Conservation Genetics **4:** 501–514, 2003.

Hierarchical genetic structure in fragmented populations of the Little Pocket Mouse (*Perognathus longimembris*) in Southern California

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Received 1 May 2002; accepted 26 July 2002

Key words: cytochrome *b*, haplotype diversity, Little Pocket Mouse, mtDNA sequences, Pacific Pocket Mouse, *Perognathus longimembris*, phylogeography, Southern California

Abstract

The geographic genetic structure, based on sequence variation of an 810 base pair fragment of the mitochondrial cytochrome *b* gene, is described for populations of five subspecies of the Little Pocket Mouse, *Perognathus longimembris*, from Southern California. One of these, *P. l. pacificus* (Pacific Pocket Mouse), is listed as Endangered by the U.S. Federal Government. Sixty-two unique haplotypes were recovered from 99 individuals sampled. Phylogenetic analyses of these variants do not identify regionally reciprocally monophyletic lineages concordant with the current subspecies designations, but most haplotypes group by subspecies in networks generated by either statistical parsimony or molecular variance parsimony. Moreover, a substantial proportion of the total pool of haplotype variation is attributed to these subspecies, or to local populations within geographic segments of each, indicating their relative evolutionary independence. The pooled extant populations of the endangered Pacific Pocket Mouse exhibit the same levels of nucleotide and haplotype diversity as other, presumptively less-impacted populations of adjacent subspecies, although the sample from Dana Point, Orange County, has markedly low haplotype diversity in comparison to all others. These populations also show a genetic signature of population expansion rather than one of decline. Both pieces of evidence are at odds with current empirical population estimates, which reinforces the fact that present-day patterns of genetic diversity are the product of coalescent history and will not necessarily reflect recent anthropogenic, or other, perturbations. Comparison of haplotype variation within and among extant populations of the Pacific Pocket Mouse with those obtained from museum samples collected more than 70 years ago suggests that the pattern of population differentiation and diversity was in place before the post-World War II exponential urbanization of Southern California.

Introduction

Population genetics and phylogenetics intersect conservation biology in a number of salient, if different, ways. These range from measures of the loss of variability due to bottleneck effects following habitat fragmentation, to consequences of inbreeding, and to the development of management plans that promote out-crossing, among others (Crandall et al. 2000; Hedrick 2001; Hedrick and Kalinowski 2000). Genetic methodologies have also proven useful in determining how genetic variation is apportioned within and among populations, races, and species, with the lineage contribution to overall genetic diversity increasingly relevant to management decisions (Moritz 1994a, b, 1995). Finally, molecular markers have been used to confirm, or reject, an extant taxonomy with respect to the uniqueness of listed

taxa, or those proposed for special protection at state, federal, or international levels (e.g., Zink et al. 2000).

In this paper, we examine the patterns of genetic variation in mitochondrial DNA sequences for populations and races of *Perognathus longimembris* (the Little Pocket Mouse) in Southern California. We use the current subspecies designations and mapped distributions (e.g., Hall 1981; Williams et al. 1993) as a point of departure, but our analyses do not depend on the taxonomic validity of any of these forms. One subspecies, *P. l. pacificus* (the Pacific Pocket Mouse) is currently listed as Endangered under the U. S. Endangered Species Act, and three others are considered of conservation concern by the State of California and the IUCN Species Survival Commission (Patten et al. 1998). The ranges of both subspecies and populations in Southern California have been strongly influenced by habitat conversion, degradation, and fragmentation as a result of intensive development, especially within the past 50 years.

We use hierarchical phylogenetic and population genetic analyses to address the question of the uniqueness of extant populations of all recognized races of the Little Pocket Mouse in Southern California. We combine data from extant populations with those from historical, but now extirpated populations, of *pacificus* to help examine the relative importance of deeper history and recent habitat fragmentation through urbanization to the observed patterns of genetic diversity. We conclude that these types of molecular studies primarily yield information about that deeper history rather than more recent, anthropogenic population perturbations. This backward look into the genetic past must be combined with future projections based on current demography and the likelihood for population interconnections in the development of any management directives.

Perognathus longimembris *in Southern California*

For the purposes of this paper, we define Southern California to include the Los Angeles Basin and coastal plain south to San Diego, was well as the western margins of the Colorado Desert in Riverside, San Bernardino, San Diego, and Imperial counties (map, Figure 1). By current taxonomy (Williams et al. 1993), four subspecies of *P. longimembris* are recognized within this region (*P. l. bangs* [Palm Springs Pocket Mouse], *P. l. brevinasus* [Los Angeles Pocket Mouse], *P. l. internationalis* [International Pocket Mouse], and *P. l. pacificus* [Pacific Pocket Mouse]). Williams (1986) noted that the systematics of the entire *P. longimembris* group is in need of revision, especially the various subspecies in Southern California. For simplicity, we use the range designations given in Williams et al. (1993) for each subspecies, yet recognize that both the validity of these taxa and their explicit boundaries remain to be firmly established. The range of *bangsi* includes in the Coachella and Imperial valleys east of San Gorgonio Pass at least to the Mexican border, *brevinasus* inhabits the arid coastal basins to the immediate west, and *internationalis* is known from the quasi-isolated interior desert valleys in eastern San Diego Co. (Figure 1). Both *bangsi* and *brevinasus* have experienced considerable habitat loss from urban development in the Palm Springs and greater Los Angeles areas, respectively (Brylski et al. 1994). The historic range of *pacificus* extended down the coastal strip from approximately El Segundo, Los Angeles Co., to near Tijuana, Baja California. Today, this subspecies is limited to two small and isolated areas, one at Dana Point, Orange Co., and the other at three separate nearby sites on Marine Corps Base Camp Pendleton in San Diego Co. (Spencer et al. 2000a, b, 2001). It has apparently been extirpated from the remainder of its range. Two other subspecies are distributed to the adjacent north and east, in the Antelope Valley and Mojave River region (*P. l. longimembris*, Figure 1, inset) and along the Colorado River (*P. l. bombycinus*); we include data for the former but not the latter.

Perognathus longimembris is a diminutive (7–12 grams in adult body mass), nocturnal, solitary, and semi-fossorial (living in self-dug burrows) rodent in the family Heteromyidae. It is largely granivorous, specializing on grass and forb seeds that it caches. The species inhabits desert scrub and sparse sage scrub in the southwestern U.S. and northwestern Mexico, particularly in areas of fine, sandy soils. Average life expectancy in the field is approximately one year, with survival for as long as 3–5 years common (French et al. 1967, 1974) and one exceptional record of over 8 years in captivity (Edmonds 1972). The extended longevity for such a small-bodied mouse is presumably facilitated by seasonal heterothermy (winter hibernation and facultative summer aestivation) resulting from the environmental stresses of food shortage and/or low temperatures (Chew et al. 1965; Bartholomew and Cade 1957).

