and zero weight to individuals from 1955 to 1988 to be poor at classifying individuals from the later period (see Table 3). This is indeed the case. Only 40% (29/72) of the individuals from 1955 to 1988 are correctly classified.

We compared the relative amounts of movement in morphology over time by calculating the Mahalanobis distances between old and new centroids for each island (Table 4). *P. m. elusus* and *P. m. anacapae* changed approximately the same amount, whereas *P. m. santacruzae* changed approximately 40% less. In Figure 3, arrows are drawn from the centroids of the 1897 to 1941 confidence ellipses to the centroids of the groupings of 1955 to 1988 individuals, illustrating the direction and magnitude of morphological change on the three islands. Although each subspecies exhibited rapid morphological change, they remain well differentiated, as shown by the high (96%) correct classification rate of the 1955 to 1988 group using the discriminant function from this period only.

DISCUSSION

The mtDNA haplotype analysis of California island deer mice was the first mtDNA study of an island vertebrate (Ashley and Wills 1987). Results demonstrated that mtDNA could be an extremely useful tool for recreating the

Table 3. Separate one-way ANOVA results for each subspecies, with time (year collected) as a factor. Significant values are in bold.

	P. m. elusus		***P. m. anacapae*** **(all)**		***P. m. anacapae*** **(West)**		***P. m. anacapae*** **(Middle)**		***P. m. santacruzae***	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
IW	0.9769	0.47083	**14.8872**	**0.00025**	2.9725	0.09405	0.8020	0.38301	**2.78766**	**0.02477**
LN	**2.4767**	**0.03180**	0.2181	0.64191	2.5757	0.11805	0.2235	0.64236	0.38121	0.90551
LPI	0.7172	0.67483	1.2265	0.27183	1.6303	0.21057	1.9023	0.18569	0.81073	0.58590
BR	1.6126	0.15901	**4.5765**	**0.03585**	2.4699	0.12559	0.0000	0.99696	**2.49615**	**0.03988**
AL	**4.6440**	**0.00072**	1.5690	0.21446	1.1880	0.28362	1.1557	0.29739	0.88875	0.52826
LIF	0.5461	0.81306	**5.7802**	**0.01882**	0.1762	0.67742	2.0468	0.17066	0.63852	0.72031
RW	0.7136	0.67785	**4.7508**	**0.03260**	**5.5002**	**0.02518**	1.7817	0.19955	1.16060	0.35599
ZB	0.4880	0.85583	0.5749	0.45082	3.3917	0.07453	1.4432	0.24609	0.30255	0.94678
IB	1.7040	0.13448	1.9757	0.16421	0.0279	0.86829	**6.1161**	**0.02425**	0.18545	0.98620
DBC	1.8004	0.11252	**14.1596**	**0.00034**	3.8160	0.05929	1.8585	0.19058	2.27068	0.05791
BB	1.6882	0.13843	0.2077	0.64994	0.0247	0.87612	0.7202	0.40786	1.26373	0.30349
BZP	1.1043	0.38536	**6.5223**	**0.01280**	0.5222	0.47498	**9.6398**	**0.00644**	1.07835	0.40296
TOT	**7.6623**	**0.00001**	**5.1537**	**0.02623**	0.0279	0.86841	**5.3093**	**0.03410**	**7.49666**	**0.00004**
TAIL	**6.3124**	**0.00006**	**7.4039**	**0.00818**	0.2631	0.61145	**14.8551**	**0.00127**	**4.98064**	**0.00094**
HIND	**13.7525**	**0.00000**	**72.8647**	**0.00000**	**37.9775**	**0.00000**	**6.3659**	**0.02189**	**6.89603**	**0.00008**
EAR	**9.0280**	**0.00001**	**18.2799**	**0.00006**	**14.0332**	**0.00069**	**7.3880**	**0.01461**	**4.29155**	**0.00447**

Key: IW = intermeatus width, LN = length of nasals, LPI = length of palate plus incisor (see text), BR = breadth of rostrum, AL = alimentary toothrow, LIF = length of incisive foramen, RW = rostral width (see text), ZB = zygomatic breadth, IB = interorbital breadth, DBC = depth of braincase, BB = breadth of braincase, BZP = breadth of zygomatic plate, TOT = total length, TAIL = tail length, HIND = hind foot length, and EAR = ear length.

