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Geographic genetic architecture of pocket gopher (*Thomomys bottae*) populations in Baja California, Mexico

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Abstract

Phylogenetic analyses of complete mitochondrial cytochrome *b* sequences support the monophyly of pocket gopher (*Thomomys bottae*) populations from the 1000 km length of the Baja California peninsula of Mexico, relative to other geographical segments of the species range in western North America. The Baja California peninsula is an area that encompasses considerable ecomorphological and infraspecific diversity within this pocket gopher species. However, detailed population analyses encompassing 35 localities distributed over the southern half of the peninsula reveal only trivial phylogeographical structure. Rather, most of the 72 unique 500-base pair haplotypes examined from 142 individuals is restricted to single populations, although a few haplotypes are shared broadly across geography. Individual populations are typically comprised of haplotype sets from different branches in a network of relationships. Analysis of molecular variance (AMOVA) indicates that approximately half of the total pool of variation is contained among individuals within local populations, and that only about 25% can be explained by the regional subdivisions of current subspecies distributions or physiographic realms. A hypothesized historical vicariant event that has been causally linked to the phylogeographical structure of other, codistributed species has had little influence on these pocket gopher populations, explaining only 13% of the total variation. The temporal depth, estimated by coalescence parameters, of the haplotype lineage in Baja California is relatively recent, approximately 300 000 generations; both the mismatch distribution of pairwise comparisons and a significantly positive exponential growth estimate support a recent history of expanding populations; but current, or recent past, migration estimates have remained small, are largely unidirectional from north to south, and weak isolation by distance is present. All data suggest that pocket gophers have relatively recently invaded the southern half of peninsular Baja California, with the genetic signature of expansion still evident but with sufficient time having lapsed to result in a weak isolation by distance pattern. The geographical assemblage of sampled populations thus appears as a meta-population, with limited gene flow contrasting with random haplotype loss due to drift in small, localized populations.

Keywords: AMOVA, coalescence, mtDNA, pocket gophers, population expansion, *Thomomys*

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Introduction

The degree to which species' populations are genetically structured over the geographical space that they occupy results from complex interactions among demographic attributes that influence local population sizes, ecological factors such as habitat breadth and patchiness and historical 'accidents' such as vicariant geological events. All other things being equal, one might expect those species that exhibit small effective population sizes due to low densities, asymmetrical mating, and/or limited dispersal to exhibit greater genetic structure than their codistributed counterparts with opposite demographic and population characteristics. Subterranean rodents, such as pocket gophers of the family Geomyidae, are one such group of mammalian

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species that typically exhibits strong population genetic structure (see review by Steinberg & Patton 2000). For these animals, strong structure is believed to result from the combination of relatively small and often physically isolated populations connected by limited dispersal, attributes which promote differentiation by genetic drift, and large geographical ranges encompassing a broad array of habitats, optimal conditions for divergent selection to maximize differences geographically. As a consequence, taxonomic diversity (as indexed by formally named taxa – species or subspecies) can be extremely high (e.g. the 213 subspecies recognized in the *Thomomys umbrinus* complex by Hall 1981), species boundaries are often difficult to delimit, and the establishment of phylogenetic relationships among lineages can be obfuscated by problems of lineage sorting and reticulation due to postdivergence hybridization (Patton & Smith 1994; Ruedi *et al*. 1997; Steinberg & Patton 2000).

We possess considerable detail regarding the geographical architecture of pocket gopher populations, primarily of the genus *Thomomys* and especially those of the *T. bottae* complex (subgenus *Megascapheus*). Much of this perspective is based on patterns of morphological and allozymic variation, including detailed geographical surveys as well as that of local populations (e.g. Patton & Feder 1981; Daly & Patton 1990; Patton & Smith 1990). The perspective provided by clonally inherited markers, such as mitochondrial DNA sequences, is more limited, as these markers have been applied mainly to broader phylogenetic questions rather than to the details of hierarchical population structure over limited geographical areas (Smith 1998; Wickliffe *et al*. 2004). Yet, because of the matrilineal inheritance of mtDNA and the relatively strong philopatry of female pocket gophers (Daly & Patton 1990), one might expect mitochondrial sequences to exhibit even more extensive degrees of geographical structure than the nuclear protein markers used to date.

Here we examine the fine-scale geographical relationships among populations of pocket gophers of the *Thomomys bottae* complex in the Baja California peninsula, with emphasis on those distributed over the southern half (the state of Baja California Sur). This latter region encompasses the ranges of seven subspecies (Patton 1999) that occupy habitats from xeric desert scrub to mesic thorn scrub and pine forest over an elevational gradient from sea level to above 2200 m (Rzedowski 1978). Populations of pocket gophers are patchy in their occurrence throughout the peninsula, although in areas with good soils that are now cultivated gophers are distributed considerably more densely and uniformly. We use the mtDNA cytochrome *b* (cyt-*b*) gene as a marker to examine hierarchical geographical population structure, in part because published data from elsewhere in the range of this species provide the appropriately broad phylogenetic and geographical context for the samples we examine.

We use phylogenetic analyses based on the entire cyt-*b* gene sequence (1143 bases, excluding the terminal stop codon) to establish the overall relationship of populations within peninsular Baja California to other geographical areas of the species range defined previously (Smith 1998; Wickliffe *et al*. 2004). We then examine the structure of local populations in a hierarchical analysis employing a population genetic perspective. We thus ask questions about the uniqueness of mitochondrial lineages within the peninsula and address the processes by which haplotype variation has become distributed within and among populations through this region. We suggest that findings related to this single geographical region will apply more broadly to other areas of the entire species complex, which ranges from southern Oregon to near Mexico City, and from the Pacific Ocean to the front range of the Rocky Mountains in western North America.

Materials and methods

Sampling

To determine the relationship of populations from Baja California relative to other geographical areas within the total range of *T. bottae*, we analysed 52 complete cyt-*b* gene sequences [1143 base pairs (bp), including the terminal stop codon]. These included representatives of each of the four geographical clades from the United States identified in previous analyses, as well as the single clade that linked the few samples then available from the length of the Baja California peninsula (Patton & Smith 1990; Smith 1998; Wickliffe *et al*. 2004). Our expanded analyses of the Baja California clade now include three individuals from extreme southern California (subspecies *T. b. nigricans*), two individuals each from the northern half of the peninsula in the state of Baja California (*T. b. xerophilus* from Valle de la Trinidad and *T. b. cactophilus* from El Rosarito) and 25 specimens from 23 populations sampled from throughout the southern half of the peninsula in the state of Baja California Sur. We use sequences of three species in the subgenus *Thomomys* (*T. mazama*, *T. monticola* and *T. talpoides*) as outgroups in the phylogenetic analyses, a legitimate choice as the taxonomy of these pocket gophers is unambiguous and noncontroversial (see Thaeler 1980; Patton & Smith 1981). Previously published sequences were obtained from GenBank, where we have also deposited the 23 complete cytochrome *b* sequences generated in this study (Accession nos provided in Table 1).