Few studies of these mice have been undertaken in Southern California. Anecdotal references (e.g., von Bloeker 1928, 1931) suggest that populations of *paci-*

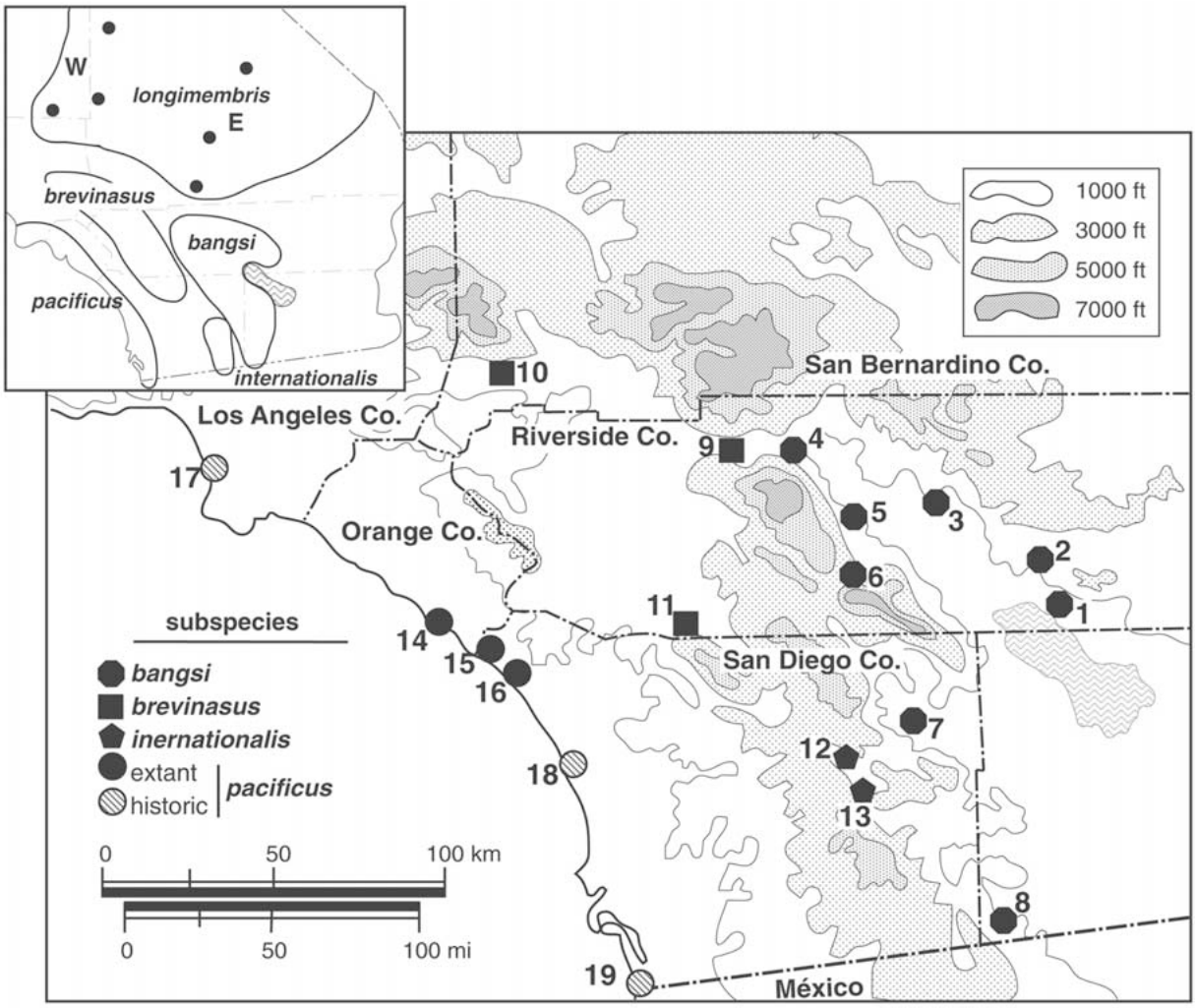

Figure 1. Map of southern California showing sample localities of four subspecies of the Little Pocket Mouse, *Perognathus longimembris*, from Southern California. Localities are numbered as listed in the Appendix. The inset map illustrates the generalized ranges of each of these subspecies as well as the range and sample sites for six localities of the nominate subspecies, *P. l. longimembris*, from the Mojave Desert north of Southern California, divided into western (W) and eastern (E) geographic units.

ficus were normally at low numbers punctuated by the occasional year with local irruptions in numbers. The only relatively long-term population studies have been at a site (Irvine Ranch, Orange Co. [M'Closkey 1972; Meserve 1976]) where pocket mice are now apparently extirpated (Brylski et al. 1994; Spencer et al. 2000a, b, 2001). Here, mice were rare and their occurrence unpredictable over a 15-month trapping study (M'Closkey 1972). Mark-and-release studies on the extant populations of *pacificus* at Dana Point and Marine Corps Base Camp Pendleton suggest current population sizes from less than 20 to about 1,000 individuals, respectively, with limited adult movement and juvenile dispersal distances (US Fish and Wildlife Service, unpublished data; Spencer et al. 2000a). Recent trapping studies of *bangsi* north of Palm Springs found it to be among the most abundant mammal species, reaching densities of about 60 to 200 individuals per hectare in creosote scrub habitat (Spencer et al. 2001).

Materials and methods

We examined variation within the first 810 base pairs of the mitochondrial cytochrome *b* (cyt-*b*) gene.

Sequence data are available for 99 specimens, including 12 of the nominate subspecies (*P. l. longimembris*) from the Mojave Desert, 24 *bangsi* from the Coachella and Imperial valleys, 9 *brevinasus* from the northern and eastern Los Angeles Basin, 6 *internationalis* from San Diego Co., and 48 *pacificus* from Dana Point, Orange Co., and three sites on Camp Pendleton in northern San Diego Co. (San Mateo North and South and Oscar One; see map, Figure 1, Table 1, and the Appendix for locality list). All preserved specimens are cataloged in the Museum of Vertebrate Zoology, and representative haplotypes are deposited in GenBank (accession numbers AY152409–AY152417).