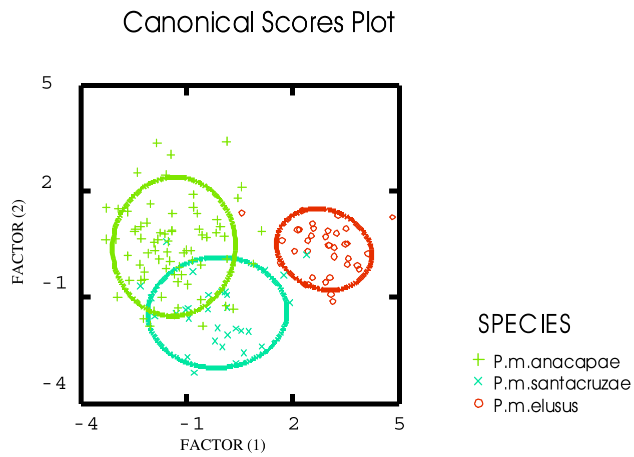

Figure 2a. Canonical scores plot for discriminant analysis of all years, 1897 to 1988.

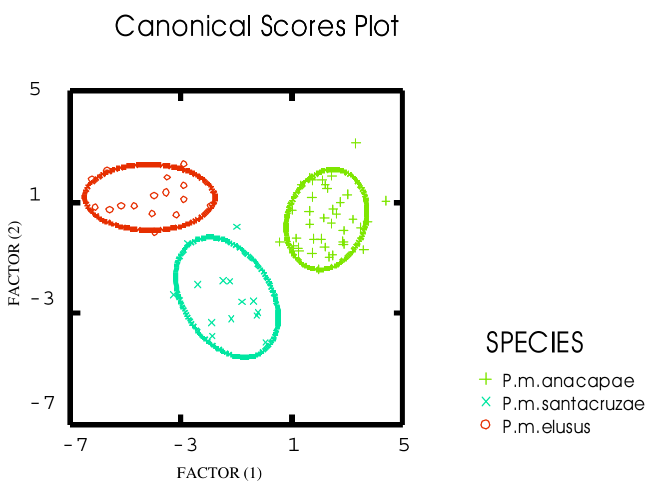

Figure 2b. Canonical scores plot for discriminant analysis of early period, 1897 to 1941.

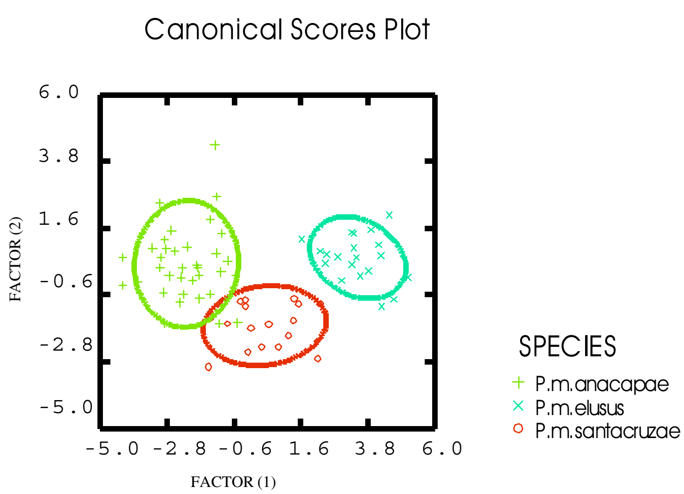

Figure 2c. Canonical scores plot for discriminant analysis of later period, 1955 to 1988.

evolutionary history of colonization and diversification of populations endemic to oceanic islands. Specifically for California Island deer mice, the islands are comprised of deer mice having haplotypes not found in mainland samples, and indicates they are genetically isolated from the mainland.

