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ENVIRONMENTAL RESPONSES TO ALTITUDINAL GRADIENTS AND SUBSPECIFIC VALIDITY IN POCKET GOPHERS (*THOMOMYS BOTTAE*) FROM BAJA CALIFORNIA SUR, MEXICO

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Dorsal pelage color, morphometry of cranial characteristics, and sequences of 500 base pairs from mitochondrial cytochrome-*b* gene of pocket gophers (*Thomomys bottae*) from the Cape region in Baja California Sur were used to evaluate how the environment influences morphologic and genetic structure in populations from habitats with altitudinal segregation. Gophers from temperate forest, tropical deciduous forest, and desert scrub were sampled. Among 80 individuals examined, 34 haplotypes were found. Specimens collected in each habitat were not monophyletic. Analysis of molecular variance indicated that more than one-half of the total pool of genetic variation was contained among individuals within local populations and that only 0.6% could be explained by the different habitats; there was no genetic structure and the populations were genetically similar. Individuals in the temperate forest were larger, darker, and more diverse in coloration; individuals from tropical deciduous forest were smaller; and those from desert scrub had lighter coloration. There were no differences among populations in cranial shape. Only the dorsal coloration of the specimens from the temperate forest matched the color of moist soil. There were no diagnostic characteristics to recognize *T. b. alticolus*, the population restricted to high-elevation temperate forests in La Sierra de La Laguna, as a valid subspecies; we consider it a junior synonym of *T. b. anitae*. The range of this subspecies is from the San Ignacio Lagoon to the southern end of the Baja California peninsula.

Key words: environmental differences, genetic homogeneity, Rodentia, subspecies, *Thomomys bottae*

Pocket gophers, fossorial rodents of the family Geomyidae, are mammals with extensive phenotypic and genetic variability (Smith 1998). For Botta's pocket gopher (*Thomomys bottae*), 195 subspecies have been recognized based mainly on morphologic characteristics such as body size and pelage coloration (Jones and Baxter 2004; Patton 1993; Wilson and Ruff 1999). The degree of differentiation among populations of pocket gophers is a reflection of diverse interacting factors including limited dispersal ability because of their subterranean habits, patchy distribution that depends on appropriate soils for burrow systems, and the large variety of habitats where they occur (Patton 1999; Patton and Smith 1989).

The capacity of pocket gophers to develop different morphotypes as a response to local ecological conditions (Davis 1938; Hadly 1997; Ingles 1950; Smith and Patton 1984) has been associated with the nutritional quality of their habitats. Morphological variation among populations of *T. bottae* that are genetically homogeneous shows that individuals that inhabit alfalfa fields are significantly larger that those that occupy areas with scarce food or poor-quality food (Patton and Brylski 1987; Smith and Patton 1988). Patton and Brylski (1987) state that it is necessary to examine cases of morphologic differentiation to determine the relationship among the phenotypic responses to different environments, because plastic responses to the environment can strongly influence divergence among local populations. On the other hand, natural selection, even in the presence of gene flow between populations, can favor morphological divergence greater than that found in populations isolated for millions of years in similar selective environments. In some cases, more divergence occurs between species within the same genus than between genera (Smith et al. 2001).

In several studies, pelage color of pocket gophers was related to moist soil color of recently excavated burrows, seemingly because the pelage acts as camouflage against predators (Ingles 1950; Kennerly 1954; Krupa and Geluso 2000; Patton 1973). Pelage color has been a critical characteristic to differentiate populations and has been the basis for taxonomy at the

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intraspecific level (Patton and Smith 1990). Descriptions of subspecies based on the morphology of a few specimens, which in many cases are subadults, have overestimated the number of differentiated populations. Such is the case for populations of *T. bottae* in California, in which Patton and Smith (1990) reduced the number of subspecies from 45 to 15 based on analyses of middorsal coloration, cranial morphology, and genetic variability (electromorphic and karyotypic). It has been suggested that subspecies of *T. bottae* must minimally exhibit cohesion in genetic characters and must maximally have cohesion in non–size-related cranial shape features (Patton and Smith 1990).

Two subspecies of *T. bottae* have been recorded in the Cape region of the Baja California peninsula: *T. b. alticolus* (Allen 1899) is restricted to temperate forests in the highlands of the Sierra de La Laguna, and *T. b. anitae* (Allen 1898) is associated with tropical deciduous forest and desert scrub at lower elevations (Fig. 1). The descriptions of both subspecies were based on morphological characteristics and pelage color with only 4 specimens in the type series for *T. b. alticolus* and 3 for *T. b. anitae*.

Our goal is to evaluate how different environments influence the genetic structure, morphology, and morphometry of populations of pocket gophers that are altitudinally segregated in temperate forests (highlands), tropical deciduous forests (middle elevations), and desert scrub (lowlands). We assessed genetic and morphologic differentiation among populations that were isolated by elevation and habitat, and evaluated the validity of the subspecies from the highland temperate forest.

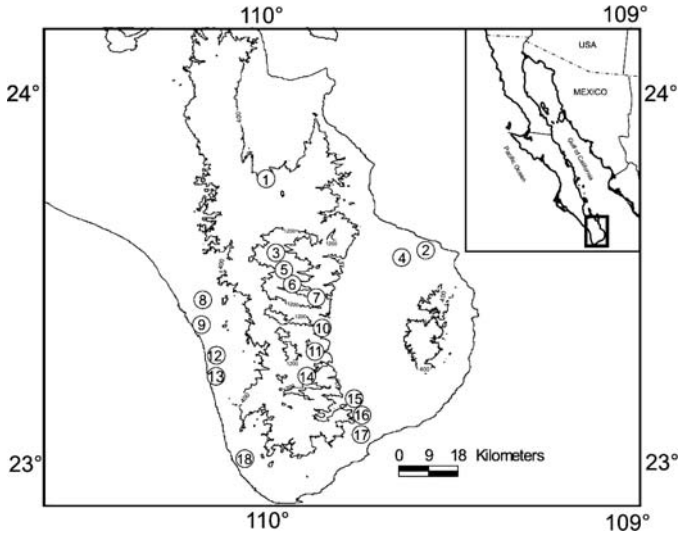

FIG. 1.—Location of samples of *Thomomys bottae* examined. Each locality is numbered as in Table 1. Desert scrub occurs from about mean sea level to 400 m of elevation, the tropical deciduous forest is between 400 and 1,200 m (contour lines), and the temperate forest is above 1,200 m.