We used liver or muscle tissue as the source of DNA for all specimens, except for most specimens of *pacificus* and a few *bangsi*, for which either hairs or a biopsied hind toe were removed from live-trapped individuals prior to their release. In addition to samples from extant populations, we also extracted DNA from 2 × 2 mm skin snips from 12 museum specimens of *pacificus* collected in the 1930s from three localities that span the historic range of this subspecies (Table 1; Appendix). We used a lysis-salt procedure (Medrano et al. 1990) or the DNAease kit (Quiagen, Inc.) for all extractions. Initially, we sequenced manually, following asymmetrical amplification and direct sequencing protocols given in earlier papers (Patton and Smith 1992; Patton et al. 1994; Smith and Patton 1993). We sequenced other specimens obtained since 1996, including all skin snips, on an ABI 377 (Applied BioSystems Inc.) automated sequencer following manufacturer protocols. For the DNA from recent specimens, we amplified the entire 810 bp fragment with primer pairs MVZ05/MVZ16 and sequenced in both directions by each primer along with the light-strand internal primer MVZ65. For DNA extracted from skin snips from museum specimens, we obtained a final 432 bp fragment by amplifying overlapping smaller fragments using primers MVZ05/MVZ06 and AS07/MVZ04. MVZ primer sequences can be found in Smith and Patton (1993); the sequence for AS07, which begins at position 14256 on the light strand of the *Mus* mitochondrial genome, is 5′-GGCTATGTCTCATTATCC-3′.

We aligned sequences either with Sequence Navigator (ABI Inc.) or Sequencher (Gene Codes Corporation) software, and identified redundant haplotypes by subjecting a file of all 99 individuals to MacClade 4.0 (Maddison and Maddison 2000). We examined the phylogenetic relationship among these unique haplotypes with PAUP 4.0b8 (Swofford 2001) using maximum parsimony. To address the question of the monophyly of the *P. longimembris* samples, we used sequences from the other two species of the "*longimembris*-group", *P. amplus* from Arizona and *P. inornatus* from the Central Valley of California (from two of the three recognized subspecies, *inornatus* and *neglectus*; see Appendix). Because of the large number of highly similar haplotypes, we limited phylogenetic analysis to a single heuristic search using TBR branch swapping. We assessed the strength of the major internal nodes by bootstrapping, with

Table 1. Sample sizes (N_i), number of separate populations sampled per pooled group (N_p), and number of haplotypes (N_h) of each subspecies of the Little Pocket Mouse, *Perognathus longimembris*. Localities are numbered as in the map, Figure 1, and as detailed in the Appendix. The date of collection of each historic sample is indicated

Subspecies: Geographic subdivision	N_i	N_p	N_h
longimembris (Little Pocket Mouse)			
west	9	4	7
east	3	3	3
brevinasus (Los Angeles Pocket Mouse)			
east (9)	6	1	4
west (10–11)	3	2	2
bangsi (Palm Springs Pocket Mouse)			
east (1–3)	9	3	9
west (4–8)	15	5	9
internationalis (International Pocket Mouse)			
north (12)	2	2	2
south (13)	4	2	2
pacificus (Pacific Pocket Mouse)			
Dana Point (14)	27	1	9
San Mateo North (15)	5	1	4
San Mateo South (15)	10	1	10
Oscar One (16)	6	1	6
El Segundo (1937) (17)	1	1	1
Oceanside (1931) (18)	6	1	3
Tijuana (1931) (19)	5	1	3

1000 replicates, on a random sample of 10,000 trees obtained from the heuristic search. Bremer support indices (Bremer 1988) were calculated using TreeRot (Sorenson 1996) on this same reduced set of trees.

We used Arlequin 2.0 (Schneider et al. 2000) for all measures of nucleotide and haplotype diversity, for hierarchical analysis of molecular variation (AMOVA), and to construct a minimum spanning network among haplotypes by the method of molecular variance partitioning. We also used TCS alpha, ver. 1.01 (Possada 2000) to construct a statistical parsimony network (Templeton et al. 1992). In both procedures, the number of mutational steps along the network branches measures the degree of evolutionary divergence between any two haplotypes in each network. To arrange the shorter museum sequences within the larger haplotype database, we truncated the 810 bp data set to the museum sequence length of 432 bp and analyzed these data separately. The AMOVA analysis used a matrix of mutation steps. We used the subspecies designation to define geographical regions, which sort taxa into those from the Mojave Desert (*longimembris*), Coachella-Imperial valleys (*bangsi*), upper Los Angeles Basin (*brevinasus*), intermontane desert valleys (*internationalis*), and coast (*pacificus*). We combined geographically adjacent localities within each subspecies to provide a measure of inter-population variation within each subspecies (see Table 1). Pooling in some cases is arbitrary but could not be helped given the difficulties in securing sufficient samples of these mice from all areas. Different pooling strategies yielded similar hierarchical patterns. For example, pooling the San Mateo samples of *pacificus* did not change the apportionment pattern obtained when each single locality of this subspecies was treated separately. We also combined historic and modern samples of *pacificus* in a separate set of hierarchical analyses for this subspecies.

We generated standard variance components at each of three hierarchical levels using the AMOVA subroutine in Arlequin: differences among the groups (subspecies or temporal samples) relative to the total haplotype pool, differences among populations within groups, and differences among individual haplotypes within populations. We used Φ_{ST} as an estimator of Wright's F_{ST} (Michalakis and Excoffier 1996) to infer levels of female gene flow (*Nm*), denoted as $\hat{M}$ (Slatkin 1993). Estimates of $\hat{M}$ provide a qualitative signal of past or recent gene flow, under assumptions of equilibrium, based on $Nm = 1/4(1/F_{ST} - 1)$ (from Slatkin 1994). Finally we used Fluctuate, version 1.3 (Kuhner 1998), to calculate the population exponential growth (or decline) parameter, *g*, from haplotype data for three single localities for which samples sizes were 10 or more (Dana Point and San Mateo, combined, of *pacificus*, and Snow Creek, of *bangsi*). This estimate can give insights into the genetic history of a population, such as the likelihood of expansion from a recent bottleneck wherein genetic diversity was reduced (Kuhner et al. 1998). Because the Markov Chain Monte Carlo simulation is based on the genealogy of haplotypes at each locality, which will necessarily be different among localities, we standardize the growth parameter by its standard deviation. Since each of these analytical procedures assumes that haplotype variants are not under selection, we used both Tajima's (1989) and Fu's (1997) tests of selective neutrality, as implemented in Arlequin.