Table 4. Centroid coordinates and Mahalanobis distances between subspecies, from 1897 to 1941 and 1955 to 1988.

	early (1897 to 1941)	late (1955 to 1988)	Mahalanobis distance
P. m. elusus	-4.148, 1.184	0.460, 2.708	4.853
P. m. anacapae	2.242, 0.390	-2.350, -1.845	5.107
P. m. santacruzae	-1.344, -2.411	-4.354, -1.847	3.062

The presence of unique haplotypes on Anacapa, Santa Barbara, Santa Catalina, and San Clemente suggests that the subspecies of deer mice on these islands have been isolated from the mainland and other islands for a period of time sufficient for genetic differentiation to occur. The distribution of a common haplotype on Santa Rosa, San Nicolas, San Miguel and Santa Cruz suggests that recent gene flow or colonization events may have connected deer mice subspecies on these islands. In part this pattern may be explained by the geological history of the islands. The four northern islands (San Miguel, Santa Rosa, Santa Cruz, and Anacapa) were joined into the superisland of Santarosae a number of times during periods of lower sea level in the Pleistocene, and thus deer mice populations would have been in contact. This history does not, however, explain the unique haplotypes found on Anacapa nor the presence of this common haplotype on San Nicolas Island, which lies 80 km south of the northern islands.

Island subspecies of deer mice exhibited much lower levels of mtDNA variability than did mainland subspecies, although allozyme variability remains relatively high in island subspecies (Gill 1980). The explanation likely lies in differing transmission genetics of mitochondrial versus nuclear genomes. Because mtDNA is effectively haploid and maternally transmitted, founder effects and population bottlenecks will have a much greater effect on mtDNA variability than on diploid nuclear genes. Although this had been predicted from theoretical considerations (Birky et al. 1983), the California Island deer mouse investigation provided the first empirical demonstration of this phenomenon (Ashley and Wills 1987).

Allozyme and mtDNA differentiation among California Island deer mice subspecies are significantly correlated. The island subspecies have apparently been isolated long enough for differentiation to have occurred in both nuclear and mitochondrial genes through processes of genetic drift or natural selection. Neither of the genetic distance measures (allozyme distance or mtDNA divergence) was significantly correlated with morphological differentiation, suggesting that the evolution of morphological traits is not closely coupled with these types of genetic markers. A significant correlation between allozyme and geographic distance likely reflects the geological history of the islands and distance-dependent dispersal ability of deer mice. The lack of such an association for mtDNA and distance may reflect the role of rare founder events and other stochastic processes that shape distributions of mitochondrial haplotypes.

The advent of the polymerase chain reaction (PCR) and more efficient methods of DNA sequencing during the late 1980s and 1990s allowed mtDNA gene sequencing studies to largely replace RFLP studies. The COII sequence results obtained here for a subset of the RFLP-analyzed subspecies were in close agreement with the RFLP results. However, additional samples from West and East Anacapa allowed resolution of the metapopulation structure of *P. m. anacapae*. As shown in Figure 1, the connectivity of *P. m. anacapae* is demonstrated by the presence of one haplotype on all three islets, although unique haplotypes on Middle and West Anacapa suggest that the subspecies is not panmictic. Estimates of 2 to 7 migrants per generation were obtained, suggesting a metapopulation structure for this subspecies. Interestingly, deer mice from East Anacapa were thought to be extinct since 1981 to 1982 (Austin, pers. comm.), and very rare since 1966 (Collins et al. 1979). Deer mice were again collected on East Anacapa in 1997, but it was not known whether these mice represented recovery from a bottleneck on East Anacapa or whether deer mice had recolonized East Anacapa from elsewhere. Finding the common Anacapa mtDNA haplotype in East Anacapa deer mice suggest that they were not recolonized from outside Anacapa, but the presence of an additional unique haplotype not found on Middle or West Anacapa may indicate recovery from a bottleneck.

Collections of specimens of island deer mice over many years provided an opportunity to determine if morphological characters had changed over time (Pergams and Ashley 1999). We found that *P. m. elusus*, *P. m. santacruzae* and *P.m. anacapae* exhibited significant temporal change in several characters. Six characters in both *P. m. elusus* and *P. m. santacruzae* had changed over time, whereas ten characters in *P. m. anacapae* had changed. External body measurements (TOT, TAIL, HIND and EAR) exhibited temporal variation in all three subspecies, and two or more cranial characters also show significant temporal variation. Figure 3 illustrates that the subspecies are not converging on a common phenotype but are remaining well differentiated. The finding that the change in several characters (ZB, TOT, TAIL, HIND and EAR) exceeded the level of differentiation between subspecies suggests that these characters are only useful for comparing specimens within a given time period. Discriminant analysis of data from the early years allowed correct classification of only 40% of the samples from the later years. Therefore the common practice of classification of modern specimens based on comparisons with much older museum type specimens is inappropriate in this case.