We then obtained a 500 bp fragment of this same gene for 142 specimens representing 35 populations to examine the pattern of hierarchical haplotype apportionment throughout the state of Baja California Sur. Sample size per population ranges from one to 11 individuals (modal number, 4). Included are representatives from each of the

Table 1 Localities sampled, sample size (N_{ind}), numbers of haplotypes (N_{hap}), haplotype diversity, nucleotide diversity and mean number of pairwise differences for sampled populations of *Thomomys bottae* from southern Baja California peninsula. Data are based on a 500 bp fragment of the cytochrome *b* gene. Localities are numbered consecutively, as in the map, Fig. 1. GenBank Accession nos for 23 complete cytochrome *b* sequences (1143 bp) representing most localities generated in this study are given

Locality (no.)	N_{ind}	N_{hap}	Gene diversity	Nucleotide diversity	Mean no. pairwise differences	GenBank Accession no.
San José del Cabo (1)	4	2	0.500	0.0110	5.50	AY589026
Santa Anita (2)	2	2	1.000	0.0180	9.00	AY589021
Migriño (3)	5	4	0.900	0.0112	5.60	AY589025
CaduaÒo (4)	4	1	0.000	—	—	AY589020
Miraflores (5)	3	3	1.000	0.0080	4.00	
Santiago (6)	4	3	0.830	0.0134	6.67	AY589018
Pescadero (7)	1	1	0.000	—	—	AY589019
Las Cuevas (8)	4	3	0.830	0.0063	3.17	
La Ribera (9)	5	3	0.700	0.0082	4.10	
Todos Santos (10)	6	4	0.800	0.0081	4.00	AY589038
La Laguna (11)	11	2	0.330	0.0052	2.62	AY589022
Los Bueyes (12)	2	2	1.000	0.0300	15.00	AY589032
El Carrizal (13)	3	3	1.000	0.0073	3.67	AY589023
El Triunfo (14)	3	2	0.670	0.0053	2.67	AY589024
Los Planes (15)	5	5	1.000	0.0172	8.60	AY589033
San Pedro (16)	2	2	1.000	0.0020	10.00	AY589037
La Paz (17)	7	6	0.950	0.0138	6.10	AY589017
Conejo (18)	3	2	0.670	0.0053	2.67	AY589035
El Cíen (19)	4	4	1.000	0.0173	8.67	AY589028
El Médano (20)	4	3	0.830	0.0060	3.00	AY589029
Punta Coyote (21)	1	1	0.000	—	—	AY589034
La Fortuna (22)	3	3	1.000	0.0187	9.33	AY589030
San Carlos (23)	5	4	0.900	0.0072	3.60	AY589036
San Buto (24)	5	1	0.000	—	—	
Magdalena (25)	2	2	1.000	0.0080	4.00	
Cd. Constitución (26)	8	6	1.000	0.0129	6.47	AY589027
La Presa (27)	3	2	0.670	0.0013	0.67	AY589031
López Mateos (28)	2	2	1.000	0.0220	11.00	
Insurgentes (29)	5	4	0.900	0.0100	4.40	
Pozo Grande (30)	5	4	0.900	0.0112	5.60	
Los Laureles (31)	7	4	0.710	0.0055	2.76	AY589039
Las Jarillas (32)	3	2	0.670	0.0040	2.00	
Emiliano Zapata (33)	4	3	0.830	0.0033	1.67	
Vizcaíno (34)	5	4	0.900	0.0112	5.60	
Guerrero Negro (35)	2	1	0.000	—	—	
Total	142	72	0.975	0.01672	8.3269	

described subspecies from this part of the peninsula (Patton 1999): 54 specimens of *T. b. anitae* from the Cape region, 11 *T. b. alticola* from the Sierra de La Laguna, 10 *T. b. imitabilis* from La Paz region, 32 *T. b. magdalenae* from the coastal plain south of Magdalena Bay, nine *T. b. litoris* from the coastal region north of Magdalena Bay, 15 *T. b. imcomptus* from the vicinity of La Purísima and 11 *T. b. russeolus* from the Vizcaíno Desert. The geographical placement of all samples available from the entire length of the peninsula are listed in Fig. 1 and listed in Table 1. All preserved specimens are cataloged in the Museum of Vertebrate Zoology (MVZ) or in the Centro de Investigaciones Biológicas del Noroeste (CIB).

Laboratory methods

Genomic DNA was extracted from liver tissue using either Chelex® (Walsh *et al*. 1991) or the DNAeasy Tissue Kit (Qiagen Inc.). We amplified the entire mitochondrial cytochrome *b* gene (1143 bp) in two phases: the first approximately 800 bp fragment, beginning with the ATG start codon, used primer pairs MVZ05/MVZ16, and the terminal but

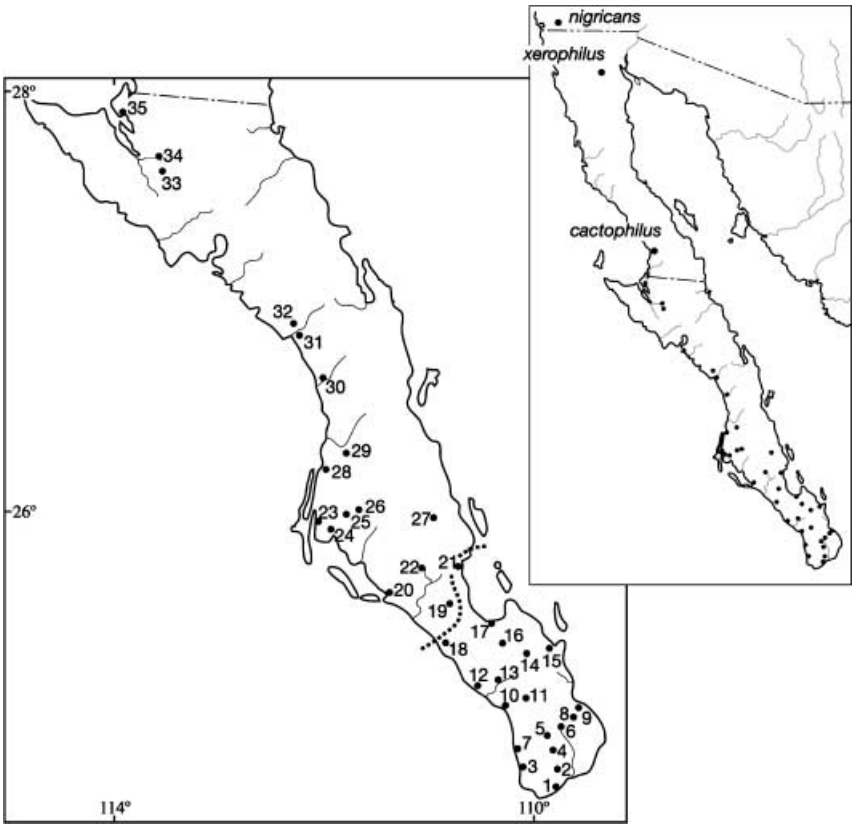

Fig. 1 Map of the southern half of the Baja California peninsula, Mexico, indicating the geographical placement of each of the 35 sampled localities, which are numbered as in Table 1 and Appendix I. Inset: geographical placement of the three samples from the northern half of the peninsula and southern California that are also part of the Baja clade of the *Thomomys bottae* complex (subspecies *nigricans*, *xerophilus* and *cactophilus*; see Figs 2 and 3). The dashed line indicates the general area where a seaway across the southern part of the peninsula has been hypothesized (see Grismer 1994; Riddle *et al*. 2000; Murphy & Aguirre-León 2002).

overlapping approximately 800 bp fragment used primer pairs MVZ45/MVZ14 (primer sequences given in Smith 1998). We used the following conditions for initial double-strand amplifications: 12.5 μL of template, 4.4 μL of ddH_2O, 2.5 μL of each primer (10 μM concentration), 0.474 μL of 0.4 μM dNTPs, 0.5 μL of 3 mM $MgCl_2$, 0.125 μL of *Taq* polymerase, and 1 × *Taq* buffer to a final volume of 25.5 μL. Amplification conditions consisted of 3 min of initial denaturation at 94 °C followed by 37 cycles of denaturation at 94 °C for 45 s, 1 min annealing at 50 °C, and 1 min extension at 72 °C. Double-strand DNA was cleaned using the QIAquick polymerase chain reaction (PCR) purification kit (Qiagen), and this template was cycle-sequenced with primers MVZ05 and MVZ67 using the dRhodamine dye terminator kit and run on an ABI 377 automated sequencer, following the manufacturer's protocol. Electropherograms were visualized and sequences aligned using the SEQUENCHER version 3.1 software (Gene Codes Corp., Ann Arbor, MI, USA). Primer sequences can be found in Smith & Patton (1993) and Patton *et al*. (1996).