MATERIALS AND METHODS

Samples.—We analyzed data from skins, skulls, and DNA sequences of individuals from 18 localities from the southern part of the state of Baja California Sur, Mexico. These localities include 3 habitats (Fig. 1; Table 1) and the type locality of both subspecies. Specimens were collected from all habitats using pocket gopher traps (Woodstream Inc., Lititz, Pennsylvania) placed inside burrows. Specimen identification numbers and localities are listed in Appendix I. Because La Sierra de La Laguna has a very nonuniform relief, the collections were only performed in reachable areas. In some places with appropriate soils for pocket gophers, specimens were not found. The museum repository is Centro de Investigaciones Biológicas, La

TABLE 1.—Sampled localities and habitat type. TF = temperate forest; TDF = tropical deciduous forest; DS = desert scrub. Number of specimens examined in each analysis. The localities are numbered as in Fig. 1.

					Specimens		
Locality	City nearby locality	Latitude	Longitude	Habitat	Skin	Skull	Cytochrome *b*
1	El Triunfo	23°48′N	110°06′W	TDF	14	10	9
2	La Ribera	23°36′N	109°33′W	DS	4	4	5
3	Valle de La Laguna	23°32′N	109°58′W	TF	38	31	18
4	Las Cuevas	23°32′N	109°39′W	DS	3	2	4
5	Agua de San Antonio	23°31′N	109°57′W	TF	2	2	2
6	Palo Extraño	23°31′N	109°56′W	TF	2	2	2
7	El Vergel	23°29′N	109°49′W	TDF	18	5	11
8	Todos Santos	23°28′N	110°12′W	DS	1	—	—
9	Todos Santos	23°26′N	110°13′W	DS	3	4	6
10	Santiago	23°26′N	109°42′W	TDF	13	6	2
11	Miraflores	23°21′N	109°46′W	TDF	3	1	3
12	Pescadero	23°21′N	110°09′W	DS	6	6	2
13	Pescadero	23°21′N	110°11′W	DS	1	—	1
14	Caduaño	23°16′N	109°44′W	TDF	4	1	4
15	Santa Anita	23°10′N	109°43′W	DS	3	2	3
16	Santa Anita	23°08′N	109°42′W	DS	10	6	—
17	San José del Cabo	23°08′N	109°42′W	DS	13	10	4
18	Migriño	23°01′N	110°09′W	DS	3	4	4
Total					141	96	80

Paz, Baja California Sur, Mexico. Samples of soil from each locality were analyzed for coloration and structural composition.

Laboratory techniques.—DNA was extracted from liver tissue using DNeasy Kit protocols (QIAGEN, Inc., Valencia, California). We used polymerase chain reaction to amplify a 500-base pair (bp) fragment of the mitochondrial cytochrome-*b* gene for 71 individuals from 16 of the 18 localities surveyed, using primer pair MVZ05/MVZ16 (Smith 1998). Specimen identification numbers and localities are listed in Table 1. We used the following conditions for initial double-strand amplifications: 12.5 μl template (10 ng), 4.4 μl double-distilled H_2O, 2.5 μl of each primer (10 nM concentration), 0.474 μl (0.4 μM) deoxynucleoside triphosphates, 0.5 μl (3 μM) $MgCl_2$, 0.125 μl (5 U/μl) Taq polymerase, and 1× Taq buffer to a final volume of 25 μl. Amplification conditions consisted of 3 min initial denaturation at 94°C followed by 39 cycles of denaturation at 94°C for 45 s for each cycle, 1 min annealing at 50°C, and 1 min extension at 72°C. Double-strand DNA was cleansed with the QIAquick PCR Purification Kit (QIAGEN), and this template was cycle-sequenced with primer MVZ05 using the Big Dye Terminator Kit (Perkin-Elmer Applied Biosystems Division, Foster City, California). We only sequenced 1 strand of our polymerase chain reaction templates because other studies (Álvarez-Castañeda and Patton 2004; Trujano-Álvarez and Álvarez-Castañeda 2007) have demonstrated the efficiency of primer MVZ05 to amplify fragments of 500 bp, and the risk of mistake is minimal. The sequencing was run on an ABI 377 automated sequencer (GMI, Inc., Ramsey, Minnesota) following manufacturer's protocols. Electropherograms were obtained to edit, clean, and align 500-bp fragments of cytochrome-*b* gene using Sequencher version 4.1.4 for Windows software (Gene Codes Corp., Ann Arbor, Michigan).

Genetic analysis.—A list of the 71 specimens sequenced (500 bp) and the GenBank acronyms are included in Appendix I. Nonredundant haplotypes were obtained using the Collapse version 1.2 program (available from http://darwin.uvigo.es) with the pairwise difference method. Arlequin 2.000 (Schneider et al. 2000) was used to obtain the relative frequency for each haplotype and construct the minimum spanning network (Excoffier and Smouse 1994) for the collection of unique haplotypes with the pairwise algorithm. An analysis of molecular variance (AMOVA) was used to examine the hierarchical apportionment of haplotypes among habitats (temperate forest, tropical deciduous forest, and desert scrub), among localities within each habitat, and among haplotypes within the same locality (Excoffier et al. 1992). The genetic structure was estimated by means of F_{ST} (Weir and Cockerham 1984). Values of nucleotide diversity were obtained for the populations in each habitat type.