Results and discussion

In what follows, we present the general trends among all sampled populations from Southern California and follow these by consideration, where appropriate, of the truncated dataset involving only the temporal and spatial samples of *pacificus*. Combining both sets of analyses, most (8 of 14) estimates of Tajima's *D* or Fu's F_S are negative and none are significantly different from zero (Table 2). These data thus support the assumption that observed haplotype variation has been governed by neutral processes. As a consequence, we can assume that selection has not significantly influenced the distribution of that variation during the history of the populations under study.

Haplotype and nucleotide diversity

There are 62 unique haplotypes among the 99 individuals for which the 810 bp cyt-*b* gene fragment was available (Table 1). These differ by a maximum of 20 mutational steps (2.5%, uncorrected), with a mean among all haplotypes of 11.4 steps. The truncated dataset of 432 bp for all 60 specimens of *pacificus* contained 19 haplotypes, 7 from the historic individuals and 12 from extant samples. These differed by a maximum of 12 mutational steps (2.8%, uncorrected). The pooled samples for each subspecies exhibit approximately the same haplotype and nucleotide diversities and contain similar average pairwise nucleotide differences among their included haplotypes (Table 2). The exception is the small sample of *internationalis*

Table 2. Estimates of haplotype and nucleotide diversity, average number of pairwise differences, and measures of the intensity of selection (Tajima's *D* and Fu's *Fs*) for pooled samples of each subspecies of *Perognathus longimembris*. Measures are means ± one standard deviation; ns = non-significant ($p >> 0.05$). The extant and historic samples of *pacificus* include 432 bp of sequence; all other measures are based on the complete set of 810 bp.

Subspecies	Haplotype diversity	Nucleotide diversity	Mean # pairwise differences	Tajima's *D*	Fu's *Fs*
longimembris	0.9636 ± 0.0510	0.00969 ± 0.00552	7.8545 ± 3.9609	0.00114^{ns}	-1.48109^{ns}
brevinasus	0.9167 ± 0.0725	0.01163 ± 0.00669	9.4767 ± 4.7775	-0.44353^{ns}	1.42422^{ns}
bangsi	0.9601 ± 0.0252	0.01972 ± 0.00582	8.8877 ± 4.2450	-0.09066^{ns}	-3.78438^{ns}
internationalis	0.7333 ± 0.1552	0.00263 ± 0.00196	2.1333 ± 1.3728	-0.14427^{ns}	1.14056^{ns}
pacificus	0.9521 ± 0.0152	0.00940 ± 0.00495	7.6117 ± 3.6134	-0.45731^{ns}	-6.43940^{ns}
extant	0.8511 ± 0.0324	0.00842 ± 0.00482	3.6374 ± 1.8755	0.46765^{ns}	-0.92524^{ns}
historic	0.8718 ± 0.0670	0.01229 ± 0.00713	5.3077 ± 2.7403	0.40958^{ns}	0.42399^{ns}

that is lower in all measures (Table 2); whether or not this difference is real or only results from the limited sample remains to be determined. Importantly, the endangered Pacific Pocket Mouse (*pacificus*) is not singularly different in any of these measures of diversity or inter-population differentiation in comparison to the other subspecies. For this subspecies, however, historic haplotypes were slightly more differentiated among themselves than were those from extant populations (means = 5.308 steps versus 3.637 steps, respectively, although this difference is not significant at $p = 0.05$; Table 2). This slight difference is also not surprising given the much larger portion of the original subspecies range represented by the historic samples (linear distance of approximately 120 versus 20 miles, respectively; Figure 1).

For the most part, it was not possible to calculate reliable measures of haplotype and nucleotide diversity for single population samples, as the number of individuals examined in each was limited. However, we did estimate these parameters for the five populations that had sample sizes between 6 and 27 (Dana Point, San Mateo, and Oscar [*pacificus*]; Cabazon [*brevinasus*]; and Snow Creek [*bangsi*]). Of these, the sample from Dana Point was notably low in haplotype diversity (0.8661 ± 0.0362) in comparison to this measure for the other four samples (range, 0.9333 [Snow Creek] to 1.0000 [San Mateo and Oscar]). Nucleotide diversities for all five samples, however, were similar, with that for Dana Point (0.00699 ± 0.00386) intermediate between those for the other samples (range, 0.00518 [Oscar] to 0.00946 [Cabazon]).

Phylogeny and phylogeography

The related species, *P. amplus* and *P. inornatus*, differ from *P. longimembris* by 103 and 61 steps, on average, respectively. Phylogenetic analyses strongly associate all sequences of *P. longimembris* into a monophyletic clade in relation to the two out-group species, with 100% bootstrap support and a Bremer index of 10 (Figure 2, inset). Within the *longimembris* clade, however, there is little phylogenetic structure among the 62 haplotypes. Although several distinct clusters are evident, only one has bootstrap support above 50% (Figure 2). This particular cluster includes haplotypes from western samples of *bangsi* (localities 4 through 8, Figure 1), those of *brevinasus* (localities 9 and 10), and two haplotypes of *pacificus* (both from Dana Point). Haplotypes of *longimembris* from the Mojave Desert (Figure 1, inset), eastern *bangsi* (localities 1–3), and *internationalis* (localities 12 and 13) form a weakly associated cluster (bootstrap < 50%) at the base of the tree. Importantly, haplotypes of *pacificus* are scattered throughout the tree, except among those of the basal cluster. The subspecies are not reciprocally monophyletic with regard to the genealogy of their respective haplotypes although, with the exception of *bangsi*, most haplotypes of single subspecies are closely united.

Haplotype networks generated by statistical parsimony and molecular variance parsimony were identical in the placement of individual haplotypes. Differences between the two methods were only in the identification of missing haplotypes in the network that are estimated by statistical parsimony. For simplicity, we illustrate the network without the placement of missing haplotypes (Figure 3). Four general