Phylogenetic and population analyses

We identified redundant sequences in both data sets with MACCLADE 4.0 (Maddison & Maddison 2000). For the complete cyt-*b* data set, we then assessed the phylogenetic relationship of haplotypes and populations by maximum parsimony (MP) and estimated the strength of internal nodes by bootstrapping (1000 replicates), using PAUP* 4.0b10. We used the Kimura two-parameter distance (K2p) to provide a measure of genetic distance among clades comparable to that used previously (Smith 1998). The MP analysis weighted all characters equally and rooted the trees with sequences from three species of the subgenus *Thomomys* (*T. mazama*, *T. monticola* and *T. talpoides*) as outgroup taxa. We also included in this analysis representative sequences from each of the phylogeographical units of *T. bottae* identified by Smith (1998), namely: Northern California, Central California, Great Basin, Basin and Range, Baja California, and the unique subspecies *T. b. awahnee* from Yosemite Valley, CA.

For the 500 bp data set, we used ARLEQUIN 2.001 (Schneider *et al*. 2000) to calculate standard diversity indices (haplotype diversity, nucleotide diversity and mean pairwise divergence, based on a matrix of the number of pairwise differences) and to construct a minimum spanning network for the set of unique haplotypes by the method of molecular variance partitioning. We also used the analysis of molecular variance (AMOVA) routine in ARLEQUIN to determine the hierarchical apportionment of haplotypes among predefined geographical regions, within regions and within populations of each region. For the latter, we ran three

Table 2 Groupings of population samples (numbered, as in Table 1) in each of the three AMOVA analyses that examine the hierarchical apportionment of sequence divergence as a function of subspecies (following Patton 1999), physiographic unit (Alvarez-Castañeda *et al*. 1995), and the hypothesized vicariant La Paz seaway (Grismer 1994; Riddle *et al*. 2000; Murphy & Aguirre-León 2002). The right-hand column provides the regional sample groupings used in the analysis of migration rate estimates

Subspecies	Physiography	Vicariant model (La Paz seaway)	Migration rate groupings (north to south)
alticolus: 11	Región de los Cabos: 1–2, 4–6, 8–9	north: 18–20, 22–35	A: 33–35
anitae: 1–10, 12–15	Pacifico sur: 3, 7, 10, 12–13, 18	south: 1–17, 21	B: 30–32
imitabilis: 16–17, 21	Sierra, golfo sur: 11, 14–15	C: 28–29	
incomptus: 30–32	Cuenca de La Paz: 16–17, 21	D: 23–26	
litoris: 28–29	Pacifico medio: 20, 22–25, 28	E: 19–20, 22	
magdalenae: 18–20, 22–27	Centro base do la sierra: 19, 26–27, 29	F: 13–15, 17	
russeolus: 33–35	Pacifico norte: 30–32	G: 1, 3–6, 8–9	
	Vizcaíno desierto: 33–35		

separate analyses, each dividing population samples into different geographical groupings (Table 2) based on (1) their designated subspecies (following Patton 1999), (2) their location within each of the nine physiographic regions of the southern peninsula (Alvarez-Castañeda *et al*. 1995) and (3) the position of a hypothesized cross-peninsula seaway barrier through the La Paz region (position marked in the map, Fig. 1, as a dashed line; Grismer 1994; Riddle *et al*. 2000). We tested for the possibility of isolation-by-distance by comparing the natural logs of pairwise population estimates of F_{ST} (derived from ARLEQUIN) and geographical distance using the IBD 1.52 program (Bohonak 2002). We estimated migration rates ($M = 2mN_f$), using the Markov chain Monte Carlo coalescent approach in the program MIGRATE 1.7.3 (Beerli 2003; see Beerli & Felsenstein 2001), with both empirical transition–transversion (Ti–Tv) ratio and base frequencies. Otherwise, the default parameters of MIGRATE were in effect. Because of low sample sizes for most individual localities, we grouped adjacent localities into seven geographical units with approximately equal numbers of individuals and haplotypes (Table 2) that are distributed sequentially from north to south along the peninsula. These groupings are nearly identical to those based on physiographic regions. Finally, we examined the demographic history of populations and geographical units by constructing a distribution of pairwise differences, the 'mismatch' distribution, which aids in to distinguishing between populations that have been stable over time with those that have experienced recent expansion or reduction. This method is based on an assumed stepwise growth model only and does not consider alternatives (e.g. exponential or logistic; Rogers 1995; Polanski *et al*. 1998). We also used the program FLUCTUATE, version 1.3 (Kuhner *et al*. 1998) to generate joint estimates of the coalescent parameter $\Theta = 2\mu N_f$ (for mtDNA sequences) and an exponential growth parameter g (in units of μ^{-1}) for the combined set of 500 bp sequences. This analysis also uses a Markov chain Monte Carlo approach. We again used the empirical Ti–Tv ratio and base frequencies, with possible genealogies and model parameters sampled with 10 short chains (sampling increments of 10; 1000 steps per chain), 10 long chains (sampling increments of 10; 20 000 steps) and a random starting tree. We used a likelihood ratio test to determine the significance of the exponential growth rate g by comparing the log-likelihood of a model in which g was set to 0 (representing a stable population) and 1 where g was allowed to vary (following Rawson *et al*. 2003). We calculated the test statistic as twice the difference in log-likelihood scores for each model and compared this value to a χ^2 distribution with 1 degree of freedom.

Results and discussion

Phylogenetic relationships and phylogeographical structure

The strict consensus tree of the maximum parsimony analysis illustrates the relationships among the 55 complete cyt-*b* haplotypes examined here (Fig. 2). With the exception of two tritomys within the large group of sequences from Baja California, this tree is fully resolved and we illustrate it as an unrooted phylogram solely for visual simplicity. Because the purpose of our analysis was to determine only the relationships among, and phyletic placement of, samples from Baja California with respect to *T. bottae* throughout the remainder of its range, our inclusion of taxonomic and geographical 'outgroups' was limited to representatives of the five major lineages of this species that had been identified previously (Patton & Smith 1990; Smith 1998). As is evident in Fig. 2, this group of five geographical lineages of *T. bottae* is both a deeply divergent (mean K2p distance = 0.2337) and a strongly supported