Morphometric analysis.—We examined 72 females and 24 males. Only skulls of adult specimens that were not broken were evaluated following the criteria of Daly and Patton (1986); 13 cranial measurements (Patton and Smith 1990) were taken with digital calipers (0.01-mm resolution) by the same person. The measurements include the following lengths: occipitonasal, basilar, rostral, nasal, diastemal, maxillary toothrow, and bullar; widths: zygomatic, mastoid, least interorbital constriction, and rostral; and rostral and cranial depths. Because *T. bottae* has marked sexual dimorphism (Patton and Smith 1990) our small sample size of males was excluded from the analysis. Data on females were grouped by habitat and analyzed by 1-way analysis of variance (ANOVA) on individual characters, Tukey's post hoc test, principal component analysis, and discriminant function analysis. The statistical tests were applied with JMP (SAS Institute Inc., Cary, North Carolina) and Statistica (StatSoft, Inc., Tulsa, Oklahoma) computer software. Euclidian distance was determined with NTSYS version 4.0 (Applied Biostatistics, Inc., Setauket, New York), which considers means for each cranial measurement for each type of environment.

Coloration analysis.—Pelage color of 141 adult specimens was determined employing an X-Rite Digital Swatchbook spectrophotometer (X-Rite, Inc., Grandville, Michigan) and compared to the Commission Internationale d'Eclairage (CIE, Vienna, Austria) Standard Illuminant F7 for fluorescent illumination, which represents a broad-band daylight fluorescent lamp (6,500 K). We chose this standard because all measurements were taken indoors under fluorescent ambient lighting. The instrument provides a reflectance spectrum (390–700 nm) of the object being measured as well as tristimulus color scores (CIE x, y, and z). Individuals in molt were excluded. Color was measured with a 3-mm-diameter port placed on 2 topographic positions on the dorsal surface of each individual specimen: on the back and at midrump. Because of the small area actually measured, 3 separate measurements were taken at each position and averaged. Soil color of the locality where pocket gophers came from also was registered. Each soil sample was measured by its color in dry and moist (not wet) condition with the spectrophotometer, and then it was compared with the specimens of the respective locality, for each habitat type.

To determine if differences in color among individuals from different habitats was present, a principal component analysis was used with the 6 variables (x, y, and z for the back and the rump), using the statistical package Statistica version 5.0. An ANOVA of the residuals from the principal component analysis data also was done using the software JMP version 3.1.6.2. The x, y, and z variables for soil and pelage coloration were averaged separately for subsequent analyses. To establish if pelage coloration was associated with soil color from its habitat (both moist and dry), we used Student's *t*-test with JMP software. The null hypothesis was that the pelage coloration of the back or rump was similar to that of dry or moist soil. We also determined dorsal pelage and soil color with Munsell soil color charts (Munsell 1975) to document the color frequency for each habitat type.

Soil structural composition.—The percentages of sand, silt, and clay, and the approximate diameter of particles were obtained from the soil of each locality with the method of Bouyoucos (1951).

RESULTS

Genetics.—Thirty-four unique haplotypes, including 9 that were previously deposited in GenBank, were found among the individuals analyzed. The maximum number of substitutions between any 2 of these haplotypes was 7. The haplotype network showed that the most distant haplotypes were found in the same locality in the tropical deciduous forest (El Triunfo; Fig. 2), and the greatest number of haplotypes (17) was found in the desert scrub. A decreasing diversity of haplotypes was associated with increasing altitude: 12 were found in the tropical deciduous forest and only 7 in the temperate forest. The most frequent haplotype was present at 1 locality in the temperate forest (Valle de La Laguna, with a relative value of 0.13; type locality of *T. b. alticolus*), and in the desert scrub (Santa Anita; type locality of *T. b. anitae*). Other shared haplotypes occurred between the tropical deciduous forest and desert scrub. However, no shared haplotypes were found between the temperate forest and the tropical deciduous forest. No monophyletic pattern was found related to any habitat (Fig. 2).

Values of nucleotide diversity for populations in each habitat exhibited a similar trend, with nucleotide diversity $\pm$ *SD* = 0.008 $\pm$ 0.004 for temperate forest, 0.013 $\pm$ 0.007 for tropical deciduous forest, and 0.014 $\pm$ 0.007 for desert scrub. AMOVA indicated that the percentage of variation among the 3 habitats was 0.65, within the localities of the same habitat type was 33.11, and within the same locality was 66.24. The genetic variance among habitats was much smaller than within each habitat across all localities pooled. In the analysis of genetic structure, the genetic distance between temperate forest and tropical deciduous forest was $F_{ST} = 0.162$, that between temperate forest and desert scrub was $F_{ST} = 0.136$, and that between tropical deciduous forest and desert scrub was $F_{ST} = 0.057$.

Morphometrics.—One-way ANOVA of cranial measurements from adult females indicated that the specimens from the temperate forest were significantly larger in nasal length ($F = 5.31$, *d.f.* = 2, 63, $P < 0.01$) and rostral length ($F = 5.57$, *d.f.* = 2, 63, $P < 0.01$) than those from the desert scrub population, and in rostral width ($F = 5.80$, *d.f.* = 2, 63, $P < 0.01$) and cranial depth ($F = 4.01$, *d.f.* = 2, 61, $P < 0.05$) than those from the tropical deciduous forest. Bullar length of the specimens from the tropical deciduous forest was significantly smaller ($F = 5.21$, *d.f.* = 2, 62, $P < 0.01$) than that of specimens from the desert scrub and temperate forest.