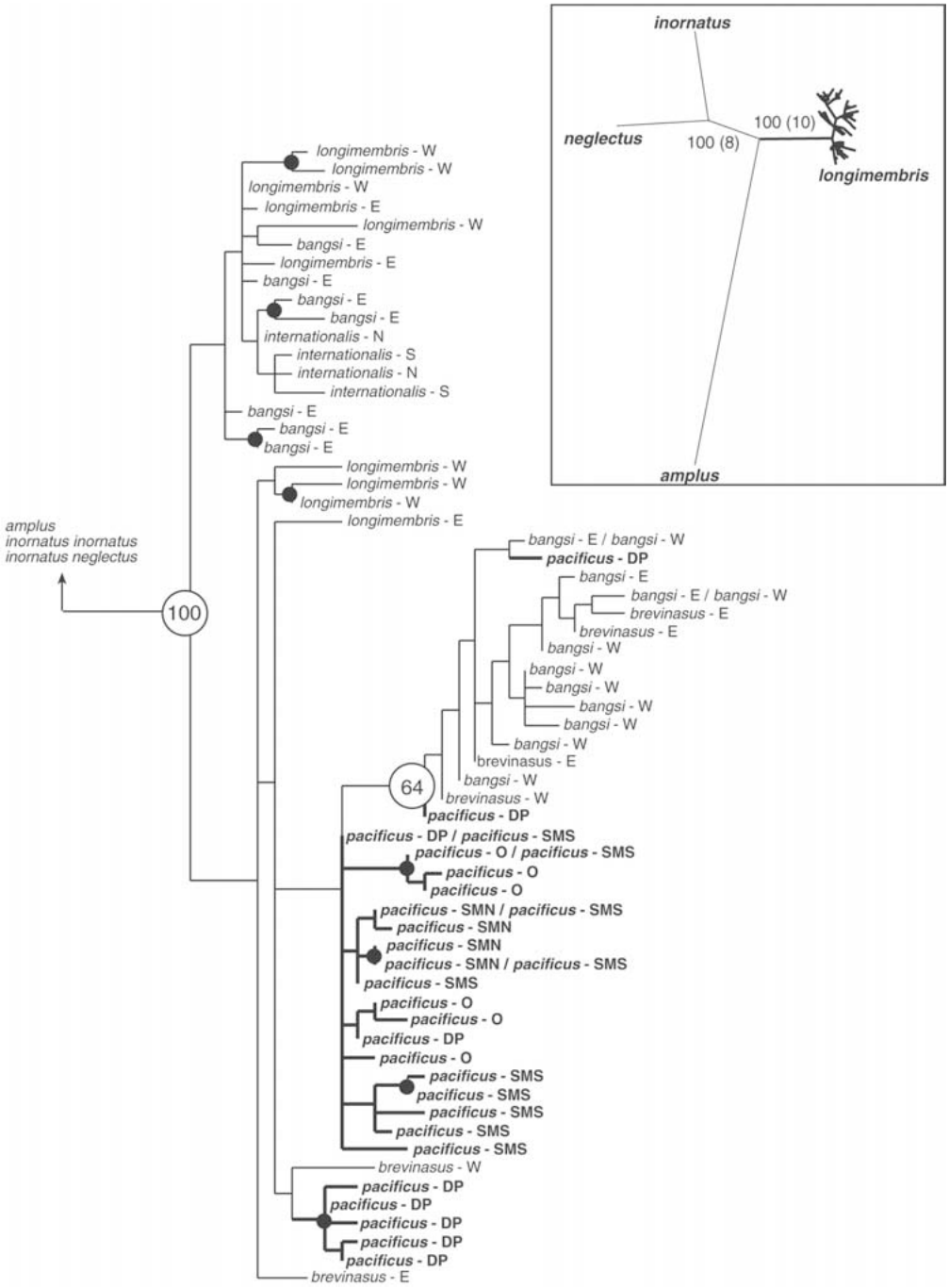

Figure 2. Strict consensus maximum parsimony tree illustrating the phylogenetic relationships of 62 unique cytochrome *b* haplotypes of *Perognathus longimembris* in Southern California and rooted by comparison to the other two species in the *longimembris*-group, *P. amplus* and *P. inornatus* (subspecies *inornatus* and *neglectus*) (of 138,395 trees, each 257 steps long; 810 total characters, 608 constant, 109 parsimony informative; consistency index = 0.665). Bootstrap values linking haplotype clusters > 50% are indicated at internal nodes. Inset illustrates the relationship of the outgroup taxa to *P. longimembris*; bootstrap and Bremer support values at nodes supporting the monophyly of the 62 haplotypes of *longimembris* are indicated.

haplotype clusters are apparent: one of these groups all *longimembris* from the Mojave Desert, *internationalis*, and eastern *bangsi* and a second groups western *bangsi*, most *brevinasus*, and one *pacificus* haplotype (from Dana Point). The remaining *pacificus* and one *brevinasus* haplotype (that from locality 11, Figure 1) belong to separate but united clusters that form the nexus between these other two groupings. Generally, each cluster of haplotypes in the network is coincident with geographic relationships of the respective samples, with one group to the north and east of the Salton Sea and a second that combines all interior and coastal Southern California samples. The exceptions are the haplotypes of *internationalis* from southeastern San Diego Co. that group with those to the east and north rather than to coastal forms (contra expectations, see Williams et al. 1993).

There is also limited haplotype sharing among the extant samples, as only one haplotype is shared between samples of the eastern and western groups of *bangsi* (between localities 3 and 4–5; Figures 1 and 3), and four haplotypes are shared among samples of *pacificus* (one between Dana Point and San Mateo South, one between San Mateo South and Oscar One, and two between the separate San Mateo samples). The Dana Point sample includes at least one haplotype positioned in each cluster in the network where *pacificus* haplotypes are found. In contrast, all haplotypes from Camp Pendleton group collectively into the single, larger cluster. Interestingly, the single haplotype of *brevinasus* from the Pauba Valley on the border of Riverside and San Diego counties, while relatively divergent from all others, groups with those of *pacificus* rather than with others of this subspecies. Thus, of the five subspecies examined, as their distributions are currently understood, *bangsi* clearly represents two historical units (eastern and western margins, respectively, of the Coachella and Imperial valleys) while *brevinasus* is the least inclusive and thus apparently the most polyphyletic. The remaining three subspecies exhibit cohesion in the network, even if they do not form reciprocal monophyletic groupings.

All historic haplotypes of *pacificus* (those with 432 bp) are tied to one of two common haplotypes among the extant samples (arrows, Figure 3). The single individual from El Segundo (locality 17) and all those from Oceanside (locality 18) group with a haplotype shared between Dana Point (locality 14) and San Mateo (locality 15) while all haplotypes from Tijuana (locality 19) stem from another Dana Point haplotype. Each of these differs by no more than 3 mutational steps (0.69%) from the closest haplotype among extant individuals.