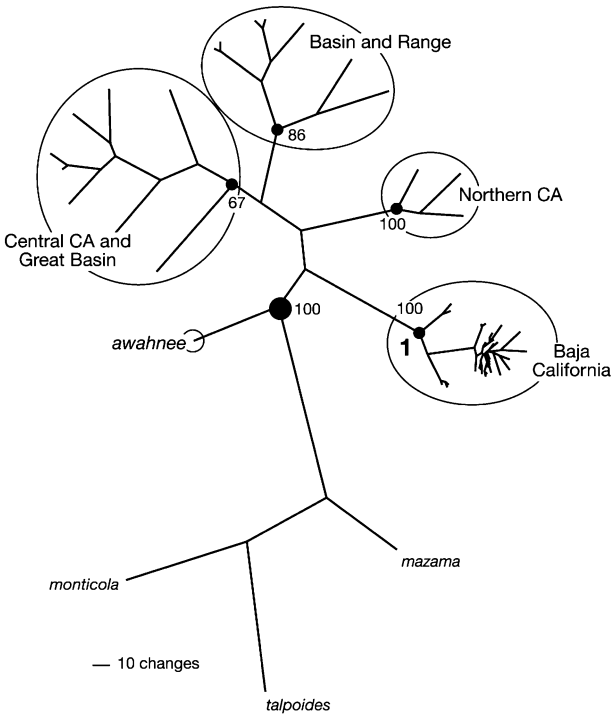

Fig. 2 Unrooted consensus maximum parsimony phylogram of 52 complete cytochrome *b* gene sequences representing each of five geographical segments of *Thomomys bottae* (subgenus *Megascapheus*) identified in previous publications (Patton & Smith 1990; Smith 1998) and sequences representing each of three species from the subgenus *Thomomys* (*mazama*, *monticola* and *talpoides*). Nodes supporting the monophyly of the clades within *T. bottae* are identified (solid circles); bootstrap values are provided for each node. The node connecting all sequences from the Baja California clade is identified by number (see Table 3). The analysis is based on 1143 characters, 418 of which are parsimony informative (633 are constant and 92 are parsimony uninformative). The consensus tree is derived from 9703 equal length shortest trees, each 2142 steps long; consistency index is 0.362.

monophyletic assemblage relative to the three outgroup species of the subgenus *Thomomys (*bootstrap support = 100), which is not surprising as the *bottae* complex is placed taxonomically in the separate subgenus *Megascapheus*. Furthermore, of the five major clades of *T. bottae* recovered, all but one has strong bootstrap support (86–100). These are also markedly divergent among themselves [mean 0.1684 ± 0.00046 (standard error) K2p distance], although the relationships among them are poorly resolved. These clades conform to the geographical genetic units defined by allozyme and morphological analyses (Patton & Smith 1990) as well as to the mtDNA lineages identified previously (Smith 1998; Wickliffe *et al*. 2004).

We are not concerned with the relationships among this complex of major clades of *T. bottae* depicted in Fig. 2, and thus we made no attempt to test alternative topologies. Rather, for the purposes of the present paper we are only interested in the relationships among the sequences from Baja California relative to this larger geographical and taxonomic group. Here, we note that all individuals from the peninsula form a strongly supported monophyletic lineage (bootstrap = 100) that is well differentiated from all other lineages within this complex of pocket gophers (mean of 0.1563 K2p distance; node 1 in Fig. 2 and Table 3). This group of sequences includes those from southernmost California (subspecies *nigricans* from San Diego Co.) and all those from the 1000 km length of the Baja California peninsula, from the border with the United States to the Cape region (Fig. 1).

Within the Baja California clade, the two samples from the extreme northern part of the range in northern Baja (*xerophilus*; node 2, Fig. 3) and adjacent United States (*nigricans*; node 3, Fig. 3) are both basal to and substantially different from all of those from the southern half of the peninsula (all samples from Baja California Sur plus the subspecies *cactophilus* from north of the Vizcaíno desert in the state of Baja California (Fig. 1) — bootstrap = 95; node 4, also marked by the arrow in Fig. 3). The Kimura two-parameter distance of these two lineages relative to those further south is 0.0496 and 0.0521 (Table 3), respectively, twice that between *cactophilus* and all samples from Baja California Sur (0.0272; Table 3) or among the latter samples (0.0176; Table 3). The relative phylogenetic uniqueness of the northern *xerophilus* and *nigricans* may identify true phylogeographical structure in the northern half of the peninsula, or it may simply reflect the extensive geographical

Table 3 Bootstrap values and average Kimura 2-parameter distances (± 1 SE) for successive nodes in the maximum parsimony trees (Figs 2 and 3)

Node	Bootstrap value	Mean K2p
1 [other *T. bottae* (all Baja California samples)], Fig. 2	100	0.15630 ± 0.00037
2 [*xerophilus* (other Baja samples)], Fig. 3	100	0.04961 ± 0.00055
3 [*nigricans* (southern Baja samples)], Fig. 3	56	0.05206 ± 0.00059
4 [*cactophilus* (Baja California Sur samples)], Fig. 3	95	0.02717 ± 0.00068
5 (all samples from Baja California Sur), Fig. 3	< 50	0.01757 ± 0.00041