The first 3 principal components (PCs) of the skull measurements explained 73.5% of the total variation (PC I = 57.4%, PC II = 8.8%, and PC III = 7.2%). In PC I the measurement that contributed the most to variation was diastemal length, in PC II it was rostral length, and in PC III it was the least interorbital constriction. The graph of PC I and PC II (Fig. 3) showed the overlap of specimens among the 3 habitats; however, the tendency of the individuals from temperate forest to be larger was clear in contrast to specimens from tropical deciduous forest, which are smaller. No difference among the populations was observed in the graph of PC II and PC III (not shown).

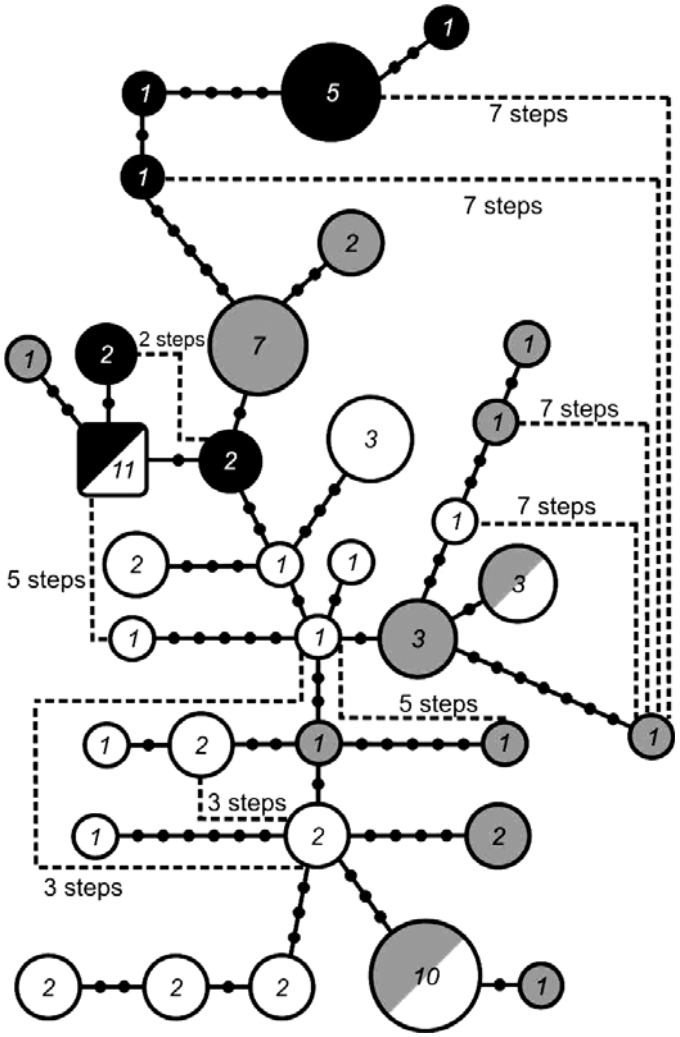

FIG. 2.—Minimum spanning network of 34 haplotypes of *Thomomys bottae* recovered from the 500-base pairs data set that included the sampled habitats: black = temperate forest; gray = tropical deciduous forest; and no shading = desert scrub. Haplotypes with 2 colors are shared between habitats. The area of each circle indicates the proportional representation of each of the respective haplotypes; the inner number indicates the frequency of each haplotype. Squares represent the ancestral haplotype of the network. Small filled circles separating haplotypes represent a single nucleotide substitution difference. Dashed lines show alternate connections with the mutational steps between haplotypes.

The percentages of correct assignments of the specimens obtained through the discriminate analysis are 87.5% for temperate forest, 71.4% for tropical deciduous forest, and 72.0% for desert scrub. All the larger specimens from the 3 habitats were assigned to the temperate forest.

The Euclidian distance of cranial measurement between temperate forest and tropical deciduous forest was 2.41, that between temperate forest and desert scrub was 1.83, and that between tropical deciduous forest and desert scrub was 1.03. A larger morphometric distance existed between the populations from temperate forest and tropical deciduous forest.

Coloration.—The analysis of dorsal pelage color according to habitat showed that the color of the pelage on the rump was

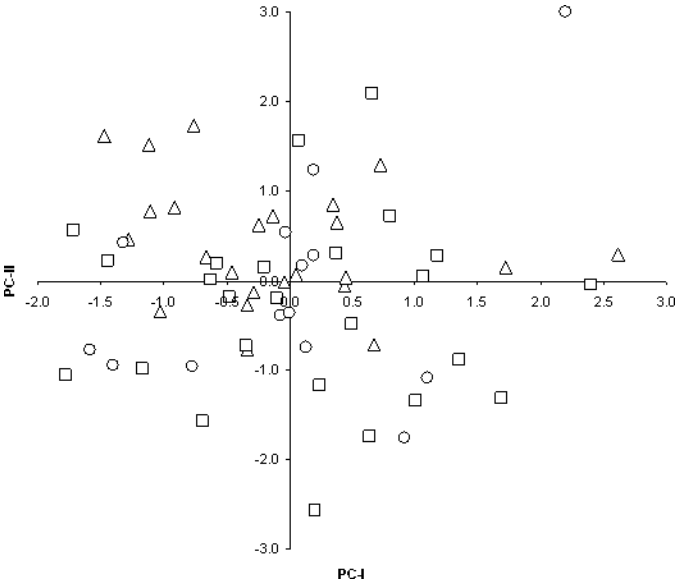

FIG. 3.—Graph of principal component I versus principal component II extracted by principal component analysis of the skull measurements of *Thomomys bottae*. Triangles correspond to specimens from temperate forest, circles to specimens from tropical deciduous forest, and squares to specimens from desert scrub.