Table 3. Hierarchical analysis of variance across population samples of *Perognathus longimembris* from Southern California. The percentage variance explained and probability estimated from permutation tests are given at each hierarchical level (see Excoffier et al. 1992)

Variance component	Subspecies		Temporal samples of *pacificus*	
	%	p	%	p
Among groups (Φ_{ST})	23.65	<0.001	6.49	>0.302
Among populations within groups (Φ_{PS})	30.83	<0.001	54.37	<0.001
Within populations (Φ_{IP})	45.52	<0.001	39.14	<0.001

Hierarchical apportionment

Only a limited amount (23.7%) of the total pool of haplotype variation is attributed to each subspecies in the AMOVA analysis (Table 3), as might be expected by visual examination of both the phylogenetic tree (Figure 2) and haplotype network (Figure 3). Rather, most variation is found within populations (45.5%) and an intermediate amount is distributed among populations within subspecies (30.8%). The structure present is significant, by permutation test, at each hierarchical level ($p < 0.001$ in all cases; Table 3). This same pattern of apportionment is present in other analyses (not shown) that placed samples into different hierarchical groupings (such as Mojave Desert, Colorado Desert, and coastal California). Hence, the patterns presented here are not merely an artifact of arbitrary grouping. The limited "subspecies effect" reflects the lack of reciprocal monophyly but is nevertheless significant and results from the general grouping of haplotypes belonging to single subspecies into unified clusters within the network. Similarly, individual populations within each subspecies or geographic group retain a substantial amount of the total pool of variation. In general, therefore, these samples do exhibit a degree of geographic structuring perhaps larger than might be expected, given the rather limited geographic scale over which our samples were collected. This grouping is evident in the haplotype network (Figure 3).

We employed a separate AMOVA analysis comparing the temporal samples of *pacificus* to determine if there has been any change in structure among

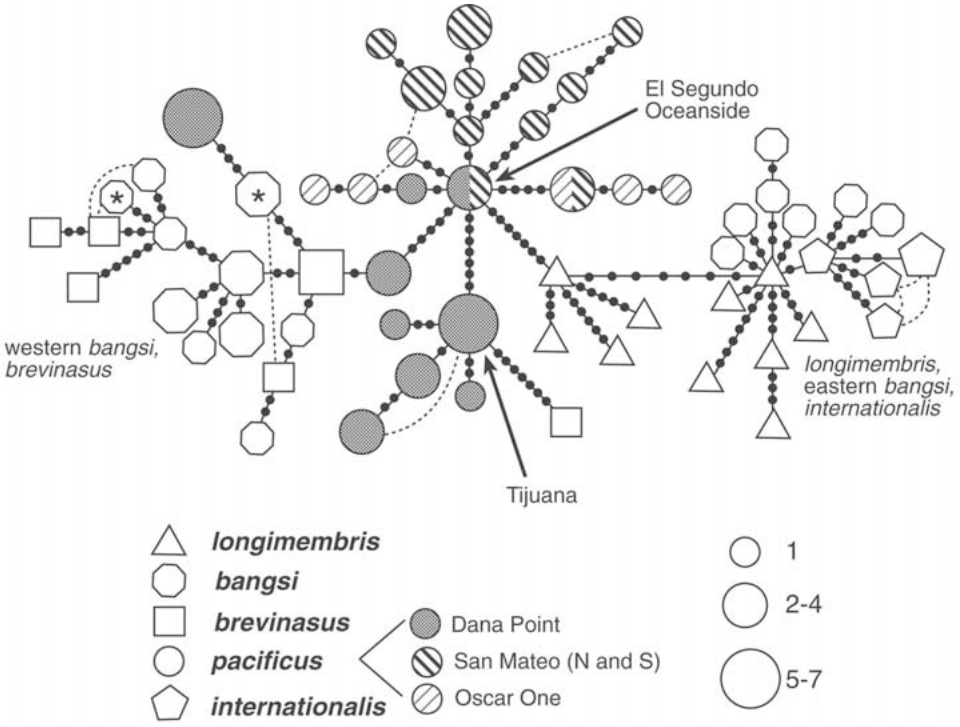

Figure 3. Parsimony network illustrating relationships of the 62 haplotypes (810 bp) of *Perognathus longimembris* samples from Southern California. Separate symbols are used for each subspecies, and for the three separate population samples of the endangered *P. l. pacificus* (Pacific Pocket Mouse); the size of the symbol indexes the number of individuals with that particular haplotype. Small solid circles along interconnecting lines are the number of mutational steps separating adjacent haplotypes in the network, and dotted lines identify alternative network connections. Arrows indicate the placement of the 432 bp haplotypes recovered from the historic samples of *pacificus* in the collection of the Museum of Vertebrate Zoology relative to the larger network.

populations over the past 70 years (Table 3). No demonstrable temporal effect is apparent, however, as only 6.5% of the total pool of haplotype variation can be attributed to comparisons between the historic and extant samples, an amount that is not significantly different from zero (p = 0.302). Most of the variation is again found among populations within each temporal period (54.4%) or is apportioned to individual differences within populations (39.1%).

Gene flow, past and present

As emphasized by Moritz (1994a), even with statistically adequate sample sizes it is difficult to obtain estimates of population size or migration rates that are accurate in the short term through the use of any set of genetic markers. This is particularly true for mtDNA genes, which are more prone to the stochastic effects of drift because of their smaller effective size relative to nuclear markers. Moreover, the maternal inheritance of mtDNA means that any gene flow estimates are those of females only, and are thus likely to be biased towards low levels in organisms, like mammals, where female philopatry and male dispersal are typical. Given these caveats, therefore, our data at best provide a qualitative perspective on the historical connectedness among samples of female pocket mice. Certainly, the exact numbers should be accepted with care, and Y-chromosome markers would be needed to obtain complementary measures for males.

For populations of *P. longimembris* of Southern California, pair-wise $\hat{M}$ values, Slatkin's (1993) estimate of *Nm*, range from 0.112 (between the Dana Point sample of *pacificus* [locality 14] and *internationalis* [locality 12]) to 10.999 (between *internationalis* and eastern *bangsi*), with a mean of 1.175 for the 78 comparisons. All samples of *P. longimembris* from the Mohave Desert north of our major sample area (Figure 1) are "connected" by relatively large $\hat{M}$ values (5.5 to 7.6) despite quite large straight-line distances among them (maximum approximate distance = 130 miles, average distance = 69.6 miles).