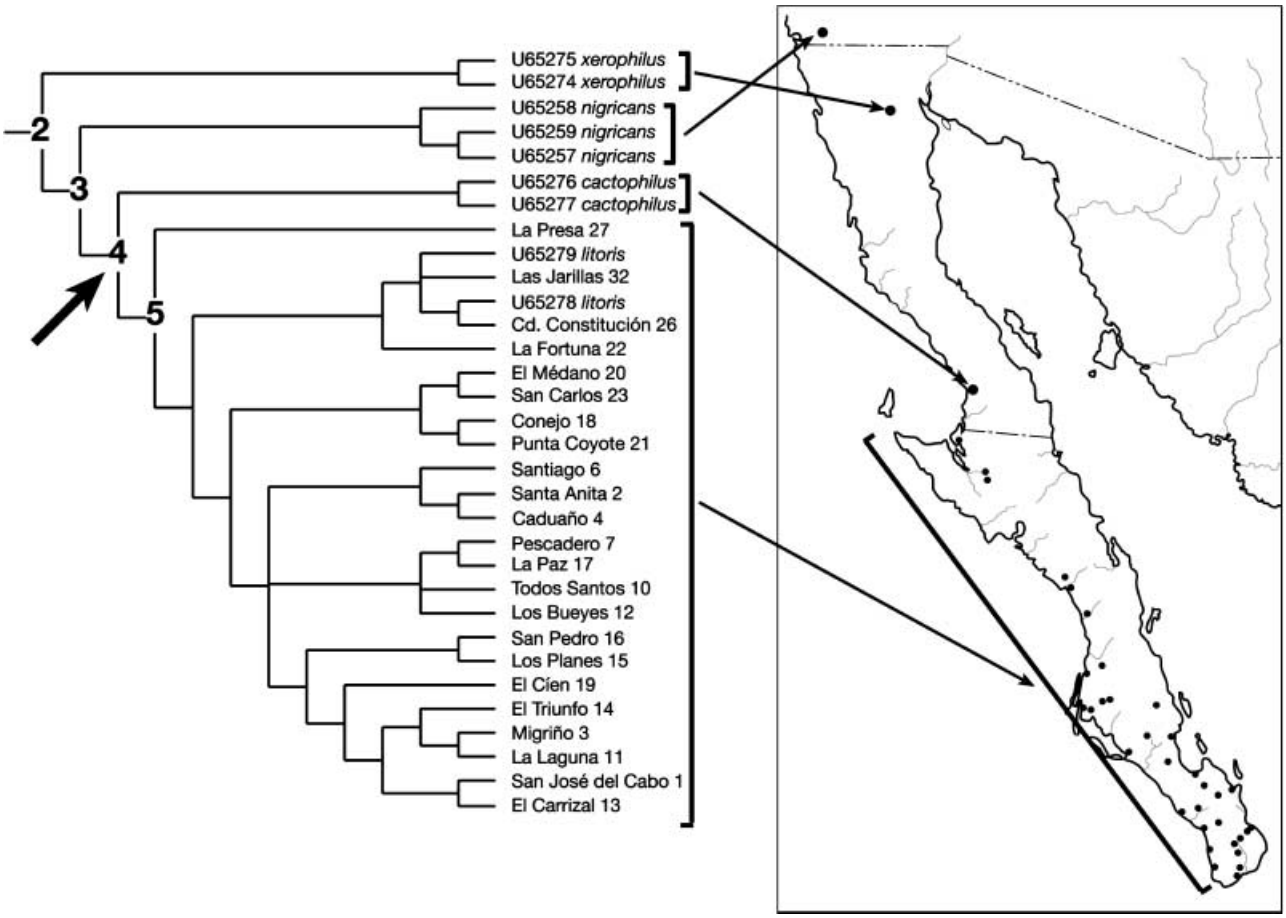

Fig. 3 Left: consensus maximum parsimony cladogram of all 1143 bp sequences of the Baja California peninsula clade, illustrating the monophyly of samples of *Thomomys bottae* from the southern half of the peninsula (node 4, arrow; bootstrap = 95). This tree is based on the same 55 sequences illustrated in Fig. 2 but pruned to include only those from the Baja California clade. Nodes are numbered as in Table 3. Those sequences obtained from GenBank are identified by Accession nos; sequences generated in this study are identified by locality name and number (see map, Fig. 1, and Table 1). Right: geographic placement of the three northern Baja–southern US samples and the single clade that represents all of the southern half of the peninsula.

gap in our sampling from those localities in the extreme north relative to those from the southern half of the peninsula. Adding samples from geographically intervening areas may yield therefore a pattern of isolation-by-distance along the entire length of the peninsula rather than the geographical structure apparent in the present geographically limited analysis (Fig. 3). Finally, there is a complete lack of any apparent phylogeographical structure among the entire set of samples from the southern half of the Baja peninsula. The 25 sequences from populations sampled in the state of Baja California Sur differ among themselves by an average of only 0.0176 K2p and collectively form a poorly resolved clade (bootstrap support < 50) relative to *cactophilus* from southern Baja California (Fig. 3; Table 3).

The overall hypothesis of phylogenetic relationship among members of the *bottae*-complex presented in Fig. 2 is completely concordant with the results of both Smith (1998) and Wickliffe *et al*. (2004), studies that emphasized other geographical regions within the complex but which included a few samples from Baja California. Both these studies identified a well-differentiated lineage that is distributed along the peninsula and that extends to extreme southern California, although the samples available in those two studies were much more limited in number and geographical extent than ours. It thus seems clear that all populations of pocket gophers within the Baja California peninsula share a more recent common ancestor among themselves than the lineage as a whole does with other geographical and/or taxonomic segments of the *T. bottae* species complex. Understanding the deeper vicariant and/or population history of pocket gophers within Baja California relative to those to the north and east in the United

States remains an area of important research. This pattern of limited to no phylogeographical structure along the Baja California peninsula is in sharp contrast to that of many codistributed species of vertebrates where substantial phylogeographical depth as well as well supported regional reciprocally monophyletic clades are present (summarized in Riddle *et al*. 2000; Murphy & Aguirre-León 2002). The lack of phylogeographical structure in these pocket gophers is all the more surprising given their taxonomic diversity over this range (27 subspecies; summarized in Patton 1999) and propensity elsewhere in the species range for extensive degrees of local geographical differentiation of high order (Patton & Smith 1990).

Haplotype diversity, genealogy and population history

Because of the limited overall sequence divergence (mean 0.01757 ± 0.00041 se K2p distance) and the general lack of phylogenetic resolution among the 25 unique haplotypes from the southern half of the peninsula (excluding cactophilus; node 4 in Fig. 3), as well as their association into a single monophyletic clade, we expanded our samples from each locality and used population genetic methods to examine the potential processes that may underlie the patterns of molecular diversification across this large region. These analyses used a truncated 500 bp fragment for each of the 142 individuals that we collected at 35 different localities (Fig. 1). Sample size varied from a single individual [Pescadero (locality 7) and Punta Coyote (21)] to as many as 11 per locality [Sierra de La Laguna (11)], with a mode of four specimens from each population (Table 1).

There are 72 unique haplotypes among the 142 individuals sampled; their genealogical relationships are estimated in the minimum spanning network illustrated in Fig. 4. Most haplotypes (56 of 72; 78%) are apparently limited to single populations. Twelve of the 16 haplotypes found at more than one locality are present in only two populations (boxes in Fig. 4); the remaining multiply distributed haplotypes are present in three (one haplotype; shaded box B in Fig. 4), four (two haplotypes; shaded hexagons C and D in Fig. 4), and eight populations (one haplotype; shaded circle A, Fig. 4), respectively. The majority (10 or 16) of these multiply distributed haplotypes occur in geographically adjacent localities and are found either at the tips or only one step away from a tip in the network (Fig. 4). However, the most common haplotype (A) was present at eight localities that span nearly the entire sampled distribution [from the Vizcaíno desert (locality 34) in the north to Todos Santos (10) on the southwest coast (see map, Fig. 1)]. Localities that share haplotypes, even if geographically nearby, do not group readily by habitat or physiographic province. For example, three of these shared haplotypes are found in the middle portion of the sampled range, from the agricultural fields at Ciudad Insurgentes (29) and Pozo Grande (30) to coastal scrub at Los Laureles (31) and Las Jarillas (32). Another is found at high elevation in the Sierra de La Laguna (11) as well as near the southern coast at Santa Anita (2) and Santiago (6).

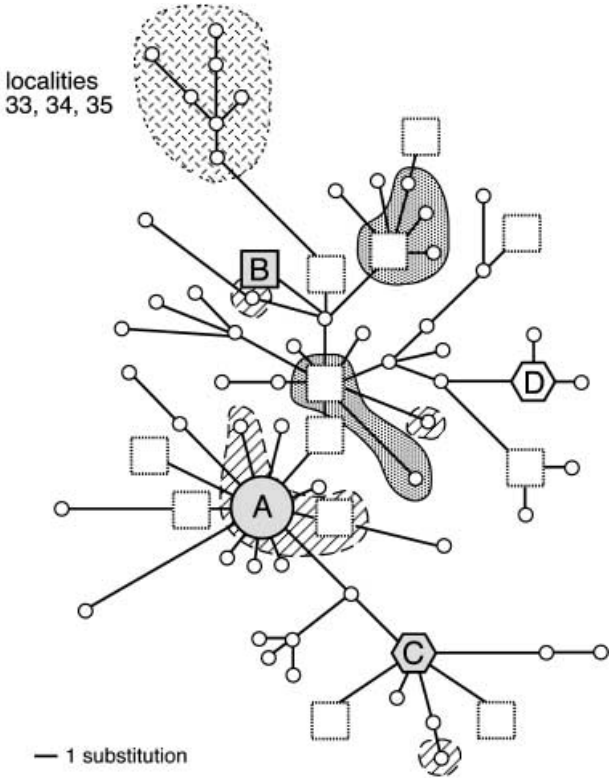

Fig. 4 Haplotype network of 72 unique haplotypes recovered from the 500 bp data set that included all sampled populations of *Thomomys bottae* from the Vizcaíno desert to the Cape (Fig. 1). Lines connecting haplotypes are scaled to the number of base substitution differences. Individual haplotypes found in more than a single population are enclosed in a symbol: dotted box = 2 populations; solid box (haplotype B) = three populations; hexagon (haplotypes C and D) = four populations; circle (haplotype A) = eight populations. Unrelated haplotypes that occur within single populations include those from La Paz (locality 17, stippled clusters that identify the six haplotypes recovered from seven individuals sampled, Table 1) and Cd. Constitución (locality 26, diagonal hatching clusters of six haplotypes among the six individuals sampled). The cluster of haplotypes that occur collectively in the northwestern sampled region in the Vizcaíno desert (localities 33–35) is cross-hatched.