more variable than that on the back, which was more homogeneous. In the temperate forest both body regions of individuals had 8 types of coloration; however, in the tropical deciduous forest the rump of the specimens had 6 different colorations contrasting with 4 on the back, and in the desert scrub, the gophers had 7 types of coloration on their rump, but only 4 on their back (Fig. 4). The dark yellowish brown (Munsell chart 10YR 3/6—Munsell 1975) dorsal pelage color was a general characteristic for individuals in the temperate forest and desert scrub. In the tropical deciduous forest this coloration also was present, but the predominant colors were dark brown (7.5YR 3/4) and strong brown (7.5YR 4/6). Soil coloration in the desert scrub was more diverse than the pelage color of pocket gophers from that habitat; on the other hand, in the temperate forest the pelage coloration of the pocket gophers was more diverse than the color of the soil. The soil coloration in each habitat was lighter than the pelage coloration of the gophers in that habitat. In the temperate forest, soil coloration ranged from very dark brown (10YR 2/2) to light olive brown (2.5Y 5/4); in the tropical deciduous forest it ranged from very dark grayish brown (2.5Y 3/2) to light olive brown, and in the desert scrub it ranged from dark grayish brown (2.5Y 4/2) to light yellowish brown (2.5Y 6/4).

The first 3 PCs of the analysis of pelage color among habitats explained 98.4% of the variation (PC I = 64%, PC II = 31.4%, and PC III = 2.9%). The biplot of PC I and PC II (Fig. 5) indicated that specimens from the 3 habitats overlapped; however, the 3 groups could be differentiated. PC I was mainly influenced by coloration of the back and PC II by coloration of the rump. ANOVA of the residuals from the PC I data showed that coloration of the back of pocket gophers from the 3 habitats was significantly different ($F = 34.13$, $d.f. = 2, 137$, $P < 0.01$). Analysis of the residuals from the PC II data did not show significant differences in coloration of the rump.

Coloration of the back of the specimens collected in the temperate forest was not significantly different from the coloration of moist soil. However, for the tropical deciduous forest ($t = 3.72$, $d.f. = 55$) and the desert scrub ($t = 3.34$, $d.f. = 54$) the differences between the specimens and the moist soil were highly significant ($P < 0.01$; Table 2). Coloration of the back of the pocket gophers was similar to that of dry soil in the tropical deciduous forest. In the desert scrub, coloration of the back was significantly different from coloration of both dry ($t = 3.23$, $d.f. = 54$, $P < 0.01$) and moist soil. Coloration of the rump was more diverse (Fig. 3); it was similar to that of dry soil in the temperate forest and desert scrub, but was different from that of dry ($t = 2.06$, $d.f. = 55$, $P < 0.05$) and moist soil ($t = 5.50$, $d.f. = 55$, $P < 0.01$) in tropical deciduous forest.

Soil structural composition.—Soils from the 3 habitats were loamy sand type (Soil Survey Division Staff 1993). The temperate forest soil had a very coarse texture with 39% (31.1–49.1%) of particles > 2 mm in diameter (fine gravel), with 14% (8.0–20.0%) silt and 13.5% (12.0–16.0%) clay (Table 3); the soil also was moister than that from the other habitats. The soil from the tropical deciduous forest was mainly fine ground with only 2.2% rock fragments > 2 mm of diameter, and was easily compactable, with 15.6% (8.0–30.0%) silt and 12.2% (10.0–17.2%) clay. In the desert scrub, the soil also was fine ground, with fine, medium, and coarse grains; there was a variable percentage of silt and clay depending on the locality of origin: 13.2% (2–36%) silt and 12.4% (7.2–21.2%) clay; the soil varied among localities from light and coarse in streams to fine and dark in farmland areas.

DISCUSSION

The genetic analyses showed that the populations of *T. bottae* from the Cape region of the Baja California peninsula were genetically homogeneous with greater variation within than among populations for the 3 habitats. In spite of the spatial isolation caused by habitat and elevation differences, no genetic differentiation was found; the degree of genetic variation at the regional level, among populations of different habitats, was smaller than at the intrapopulational level. Therefore, among individuals from all 3 habitats (temperate forest, tropical deciduous forest, and desert scrub) there was a reduced genetic variability, as observed in the haplotype analysis. The haplotype net showed that the haplotypes present in the temperate forest are grouped with those from the tropical deciduous forest. However, the haplotypes of the temperate forest were on terminal branches, which indicates that they are descendent haplotypes. We assumed that the haplotypes from the desert scrub are the ancestral ones because these had more mutational connections (they are related to more haplotypes), occurred in greater frequency, and were more widely distributed geographically (Posada and Crandall 2001).

Most of the haplotypes represented by a single individual were connected to the most frequent haplotypes of the same habitat. This is in agreement with coalescent theory, which