Clearly, populations of this species have the capacity to exchange genes, or at least be genealogically connected, over respectable distances. The relatively high connectedness among samples from the Mojave Desert contrasts sharply with data for samples from Southern California. In this focal sample area, the mean $\hat{M}$ value is 0.814, only 8 pairwise comparisons are above 1.0, and those that are range to a maximum of 2.71 (between western *bangsi* and eastern *brevinasus*, sample sites that are approximately 6 miles apart). For samples of *pacificus*, all $\hat{M}$ values are less than 1.0, with the exception of those between the two San Mateo samples ($\hat{M}$ = 4.1379) and between San Mateo North and Oscar One ($\hat{M}$ = 1.6588). The Dana Point sample exhibits uniformly low $\hat{M}$ values to other samples (0.4956 to 0.6069), suggesting that it has been relatively isolated from these for a period of time. Similarly, estimates of $\hat{M}$ among the historic samples are all below 1.0 (range, 0.1696 to 0.4170), indicting that the pattern of relative isolation between the extant samples at Dana Point and Camp Pendleton is qualitatively the same as that among population samples taken 70 years ago. Assuming that these estimates of $\hat{M}$ are accurate, then values among the sampled populations of *P. longimembris* throughout Southern California are, for the most part, below the threshold of 1.0 whereby populations are likely to diverge by genetic drift alone (Mills and Allendorf 1996).

Historical population demography

Estimates of g, the population growth parameter, for Dana Point (g = 664.5496, sd = 84.3272), San Mateo (g = 1206.3342, sd = 203.1894), and Snow Creek (g = 3359.2727, sd = 414.8081) are each positive and significantly different from zero (using a conservative estimate of $g > 3 \times$ standard deviation). This suggests that each population has experienced population expansion since the last coalescent event in their respective haplotype genealogies (Kuhner et al. 1998). This conclusion is consistent with the relatively large negative estimate of Fu's (1997) F_S, especially so for samples of *pacificus* (–6.4394; Table 2), which is nearly significant and also in the direction of expected population expansion (p = 0.024; statistical significance at the a = 0.05 level for Fu's test is a p-value of 0.02; see Schneider et al. 2000: 90). The standardized estimates of g are two to three times higher for the Snow Creek sample than for either sample of *pacificus* (7.182 versus 2.687 and 1.979, respectively).

The indication of exponential growth for samples of *pacificus* is at odds with actual estimates of population size based on current demographic studies. Spencer et al. (2000a) summarized trapping studies performed at Dana Point from 1993 to 1998 and estimated the total population at approximately 25 to 36 individuals. More recently, an intensive trapping effort during 2001 that was designed to obtain a complete population count captured only 4 individuals at Dana Point (U.S. Fish and Wildlife Service, unpublished data). Spencer et al. (2000a) also estimated the two San Mateo sites to support no more than 50 individuals each and the largest extant population (Oscar) to support in the neighborhood of 1,000. Clearly, growth estimates based on our genetic samples reflect a longer history than the likely demographic impacts of severely restricted habitat that characterize these populations today. Rogers and Harpending (1992), for example, have shown that the time scale for populations to reach genetic equilibrium after a decline is an evolutionary one, measured in units of hundreds or thousands of generations ($1/2\mu$, where μ is the mutation rate). Several empirical studies, including that of the endangered Stephens' Kangaroo Rat (*Dipodomys stephensi*; Metcalf et al. 2001) distributed over much of the same region in Southern California, and coconut crabs from the Pacific (e.g., Lavery et al. 1996), have documented genetic signatures of expansion even in severely declining populations of endangered species. Consequently, the genetic data we report here for *pacificus* should be taken in this light, and certainly do not suggest that the species is currently enjoying a population expansion or is unworthy of protection.

Conclusions and prospectus

The combination of (1) most samples with unique haplotypes that generally group into genealogical clusters (Figure 3); (2) lack of haplotype sharing among any subspecies or between most populations, even those geographically adjacent; (3) a substantial degree of apportionment at the level of geographic area (Table 3); and (4) low estimates of inter-population gene flow suggests that each subspecies of *P. longimembris* in Southern California and most of their member geographic units and/or populations have been genetically relatively independent for a reasonable period of time. Samples from the Mojave Desert where gene flow rates, as measured by $\hat{M}$ values, are uniformly high offer exceptions to this

general trend. Nevertheless, either local populations must have remained sufficiently large to prevent the loss of genetic variation, despite opportunities for drift, or these populations have remained of similar size regardless of the degree of habitat modification they have experienced. Indeed, historical population expansion is supported by the few available estimates of the genealogical growth parameter, *g*. Otherwise, with such low levels of gene flow among populations, both the different subspecies, if not many local populations, might be expected to exhibit a greater degree of reciprocal monophyly of haplotypes then they do. While it does seem clear from the network (Figure 3) that the subspecies are "moving" in the direction of reciprocal monophyly, none have reached this degree of differentiation. The anecdotal record of occasional population outbreaks (which would presumably lead to momentary increases in interpopulation "connectedness") interspersed temporally with more typically small-sized but persistent local populations (von Bloeker 1928) is fully consistent with these observations.

We offer two concluding comments on the utility of these types of data relative to the conservation of these mice, and other taxa. The first concerns whether or not any of these populations or subspecies of *P. longimembris* represent an Evolutionarily Significant Unit (ESU) as applied under the federal Endangered Species Act. Although each population is generally separable in the haplotype network (Figure 3), none is reciprocally monophyletic for their mtDNA genealogy, and hence none meet a strict definition of an ESU based purely on neutral genetic markers (e.g., Moritz 1994b). However, such a stringent criterion has been strongly debated and is being replaced by an emerging consensus that ESUs should be evaluated along two axes – molecular genetic diversity and adaptive diversity (Crandall et al. 2000; DeWeerdt 2002; Frazer and Bernatchez 2001; Moritz 2002). Neutral genetic markers (e.g., mtDNA) reflect the evolutionary history of a population and remain a primary method of measurement along the first axis. The second axis, adaptive diversity, can be measured either by investigating the genetic bases of adaptive traits (e.g., studying functional nuclear genes) or by investigating meaningful ecological differences between populations. The overall goal of defining ESUs thus becomes one that conserves both the products and the processes of evolution (Moritz 2002), a shift in focus from defining isolated products of evolution to investigating and protecting historical levels of gene flow between them (DeWeerdt 2002).