Although most haplotypes were found only at single localities, individual population samples do contain proportionately large numbers of haplotypes (average haplotype diversity is 0.776, with only three of 35 localities apparently fixed for a single haplotype; Table 1). More interestingly, haplotypes that co-occur in single populations are not highly similar, as there is an average of 5.23 mutational steps among haplotypes contained within single polymorphic

populations. Populations with the most divergent haplotypes are those at Los Bueyes (locality 12; 15 mutational steps between the two haplotypes recovered, 18 steps in the network, Fig. 4), Lopez Mateos (locality 28; 11 mutational steps, 15 steps in the network) and San Pedro (locality 16, 10 mutational steps, 12 steps in the network). The two localities with both the largest sample sizes of individuals and haplotypes [Table 1; Cd. Constitución (locality 26) and La Paz (locality 17)] are both comprised of genealogically unrelated sets of haplotypes (Fig. 4; diagonal hatching and stippling, respectively). To put these numbers in perspective, the maximum number of mutations separating any pair of the 72 unique haplotypes is 21, with the mean number 8.3 (Table 1). Haplotypes present within the average population thus differ by 63% of the total level of differentiation found among all haplotypes from throughout the sampled range. Only the cluster of three populations at the northwestern region of our sampling [Emiliano Zapata (locality 33), Guerrero Negro (34) and Vizcaino (35)] share a common set of haplotypes that form a genealogical lineage (Fig. 4, cross-hatching).

The general pattern of haplotype distribution is thus one of the relative uniqueness of individual populations with few sharing the same haplotypes. Where sharing does occur, it is limited largely to sets of rather nearby localities. Nevertheless, populations maintain a reasonable level of haplotype polymorphism, although these sets of co-occurring haplotypes are typically not genealogically close. This pattern is also readily apparent in the three separate analyses of molecular variance that we performed. Here, we portioned the sampled populations into three groups (Table 2): (1) by their subspecies (following the mapped ranges in Patton 1999), (2) by the physiographic province of Baja California Sur (Alvarez-Castañeda *et al*. 1995) within which each locality is found and (3) by the hypothesized cross-isthmian seaway in the general vicinity of La Paz that has been identified in the phylogeographical history of other vertebrates (Grismer 1994; Riddle *et al*. 2000; Murphy & Aguirre-León 2002). Each of these analyses was undertaken to determine the proportion of the total pool of variation among the 72 unique haplotypes that could be explained by these predetermined hierarchical relationships among their member populations.

The combination of among-populations within groups, or within-population components explained more than 70% of the total pool of variation (Table 4). Importantly, regardless of the hierarchical grouping of samples, the largest component of variation is found among individuals within populations (~50% in each case). The 'subspecies' and 'physiographic' effects were substantial and nearly the same, explaining 25–29% of the variation, but a vicariance model of a cross-peninsula seaway explains only 13% of the total variance. The similarity in partitioning pattern between the subspecies and physiographic designations is not surprising, as the two sets of geographical subdivisions are similar (Alvarez-Castañeda *et al*. 1995; Patton 1999), and the different subspecies, defined mainly on the basis of overall body size and pelage colouration, directly reflect the habitat in which each occurs (Smith & Patton 1988; Patton & Smith 1990).

Much of the apparent among-groups structure at both the subspecies and physiographic levels is due, however, to the inclusion in our analyses of the phylogenetically unified samples from the Vizcaíno desert (Fig. 4). If, for example, the AMOVA analyses exclude those samples, the 'subspecies' and 'physiographic' effects decrease by nearly half, to 14.6% and 17.6%, respectively, with a corresponding increase in the within-population component. The lack of a substantial relationship between haplotype partitioning and the hypothesized historical vicariance event of a trans-isthmian seaway does not refute the existence of such a seaway. Rather, our data suggest only that, if this seaway did exist at some time in the past, it was unimportant in the diversification of pocket gophers in the southern part of the peninsula.

Most variation, regardless of how the total pool of sampled localities is hierarchically organized, is therefore found at the individual level within populations, supplemented by a limited geographical influence of clustered samples. This result is wholly consistent with the lack of nearly any genealogical hierarchy of the haplotypes recovered (Fig. 3) and the lack of any geographical pattern to the distribution of individual haplotypes (Fig. 4). It is also consistent with the pattern of distribution of pairwise differences between all haplotypes (the 'mismatch distribution' of Rogers & Harpending 1992), which is presented

Table 4 Proportion of variance in haplotype diversity explained at each of three hierarchical geographical levels, for each of three 'treatments' of localities: grouped by the seven subspecies (following Patton 1999), by the eight physiographic regions (Alvarez-Castañeda *et al*. 1995), or by north and south of the presumptive trans-peninsula seaway through the La Paz basin (Grismer 1994; Riddle *et al*. 2000). Each level is significantly different from zero, by permutation tests, in all three analyses

Hierarchical level	Subspecies	Physiography	Vicariance model
Among groups	26.54	28.53	13.36
Among populations within groups	24.12	20.78	37.92
Within populations	49.34	50.69	48.72

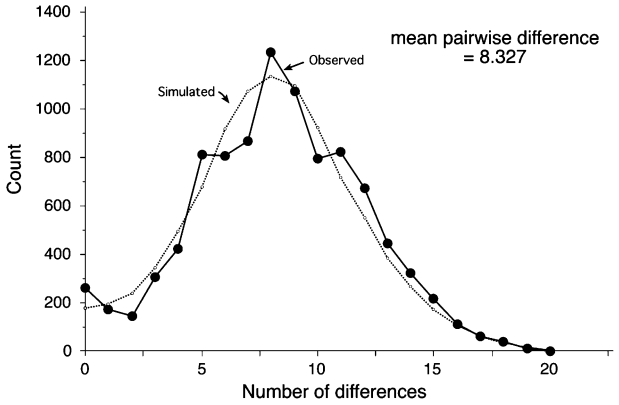

Fig. 5 Mismatch distribution, the frequency of pairwise differences between all 72 unique 500 bp haplotypes recovered from all populations sampled. The solid circles represent the empirical distribution; open circles represent the expected distribution, assuming a stepwise population expansion following a Poisson distribution.

visually in Fig. 5. This empirical distribution is clearly unimodal and is statistically identical with that produced from an expanding population based on an expected Poisson distribution (the probability that the variance of the simulated distribution is greater than the empirical one is 0.5899). The close fit between empirical and simulated distributions suggests that the group of sampled populations over the southern half of the Baja California peninsula has experienced an expansion over the coalescence time of their included haplotypes. The results from the coalescence-based analysis of the program FLUCTUATE provide further support for population expansion. Here, the null hypothesis of a stable population was rejected and the estimated population growth rate, based on all haplotypes considered as a single population, was high [g = 515.88, 20.438 standard deviation (SD); ln(L) values for the 0 growth model = 0.02284 and for the exponential growth model = 2.7677; $\chi^2 = 5.4897$, $P < 0.05$ with 1 d.f.].