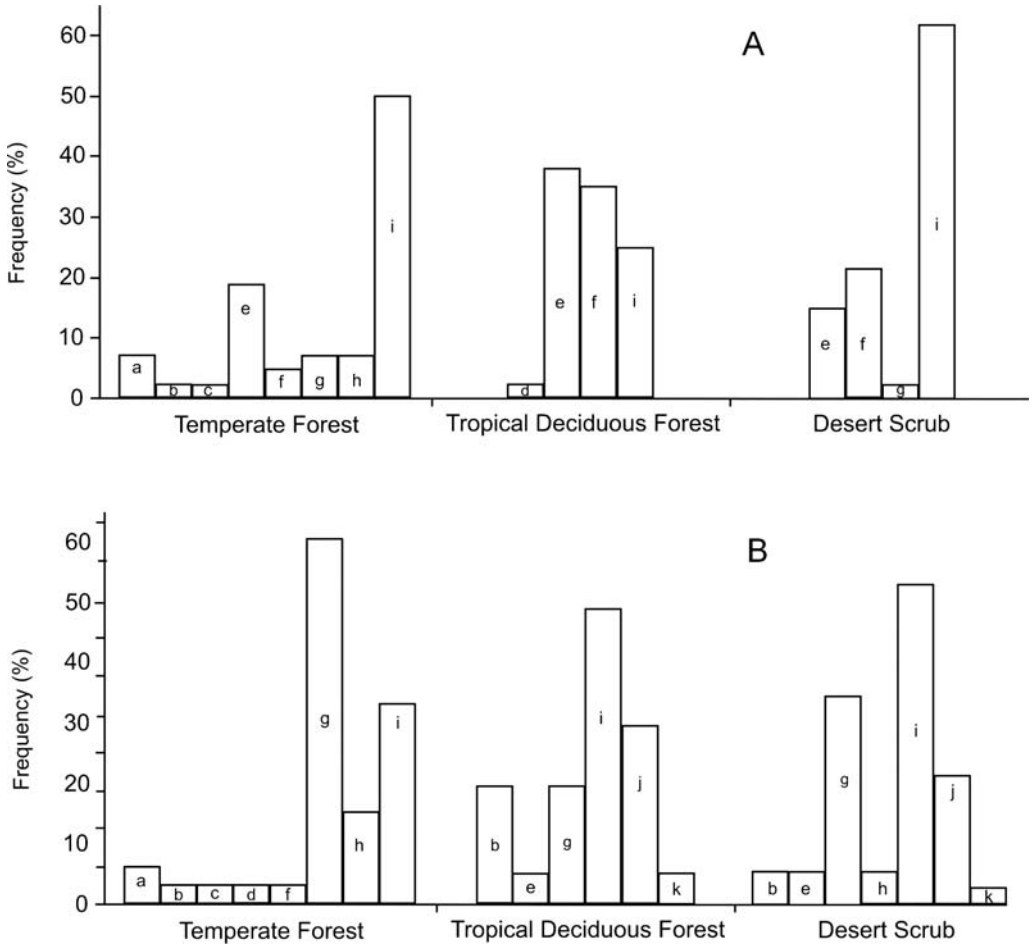

Fig. 4.—Pelage color patterns of *Thomomys bottae* found in each habitat based on a comparison with Munsell charts. The colors are represented in frequency percentages. A) Dorsal coloration: a = black, b = very dark gray, c = dark gray, d = very dark brown, e = dark brown, f = strong brown, g = brown, h = very dark grayish brown, i = dark yellowish brown. B) Rump coloration: a = very dark gray, b = strong brown, c = brown, d = pale brown, e = light brown, f = dark yellowish brown, g = yellowish brown, h = light yellowish brown, i = brownish yellow, j = reddish yellow, k = yellow.

implies that the immediate descendents of a new mutation are more likely to remain in the original population than to move to a distant population (Posada and Crandall 2001).

The most distantly related haplotypes were found in a single locality within the tropical deciduous forest, although this may be an artifact of sampling—that is, intermediate haplotypes exist but were not collected. Values of nucleotide diversity also were low, similar to published values for other pocket gopher populations in Baja California Sur (Álvarez-Castañeda and Patton 2004; Trujano-Álvarez and Álvarez-Castañeda 2007), where a lack of phylogeographic structure among samples also was found. The genetic distance (F_{ST}) between the tropical deciduous forest and the desert scrub was very low, relative to the temperate forest. This implies more gene flow between the populations of these 2 habitats than with the temperate forest.

Although the pocket gophers were genetically similar, there was some morphologic variation among populations. The analyses showed significant differences in the coloration of the dorsal pelage among individuals from the 3 environments. Color variation of the pocket gophers was related to the different habitats, with a general pattern of dark specimens in highlands and lighter ones in lowlands. Although soil coloration also showed this altitudinal pattern, soil coloration in each habitat was lighter than pelage coloration. Contrary to the results of studies with pocket gophers in other regions, which also included temperate and desert habitats where there is a direct correlation between pelage color and the moist soil color in which they live (Krupa and Geluso 2000; Patton 1973), only the specimens from the temperate forest in our sample matched the moist soil. The dorsal coloration of the individuals from tropical deciduous forest was similar to the color of dry soil. However, gophers from the desert scrub showed no direct relationship between pelage color and the soil color in which they were collected. In other studies, it has been reported that in highlands the soil and the pelage of pocket gophers tend to be darker because of greater rainfall, whereas in lowlands the pelage and soil are paler and lighter (Getz 1957; Sumner 1921). Our results show the same. When the soil has greater moisture, it contains more humus and is darker, in agreement with the color of the analyzed soil and pocket gophers from temperate forest. Although the pelage of individuals from tropical deciduous forest and desert scrub did not match the moist soil

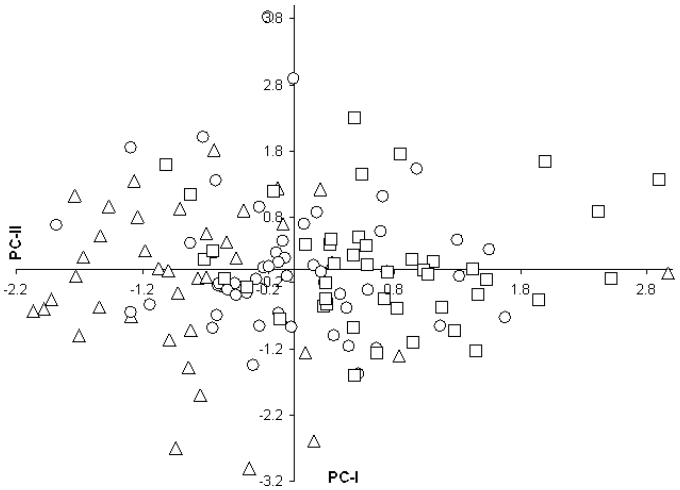

FIG. 5.—Graph of principal component I versus principal component II extracted by principal component analysis of the dorsal pelage coloration. The triangles correspond to specimens from temperate forest, circles to specimens from tropical deciduous forest, and squares to specimens from desert scrub.