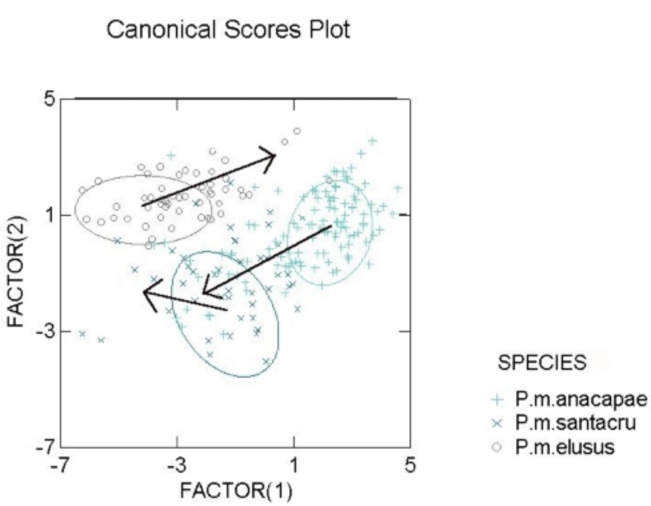

Figure 3. Canonical scores plot for discriminant analysis of all years, 1897 to 1988, but with discriminant function defined only by years 1897 to 1941.

Clearly these island subspecies have undergone rapid phenotypic change during this century. The rate of change dramatically exceed those estimated from paleontological records (Gingerich 1983; Pergams and Ashley 1999) and are even higher than those reported in experimental selection studies (e.g. Losos et al. 1997). Although the changes were not associated with known selective forces, natural selection may be the most likely explanation for the observed morphological changes. Many changes have occurred on these islands, especially in the biotic components. For example, Santa Barbara had feral goats (Remington 1971), and sheep were introduced to all three islands in the 1800s (Brumbaugh 1980; Doran 1980; Federal Register 1997). European rabbits were introduced to Santa Barbara, and there were reports of feral pigs on Santa Cruz (Remington 1971, Federal Register 1997). Black rats (*Rattus rattus*) were accidentally introduced to Anacapa. Rats may have large effects on Anacapa mice because of their abundance and because they are both competitors and predators of deer mice (Collins et al. 1979). The introduction of exotic grasses for fodder, along with overgrazing, caused the extirpation of some native plants (Banks 1966). Examination of stomach contents of Anacapa and Santa Barbara deer mice showed that they were eating a mixture of native and exotic plants (Collins et al. 1979; Philbrick 1980; Federal Register 1997; CalFlora 8/2/98), thus dietary changes have occurred. However, none of these factors clearly stands out as a likely causal explanation for rapid morphological change. The island deer mouse populations are likely responding to differing combinations of stochastic and environmental factors that have resulted in rapid phenotypic change.

To summarize our findings on the evolution of California Island deer mice, it is clear that they represent a rich history of colonization, isolation, and evolutionary divergence in both genetic and morphological characters. Several of the islands have populations of deer mice that are morphologically and genetically distinct and have been following evolutionary trajectories independent of the mainland populations and other island populations. Nevertheless, they retain enough similarities for comparisons to be made and informative differences to be found. Our data also indicates recent contact among some of the island populations, including those of San Nicolas, San Miguel, and Santa Rosa and among East, Middle, and West Anacapa Islands. Finally, the California Island deer mice have served as an excellent “natural laboratory” for new and substantive evolutionary findings, including different founder effects on nuclear and mitochondrial genomes and three of the most rapid cases of morphological evolution ever reported in natural populations. Now that we are entering the second century of research on California Island deer mice, we are certain that additional discoveries will emerge.

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