Both the mismatch distribution and population expansion analysis based on the growth parameter, g, are consistent with the general lack of phylogeographical structure (Fig. 3), the overall low level of sequence divergence, with the few mutational steps that are necessary to relate all haplotypes in the network (Fig. 4), and estimates of Θ_0 (population size before the expansion) and Θ_1 (the population size following expansion). These parameters were estimated as 1.374 (95% confidence limits, 0.000, 4.698) and 51.189 (24.950, 3959.939), respectively, with the large difference between them indicative of a significant increase in effective population size during their coalescence history (Schneider & Excoffier 1999).

The time period over which this expansion has taken place can be estimated from the formula $\tau = 2ut$, where u is the mutation rate for the entire sequence being analysed and t is generation time, based on the stepwise growth model and a nonlinear least-squares approach implemented in ARLEQUIN (Schneider *et al*. 2000). Using the estimate of the mutation rate for the cyt-*b* gene of 1.3×10^{-5} (Zheng *et al*. 2003) and the empirical estimate of τ for our data (7.825, 95% confidence limits, 5.634 and 12.000), the approximate coalescence time is 300 000 generations (range between 217 000 and 462 000). As the average generation time for *T. bottae* pocket gophers is somewhat more than 1 year (Daly & Patton 1990), the initiation of population expansion throughout the southern half of the Baja California peninsula is estimated to have occurred during the last quarter of the Pleistocene.

Migration among populations

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. As a consequence, we provide the migration rates estimated from MIGRATE as only a generalized approximation, and do not present the profile confidence limits provided by this program because of the recent concerns documented by Abdo *et al*. (2004).

Measured migration rates are overall quite small, with nearly half [19 of the 42 reciprocal paired comparisons (8 north to south and 11 south to north)] effectively zero (Table 5). This is particularly true for the samples from the Vizcaino Desert (Region A; localities 33–35) for which a migration connection to Region B to the immediate south is essentially nonexistent (A→B, $M = 3.30 \times 10^{-10}$; B→A, $M = 4.04 \times 10^{-13}$). This cluster of populations is comprised primarily of a phylogenetically unique set of haplotypes (Fig. 4) but also retains haplotype A, which is distrtibuted widely throughout the southern half of the peninsula. Otherwise, migration estimates between geographically adjacent regional groups, where values are sufficiently high to suggest effective genetic communication, are consistently asymmetrical and mostly biased in a north to south direction. Measureable migration rates are always present between all successive north to south pairs of grouped samples from Region B to Region G, and higher than the reciprocal direction for regional pairs C→D (M = 151.39 vs. 1.6×10^{-9}), D→E (M = 50.92 vs. 1.61×10^{-6}), and E→F (M = 347.78 vs. 88.25). Alternatively, south to north migration estimates are only measureably present between three pairs of areas (G→F, F→E, and C→B; Table 5). Migration among these populations thus appears to be relatively limited but largely directional, from north to south. Evidence for a north to south direction of movement is consistent with the

Table 5 Maximum likelihood estimates of migration rates among geographical samples of *Thomomys bottae* populations in Baja California Sur. Individual localities have been grouped into seven regional units by proximity, and are ordered generally from north to south (see text and Table 2). The reciprocal migration estimates between adjacent regions are given from north to south (above the diagonal) and south to north (below the diagonal)

Region	A	B	C	D	E	F	G
A	—	3.30×10^{-10}	1.34×10^{-9}	8.18×10^{-9}	9.52×10^{-14}	1.02×10^{-8}	2.36×10^{-71}
B	4.04×10^{-13}	—	70.5215	315.7310	5.43×10^{-6}	310.1408	5.40×10^{-12}
C	8.25×10^{-8}	261.7008	—	151.3906	6.20×10^{-6}	80.0170	39.1660
D	9.36×10^{-14}	9.40×10^{-14}	1.60×10^{-9}	—	50.9231	1179.3898	1114.8895
E	4.87×10^{-9}	119.0358	313.8393	1.61×10^{-6}	—	347.7755	173.4052
F	9.36×10^{-14}	262.4372	1093.2066	178.8674	88.2496	—	35.6124
G	6.29×10^{-9}	437.6685	1.38×10^{-5}	3.40×10^{-6}	260.3028	1115.6069	—

probable direction of historical colonization of the entire peninsula by pocket gophers, based on both the limited phylogenetic analyses presented here, where the basal groups are in the north (Fig. 3), as well as by the overall distribution pattern of the species (Patton & Smith 1990). The limited measureable migration rates are also fully consistent with both the presence of unique, 'private' haplotypes in so many sampled populations and the observation that individual populations are often collections of genealogically unrelated assemblages of haplotypes.

There is also a small, but significant, relationship between the genetic distance (as measured by F_{ST} values) and the geographical distance between pairs of individual locality samples, based on Mantel tests [$r = 0.416$, $P < 0.001$; 95% confidence intervals for both the slope (1.595, 2.802) and R^2 (0.084, 0.301) exclude zero]. This suggests slight isolation by distance over the sampled range in the southern half of the peninsula is consistent with the migration estimates of asymmetrical, directional movement.

The genealogy and geography of differentiation in pocket gophers

Haplotype distribution among populations of pocket gophers along the southern half of the peninsula of Baja California is unrelated to morphological differentiation among these same populations (as judged by current subspecific taxonomy) or to habitat occupied. It also shows no relationship to a hypothesized historical vicariant event that has been argued to have affected other codistributed vertebrates. Rather, most populations contain unique but almost genealogically random assemblages of haplotypes that are collectively present throughout the large sampled area. Migration estimates among localities are limited, but where measureable are directional from north to south, a pattern reflected in weak isolation by distance along the > 600 km sample distribution. Moreover, this collective set of populations appears to have undergone continuous expansion since the coalescent event that links all haplotypes, as suggested by the structure of the haploytype network (Fig. 4), the unimodal mismatch distribution (Fig. 5), and measures of exponential growth.

We believe that this collective set of observations is best explained by a combination of a historically rapid spread of pocket gophers throughout the region sampled following their initial entry to the area and a subsequent geographical structure of populations within a metapopulation model. We suggest that population sorting events, given the characteristically small size of local populations of pocket gophers, with periodic contact between neighbouring populations adding both old and new haplotypes, followed the rapid initial expansion. Ultimately, these populations of pocket gophers are in quasi-equilibrium between haplotype extinction by drift due to small actual numbers of individuals breeding at any one time offset by occasional migration. This general model has been argued to characterize pocket gopher populations based on both allozyme genetic markers and actual dispersal studies (Steinberg & Patton 2000). While data are limited, in one long-term study of this species in central California, for example, females were highly philopatric, an observation fully consistent with the low levels of female gene flow measured by the mitochondrial cyt-*b* gene here, but males moved much greater distances (Daly & Patton 1990). Importantly, movement of either sex into established populations is exceedingly limited on an annual basis (i.e. annual recruitment from outside the natal population is low), but dispersal into vacant habitat (created by local extirpation) was both rapid and successful for both sexes and for all age classes, adults and young of the year. Finally, geographical patterns of allozyme alleles examined by spatial autocorrelation and hierarchical *F*-statistical analyses (Patton & Smith 1990) suggested that populations might be linked by limited gene flow for linear distances up to 400 km, beyond which populations become effectively genetically independent. Both sets of observations support our view

of the history of pocket gopher populations in southern Baja California: an initial relatively recent but rapid colonization throughout this region followed by repeated random haplotype loss by drift and gain by occasional migration events. Haplotypes present in populations over large portions of the sample distribution (e.g. haplotype A, Fig. 4) are probably shared by ancestry, while those shared between neighbouring populations could result from occasional contact. A coalescence history of 300 000 generations would surely have left most populations with genealogically related and unique sets of haplotypes in the absence of some continued connectivity among them following founding.