Based on such thinking, Frazer and Bernatchez (2001) argue that an ESU should be defined as a "lineage demonstrating highly restricted gene flow from other such lineages within the higher organization level (lineage) of the species" (Frazer and Bernatchez, 2001: 2742; see also Crandall et al. 2000 and Moritz 2002). By this definition, which emphasizes the degree of both ecological and genetic exchangeability (in the sense of Templeton 1989), the Federally listed Pacific Pocket Mouse (*P. l. pacificus*) is clearly an ESU. Indeed, a case could even be made for recognizing its separate populations as ESUs. An alternative interpretation of our results – based purely on the one axis of molecular genetic diversity – supports considering a combined grouping of *pacificus*, *brevinasus*, and western *bangsi* populations as one ESU, separate from eastern *bangsi*, *internationalis*, and Mohave populations of *longimembris*. This alternative nearly meets the strict definition of an ESU based on reciprocal monophyly. Nevertheless, we strongly suspect that the yet unstudied second axis – that of adaptive variation – would support defining *pacificus*, and perhaps other subspecies and populations of *P. longimembris* from Southern California, as separate ESUs, due to the extensive ecological differences each exhibits. The Pacific Pocket Mouse, for example, lives immediately along the Pacific coastline, which differs dramatically in climate, geology, vegetation, animal communities, and seasonal phenology from the interior deserts and valleys occupied by the other subspecies (Goudey and Smith 1994; Miles and Goudey 1998). Although detailed comparative studies of adaptive variation among these populations are not available, these environmental differences are so strong that the scientific peer committee overseeing recovery research for *pacificus* concluded that no other subspecies represented a valid research surrogate for investigating its biology (Spencer et al. 2001).

Finally, we simply reiterate that the genetic "signature" of extant populations is the result of the history of those populations, including their demographic characteristics and degree of genetic connection with others. It should not be a surprise, therefore, that extant populations of *pacificus* appear little different in their degree of molecular diversity or geographic structure and relative isolation than historical samples of this taxon taken more than 70 years earlier. It should also not be unexpected to record genetic characteristics indicative of exponential growth even in

populations, such as at Dana Point, where current estimates are of exceedingly low numbers of individuals. It takes time for the measured genetic characteristics of an extant population to reflect its immediate past history, and clearly the 40 years or so of increasing isolation and reduction in habitat and population numbers at Dana Point has been insufficient to override this deeper genetic history. What population genetic theory allows us to predict with some degree of confidence, however, is that if the current demographic conditions at Dana Point continue into the foreseeable future, loss of diversity must necessarily occur (Luikart et al. 1998). Indeed, Dana Point already exhibits the lowest haplotype diversity of any sample of *P. longimembris* from Southern California, including the other two extant populations of the Pacific Pocket Mouse at nearby Camp Pendleton.

Acknowledgements

Dr. Jesus Maldonado, in the laboratory of Professor Robert Wayne at UCLA, extracted the DNA from an initial set of six individuals of the Pacific Pocket Mouse; we thank both for their generosity in providing these extracts. Vouchered specimens were collected under auspices of the California Department of Fish and Game, hair samples from released Pacific Pocket Mice were collected by personnel of the PPM Recovery Team, and funding for the laboratory analyses was underwritten by grants from the National Science Foundation (to JLP) and US Fish and Wildlife Service and California Department of Fish and Game. We are grateful to Mark Pavelka, US F&WS, for logistic and other support, and to Marjorie Matocq and Fred Allendorf, plus two anonymous reviewers, for improving the contents and presentation of the manuscript.

Appendix

List of localities from which specimens were examined, grouped into pooled samples used in all analyses. Those for the four subspecies from southern California are numbered as in the map, Figure 1; all samples of *P. l. longimembris* come from the Mojave Desert of California (inset in Figure 1). Voucher specimens, when preserved, are identified by their Museum of Vertebrate Zoology (MVZ) catalog number.

Perognathus l. longimembris. **West** – Kern Co., 6 mi N and 5 mi E Rosamond (n = 3; MVZ 182710–182712); 7 mi N and 5.5 mi E Rosamond (n = 1; MVZ 182713); San Bernardino Co., 6 mi WSW Boron (n = 1; MVZ 182347); 2 mi E Searles Station (n = 4; MVZ 145699–145702). **East** – San Bernardino Co., 5 mi ENE Yucca Valley (n = 1; MVZ 195957); Pisgah lave flows (n = 1; MVZ 195191); Halloran Spring (n = 1; MVZ 195190).

Perognathus l. bangsi (Palm Springs Pocket Mouse). **East** – Riverside Co., [1] 0.2 mi W Rancho Dos Palmas (n = 1; MVZ 195955), [2] Shavers Valley, 9 mi E Cactus City (n = 5; MVZ 195954); [3] Indio Hills, Pushawalla Canyon (n = 3; MVZ 184650). **West** – Riverside Co., [4] Snow Creek (n = 10; MVZ 184653–184661); [5] 0.5 mi W and 0.1 mi S Palm Spring Station (n = 1; MVZ 184651); [6] 0.4 mi E Dos Palmas Spring (n = 1; MVZ 184652); San Diego Co., [7] 3.3 mi S Borrego Springs (n = 2; 184663–184664); Imperial Co., [8] Crucifixion Thorn Reserve (n = 1; MVZ 184644).

Perognathus. l. brevinasus (Los Angeles Pocket Mouse). **East** – Riverside Co., [9] 1 mi E Cabezon (n = 6; MVZ 184645–184649). **West** – [10] Etiwanda Wash (n = 2; MVZ 182968–182969); [11] Temecula Creek, Pauba Valley (n = 1; MVZ 182967).

Perognathus l. internationalis (International Pocket Mouse). **North** – San Diego Co., [12] west side San Felipe Creek, 2.5 mi N S2 on Hwy 78 (n = 2; MVZ 182970–182971), San Felipe Valley, 3.9 mi S San Felipe (n = 1; MVZ 182972). **South** – [13] 6.7 mi S Jct Hwy S2 and S22 (n = 2; MVZ 182973–182974), 7.2 mi S Jct Hwy S2 and S22 (n = 1; MVZ 182975).

Perognathus l. pacificus (Pacific Pocket Mouse). **Extant samples**: Orange Co., [14] Dana Point Reserve (n = 27; MVZ 195949); San Diego Co., [15] San Mateo North (n = 5; no vouchers) and San Mateo South (n = 10; no vouchers), Camp Pendleton Marine Corps Base; [16] Oscar One, Camp Pendleton Marine Corps Base (n = 6; MVZ 195951–195952). **Historic samples**: Orange Co., [17] 0.5 mi NW El Segundo (n = 1; MVZ 74750); San Diego Co., [18] Oceanside (n = 6; MVZ 47101–47106); [19] mouth Tiajuana River (n = 5; MVZ 47312–47316).

Outgroups used in phylogenetic analyses: *Perognathus amplus pergracilis* (MVZ 149946 – Arizona: Mohave Co.; 10.2 mi NE Oatman). *Perognathus inornatus inornatus* (MVZ 182713 – California: Tulare Co., Pixley National Wildlife Refuge). *Perognathus inornatus neglectus* (MVZ 182836 – California: Contra Costa Co., Horse Valley, 4 mi S Antioch).

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