color, it had a tendency to be lighter, and the soil coloration tends to be lighter and paler when the elevation decreases. Studies of the rock pocket mouse (*Chaetodipus intermedius*) show that populations that are very different in coat coloration may still be connected by gene flow. However, selection is acting very strongly on color to maintain habitat-specific phenotypes (Hoekstra et al. 2005). Nevertheless, in *Peromyscus polionotus* no evidence was found for the role of predation as a selective force affecting pelage color (Belk and Smith 1996). Because of the larger diversity in pelage coloration of pocket gophers from the temperate forest, contrasting with low diversity in soil coloration, and the significant differences between the pelage and soil color in tropical deciduous forest and desert scrub, we conclude that selective pressures are low on pelage coloration in *T. bottae* of the Sierra de La Laguna.

Cranial size was the main morphological difference among the specimens from the 3 habitats. However, the morphometric analyses did not show variation in cranial shape. Variation in the size of specimens from the same population can be heavily influenced by food quality (Patton and Brylski 1987; Patton and Smith 1990; Smith and Patton 1988). Pocket gophers are completely herbivorous; they eat mainly bulbs, roots, tubers, and occasionally aboveground plants. It has been demonstrated that nutritional values of local foods strongly influence rates of growth (Patton and Brylski 1987). Even though we do not have data on nutritional quality for each habitat, the 3 study areas had different vegetation. Given this consideration, we suggest that size differences observed among pocket gophers are a reflection of habitat differences, with a tendency for larger size in the highlands (temperate forest) and smaller size in the middle and low elevations (tropical deciduous forest and desert scrub). Animals from the population from the tropical deciduous forest were the smallest.

The small size of the individuals from the tropical deciduous forest can probably be attributed to the quality of the habitat, mainly to the interaction of 2 factors: nutritional quality of vegetation and soil conditions. Although soils in all 3 habitats were the loamy sand type, in tropical deciduous forest the grain diameter was smaller. Therefore, in this habitat the soil had a tendency to be more compact (Duchauford 1995), and in certain areas it could be very hard for pocket gophers to dig. Moreover, the tropical deciduous forest is a very rugged area of the Sierra de La Laguna with areas of slope outcrops between canyons where the soil is more exposed to erosion and sometimes bordered by rock. Under these conditions, the body size of pocket gophers can be limited (Davis 1938; Patton 1999). In contrast, the temperate forest had soil with the largest diameter of grain, which results in a more friable soil. Pocket gophers in the temperate forest were found mainly in a valley with grassland, whereas the individuals of the desert scrub lived in diverse soils, but generally in plains. In both cases, soils were deeper and more friable than in tropical deciduous forest.

The recognition of 2 subspecies of *T. bottae* in the Sierra de La Laguna area was based mainly on color characteristics of pelage as well as on the size of the skull of a few individuals (Allen 1898, 1899). The original description for *T. b. alticolus* from the temperate forest indicated that it has a larger skull and a darker and less fulvous pelage than *T. b. anitae*. It was described as yellowish brown above, but with much variation, and with black to dark brown along the median line (Allen 1899). *T. b. anitae* was considered to be not variable in color, and easily distinguished from *T. b. alticolus* (Allen 1899). These descriptions are consistent with the results we obtained; individuals of the temperate forest showed diverse coloration, but in general, they were darker than those from other habitats. Nevertheless, some temperate forest specimens can be confused with tropical deciduous forest and desert scrub individuals because they had similar dark and light yellowish brown pelage. Therefore, pelage color is not a characteristic that could be used as diagnostic for these populations.

According to the subspecies concept proposed by Patton and Smith (1990) for pocket gophers, all members of a subspecies share close genetic relationships and diagnostic, non–size-

TABLE 2.—Mean ± *SE* of values of the brightness of coloration of specimens and soil from the 3 different examined habitats, measured by spectrophotometer. TF = temperate forest; TDF = tropical deciduous forest; DS = desert scrub. * $P < 0.05$; ** $P < 0.01$; NS not significant.

	Pelage		Soil		Student's *t*-test				
Habitat	Back (B)	Rump (R)	Dry (D)	Moist (M)	B versus D	B versus M	R versus D	R versus M	D versus M
TF	6.88 ± 0.35	12.33 ± 0.52	10.30 ± 2.23	5.03 ± 0.67	*	NS	NS	**	NS
TDF	9.01 ± 0.25	13.68 ± 0.43	10.70 ± 0.95	5.88 ± 0.21	NS	**	*	**	**
DS	10.74 ± 0.32	14.08 ± 0.38	13.29 ± 0.79	8.19 ± 0.63	**	**	NS	**	**

related features of cranial shape. We could not distinguish between *T. b. alticolus* and *T. b. anitae* based on the morphometric and genetic traits we measured. The differences we observed among populations reflected only habitat differences. Individuals from the type localities for the 2 subspecies (*T. b. alticolus*: Valle de La Laguna; *T. b. anitae*: Santa Anita—Hall 1981; Patton 1999) shared the same haplotype, in spite of coming from different habitats. Therefore, we do not recognize *T. b. alticolus* as a valid subspecies, and the name should be considered a synonym of *T. b. anitae*. The form *anitae* includes other subspecies previously recognized (Trujano-Álvarez and Álvarez-Castañeda 2007).