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Appendix I

Locality designation (listed numerically as in the map, Fig. 1), sample size, and provenance, with catalogue numbers of voucher specimens, for each individual analysed in this study; * = specimens for which complete cyt-*b* sequences were obtained. Museum repositories are the Centro de Investigaciones Biologicas, La Paz, Baja California Sur, Mexico (CIB) and Museum of Vertebrate Zoology, University of California Berkeley (MVZ)

1 San José del Cabo (4): 10 km N San José del Cabo (CIB 6654*, 6656, 6658, 6661).
2 Santa Anita (2): Santa Anita (CIB 6219, 6220*).
3 Migriño (5): 6 km SE Migriño (CIB 6638, 6639*–6641, 6644).
4 Caduaño (4): Caduaño (CIB 6215*–6218).
5 Miraflores (3): 'Internado' Miraflores (CIB 5304–5306).
6 Santiago (4): Rancho 'La Misiôn', Santiago (CIB 5295–5297*, 5298).
7 Pescadero (1); 3 km S Pescadero (CIB 6214*).
8 Las Cuevas (4): Las Cuevas, 6 km S & 8 km W La Rivera (CIB 5299, 5301–5303).
9 La Ribera (5): La Ribera (CIB 6632–6636).
10 Todos Santos (6): Todos Santos (CIB 5480–5483*–5485).
11 Sierra de la Laguna (11): Valle de la Laguna, Sierra de la Laguna (CIB 6514–6516*, 6517, 6524–6529, 6537).
12 Los Bueyes (2): Los Bueyes, 5 km E Ej. Meliton Albañez, 60 km SW La Paz (CIB 8366*); 'Los Bueyes', 5 km E Ejido Melitón Albañes (CIB 7880).
13 El Carrizal (3): El Carrizal, 39 km S & 3 km E La Paz (CIB 7622, 7623), Tres Pachitas, 14 km S & 12 km E La Paz (CIB 6548*).
14 El Triunfo (3): El Triunfo (CIB 6620*, 6624, 6626).
15 Los Planes (5): La Pimientilla, 2.5 km S Los Planes (CIB 7645–7649*).
16 San Pedro (2): San Pedro (CIB 7864*, 7865).
17 La Paz (7): La Paz (CIB 5478, 5479, 5868, 5869, 7844); Chametla, 10 km W La Paz (CIB 126); El Centenario, 14.5 km W La Paz (CIB 6665*).
18 Punta Conejo (3): Punta Conejo (CIB 7837, 7838); Ley Federal Agraria, 20 km NW La Paz (CIB 8359*).
19 El Cíen (4): 18 km S, 9 km E El Cíen (CIB 6198*–6201).
20 El Médano (4): El Médano, 6 km S & 3 km W Santa Rita (CIB 6686, 6687*–6689).
21 Punta Coyote (1): Rancho El Potrero, km 36 carretera a Punta Coyote (CIB 7608*).
22 La Fortuna (3): La Fortuna, 11 km N, 28 km E Santa Rita (CIB 6694*–6696).
23 San Carlos (5): Puerto San Carlos (CIB 6158, 6159*); 1 km S * 4 km E Puerto San Carlos (CIB 6164, 6165); 3 km E Puerto San Carlos (CIB 6163).
24 San Buto (4): San Buto, 2 km S & 4 km E San Carlos (CIB 7867–7868, 7871–7872, 7874).
25 Magdalena (2); 3 mi W Ciudad Constitución; (MVZ 153727–153728).
26 Ciudad Constitución (8): Ciudad Constitución (CIB 6167*–6170); 3 mi W Ciudad Constitución (U65278*-U65279*); 30 km S & 14 km W Ciudad Constitución (CIB 6196–6197).
27 La Presa (3): La Presa, 40 km N & 4.5 km E Santa Rita (CIB 6691, 6692*, 6693).
28 López Mateos (2): 5 km N & 16 km E Puerto López (CIB 6154–6155).
29 Ciudad Insurgentes (5): 19 km N & 9.25 km W, Ciudad Insurgentes (CIB 7670–7673); 8.5 km N, 5.5 km W Ciudad Insurgentes (CIB7677).
30 Poza Grande (5): Francisco Villa, 9 km N La Poza Grande (CIB 6670, 6671, 6673 6676); 2 km N & 5 km W La Poza Grande (CIB 6682).
31 Los Laureles (5): Los Laureles, 16.5 km S & 7.5 km W San Isidro (CIB 7657–7660, 7680*).
32 Las Jarillas (3): Las Jarillas, 13.2 km S & 13 km W San Isidro (CIB 7654, 7678–7679).
33 Emiliano Zapata (4); Ejido Emiliano Zapata, 11 km S & 6 km E El Vizcaíno (CIB 7823–7826).
34 Vizcaíno (5): Rancho Valladarez, 9 km S & 17 km W Vizcaíno (CIB 7800–7804).
35 Guerrero Negro (2): Guerrero Negro (CIB 7721, 7723).

The following three samples are from the northern state of Baja California and southern San Diego Co., USA and were obtained from GenBank (Fig. 1; see Smith 1998).
T. b. nigricans (U65257–U65259): Julian, San Diego Co., CA
T. b. cactophilus (U65276, U65277): 4.5 mi S & 14.0 mi W El Rosarito, BC, Mexico
T. b. xerophilus (U65274, U65275): 6 km S & 17 km E Valle de la Trinidad, BC, Mexico

Samples representing other subspecies of *Thomomys bottae* obtained from GenBank, with Accession nos and localities (from Smith 1998):
T. b. perpallidus (U65261) California, San Bernardino Co., Harvard
T. b. concisor (U65264) Nevada, Lander Co., Monitor Valley
T. b. bottae (U65256) California, Monterey Co., Hastings Reservation
T. b. operarius (U65262) California, Inyo Co., Keeler
T. b. canus (U65265) Nevada, Washoe Co., Deep Hole
T. b. bottae (U65252) California, Inyo Co., Coso Junction Ranch
T. b. pascalis (U65255) California, Tulare Co., 6 mi S Kingsburg
T. b. centralis (U65266) Utah, Millard Co., Skull Rock Pass
T. b. mewa (U65254) California, Fresno Co., 0.6 mi NW Academy
T. b. connectens (U65268) New Mexico, Socorro Co., La Joya

T. b. ruidosae (U65261) New Mexico Lincoln Co., Bonita Lake
T. b. grahamensis (U65261) Arizona, Graham Co., Graham Mountains
T. b. fulvus (U652271) Arizona, Yavapai Co., Wolf Creek, Bradshaw Mountains
T. b. analogus (U65273) Mexico, Coahuila, 1 mi N Bella Union
T. b. albatus (U65260) California, Imperial Co. 3 mi E Holtville
T. b. cervinus (U65267) Arizona, Maricopa Co., 5.0 mi W & 3.0 mi N Gila Bend
T. b. fulvus (U65269) New Mexico, Grant Co., Iron Creek
T. b. awahnee (U65251) California, Mariposa Co., Yosemite Valley
T. b. leucodon (U652490) California, Modoc Co., 3.5 mi SW Adin
T. b. saxatilis (U65250) California, Lassen Co., 4 mi SW Susanville
T. b. laticeps (U65247) California, Humboldt Co., Rio Dell