Thomomys bottae anitae

Thomomys bottae anitae J. A. Allen, 1898:146. Type locality. ''Santa Anita, Lower California, Mexico'' [Baja California Sur].

Thomomys fulvus alticolus J. A. Allen, 1899:13. Type locality. ''Sierra Laguna, 7000 feet, Lower California'' [Baja California Sur].

Thomomys magdalenae Nelson and Goldman, 1909:24–25. Type locality ''Magdalena Island, Lower California'' [= Baja California Sur].

Thomomys bottae incomptus Goldman, 1939:29–30. Type locality ''San Jorge, near Pacific coast west of Poza Grande and about 25 miles southwest of Comondú, southern Lower California (altitude 50 feet)'' [= Baja California Sur].

Thomomys bottae imitabilis Goldman, 1939:30–31. Type locality ''La Paz, southern Lower California'' [= Baja California Sur].

Thomomys bottae litoris Burt, 1940:1. Type locality ''Stearns Point, Magdalena Bay (west side), Lower California'' [= Baja California Sur].

Distribution.—The range of *T. b. anitae* extends from the San Ignacio Lagoon to the southern end of the Baja California peninsula, along the coast of the Cape region, and in the highlands and lowlands of La Sierra de La Laguna.

Resumen

La coloración dorsal del pelaje, morfometría craneal y secuencias de 500 pb del citocromo *b* de *Thomomys bottae* de la Sierra de Laguna, Baja California Sur, fueron evaluados para conocer la influencia del ambiente sobre la morfología y la estructura genética de poblaciones de hábitats segregados altitudinalmente: bosque templado, selva baja caducifolia y matorral xerófilo. De 80 individuos, se encontraron 34 haplotipos los cuales no se agrupan por hábitat monofiléticamente. El AMOVA indicó que más de la mitad de la varianza se encuentra entre individuos de poblaciones locales y sólo se explica el 0.6% por las diferencias de hábitat, por lo que no hay estructura genética, siendo las poblaciones genéticamente similares. Los individuos del bosque templado son de una talla mayor, con una coloración más oscura y diversa; los de la selva baja caducifolia son los más pequeños y los del matorral xerófilo más claros. No se encontraron diferencias en la forma craneal entre poblaciones. Sólo la coloración de los especímenes del bosque templado es similar a la del suelo húmedo. No hay características diagnósticas para reconocer a *T. b. alticolus* como una subespecie válida y se considera sinónima de *T. b. anitae*, con una distribución que incluye todo el extremo sur de la península de Baja California, desde San Ignacio hasta Los Cabos.

Table 3.—Soil texture in each habitat. TF = temperate forest; TDF = tropical deciduous forest; DS = desert scrub. The values are mean percentages (minimum and maximum) for each texture type.

Habitat	Soil texture %		
	Sand	Silt	Clay
TF	72.5 (66.0–80.0)	14.0 (8.0–20.0)	13.5 (12.0–16.0)
TDF	72.2 (52.8–82.0)	15.6 (8.0–30.0)	12.2 (10.0–17.2)
DS	74.4 (50.8–90.8)	13.2 (2.0–36.0)	12.4 (7.2–21.2)

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APPENDIX I

Locality (listed numerically as in Table 1 and Fig. 1), sample size, and catalogue numbers of specimens used in the analyses; all samples are housed in the Mammal Collection of the Centro de Investigaciones Biológicas. GenBank accession numbers are enclosed in square brackets.

1) El Triunfo (15): El Triunfo (6620 [AY589024], 6621–6631, 11668, 11670, 11671); 2) La Ribera (4): La Ribera (6632, 6633 [EF088484], 6635, 6636); 3) Valle de La Laguna (42): Valle de La Laguna, Sierra de La Laguna (6514, 6515, 6516 [AY589022], 6517–6538, 6540–6547, 10929–10933, 10935–10938); 4) Las Cuevas (4): Las Cuevas, 6 km S, 8 km W La Ribera (5299 [EF088485], 5301–5303); 5) Agua de San Antonio (2): Agua de San Antonio, 9 km N 26 km E Todos Santos (10941 [EF088487], 10942); 6) Palo Extraño (2): Palo extraño, Sierra de La Laguna (10939, 10940 [EF088488]); 7) El Vergel (19): El Vergel, 12 km NW Santiago (7881, 7882, 8370–8372, 8374, 8375, 11672 [EF088489], 11673–11683); 8) Todos Santos (1): 4 km N, 2 km E Todos Santos (8966); 9) Todos Santos (7): Todos Santos (5480–5482, 5483 [AY589038], 5484, 5485, 8967); 10) Santiago (13): Rancho ''La Misión,'' Santiago (5295, 5296, 5297 [AY589018], 5298, 8368, 8369, 10943, 10944, 11685, 11686, 11687, 11688, 11689); 11) Miraflores (3): ''Internado'' Miraflores (5304 [EF088486], 5305, 5306); 12) Pescadero (7): Pescadero (8968, 8969 [EF088490], 8970–8972, 8974, 8975); 13) Pescadero (1): 3 km S Pescadero (6214 [AY589019]); 14) Caduaño (4): Caduaño (6215 [AY589020], 6216–6218); 15) Santa Anita (3): Santa Anita (6219, 6220 [AY589021], 6221); 16) Santa Anita (11): San Bernabé, 3 km S, 4 km E Santa Anita (8901–8903, 8905–8908, 8910, 8911, 8917, 8918); 17) San José del Cabo (14): 10 km N, San José del Cabo (6647, 6649–6653, 6654 [AY589026], 6656–6661, 6664); 18) Migriño (6) 6 km SE Migriño (6638, 6639 [AY589025], 6640, 6641, 6644, 6645).