

PHYLOGEOGRAPHIC STUDY OF THE CALIFORNIA VOLE, *MICROTUS CALIFORNICUS*

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Many vertebrate taxa show genetic differentiation between populations in northern and southern California. This genetic pattern may reflect a common environmental history for these species. For example, a previous study of the California vole (*Microtus californicus*) showed morphological divergence between populations in northern and southern California and decreased fertility in crosses between the populations. To investigate phylogeographic differences in this species, we assessed variation in mitochondrial and nuclear DNA throughout much of its distribution from Oregon to Baja California. We generated molecular data (mitochondrial cytochrome *b* and nuclear acid phosphatase V intron [AP5]) for 178 individuals. Examination of these data suggests 2 phylogeographic groups that are largely discordant with the boundaries of 17 currently recognized subspecies. Estimates of pairwise genetic divergence between these groups for cytochrome *b* are as high as 4.46% uncorrected *p*. Sequence data for AP5 also indicate a division between populations of *M. californicus* in northern and southern California. Examination of data from the mitochondrial and nuclear markers together suggests limited gene flow between clades. These data are concordant with other studies that suggest that mountain ranges in California were important in within- and possibly between-species divergence and subsequent contact. The general distribution of each clade, combined with a geographic information system analysis of known capture sites, suggests that clade divergence may be correlated with ecological differences. Our study creates a new framework for reevaluating morphological and ecological diversity in this species, and with more diverse markers, possibly the recognition of 2 species of California voles.

Key words: California, climate, cytochrome *b*, gene flow, geographic information system, hybrid, incipient species, *Microtus californicus*, vole

Speciation is one of the most fundamental aspects of evolutionary biology. Investigating this process as it occurs provides an opportunity to observe the shift from populations that exhibit persistent gene flow to the development of higher taxonomic categories. Examining DNA sequence divergence in a phylogenetic context can identify independent lineages that may be in the process of speciation (Wiens 2004; Wiley 1978). However, corroborating data from additional data sets, such as other genetic markers, morphology, chromosome incompatibility, and ecology, are necessary to argue strongly for the recognition of diagnosable species (Coyne and Orr 2004; Dayrat 2005; Ferguson 2002; Templeton 2001). Studies that incorporate multiple data sets can be especially valuable for identifying patterns and addressing processes of incipient speciation.

Studies of mammals clearly at the cusp of speciation are few and generally report cryptic diversity within species or cryptic gene flow among closely related species (e.g., house mice [*Mus musculus*]—Britton-Davidian et al. 2000; chipmunks [*Tamias amoenus*]—Demboski and Sullivan 2003; voles [*Microtus agrestis*]—Hellborg et al. 2005; and woodrats [*Neotoma fuscipes*]—Matocq 2002). Ideally, studies of speciation that combine estimates of the degree of divergence and unique ecological characteristics will elucidate details of the mechanisms of speciation (Mayr 1963; Wiens 2004). Investigations into gene flow among genetically distinct populations also can be informative (Barton and Hewitt 1985). It has been suggested that mammals likely speciate allopatrically (Barnosky 2005; Fitzpatrick and Turelli 2006) and are distributed, in part, by climatic constraints (Grinnell 1914). Therefore, climate change and its consequences may be vital to understanding how mammals speciate.

The California vole, *Microtus californicus* (Cricetidae: Arvicolinae), includes 17 described subspecies (Hall 1981) and ranges from southern Oregon to Baja California (Fig. 1).

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Subspecific designations have been based on skull shape, body size, and pelage differences (Kellogg 1918). Previous studies have examined *M. californicus* from numerous perspectives including genetics (Bowen 1982; Bowen and Yang 1978; Gill 1977, 1984), ecology (Lidicker 1980; Ostfeld et al. 1985), and physiology (Houlihan 1963). Few studies have included broad geographic sampling; thus, most information about this species may reflect only local conditions. Despite limited geographic sampling, there have been studies that included samples from different parts of the species' range. Gill (1982, 1984) attempted to cross individuals from populations from northern and southern California and found substantial aggression between geographic groups. When individuals from the 2 groups did mate, they produced females with low fertility and infertile males. Gill (1984) also examined 30 allozyme markers from these 2 populations and found 11 polymorphic loci. Of those, 2 had alleles with fixed differences between northern and southern populations. Morphological studies also revealed differences; southern populations have longer skulls and broader maxillary bones than the northern voles, which have broader zygomatic arches (Gill 1984). These early studies indicate that key differences exist between northern and southern populations. In addition, chromosomal variation exists within populations (Gill 1982; Modi 1985), with reported diploid numbers of $2n = 52, 53,$ or 54 . However, variation between northern and southern California has not yet been investigated, and neither Gill (1982) nor Modi (1985) argued for species status of either group.

Many other vertebrate taxa also show north–south phylogeographic breaks in California (e.g., Calsbeek et al. [2003] in vertebrates and invertebrates; Burns and Barhoum [2006] in wrentits [*Chamaea fasciata*]; Sgariglia and Burns [2003] in California thrasher [*Toxostoma redivivum*]; and Feldman and Spicer [2006] in reptiles). The concordance of geographic breaks among species suggests that a common process may be structuring genetic diversity and, ultimately, speciation in vertebrates in California (Davis et al. 2007). Although there may be a common process, it may be affecting lineages at different temporal and spatial scales.

We investigated phylogeographic differentiation within *M. californicus* using 3 data sets: mitochondrial DNA (mtDNA), nuclear DNA (nDNA), and environmental variables at sites of known occurrence. We discuss the results of these analyses in the context of incipient speciation in this system.

MATERIALS AND METHODS

Tissue samples.—We obtained a total of 178 samples from throughout California (Fig. 1; Appendix I). We included samples collected for this study ($n = 87$), previously collected museum study skins and tissues ($n = 58$), and tissue biopsies from other studies that lack vouchers ($n = 33$). Examination of preliminary data indicated a potential phylogenetic break in Santa Barbara and Ventura counties. Therefore, we emphasized thorough sampling of those locales (see inset in Fig. 1). We flash-froze tissues collected in the field and later stored them at -70°C . For populations that were remote or protected, or

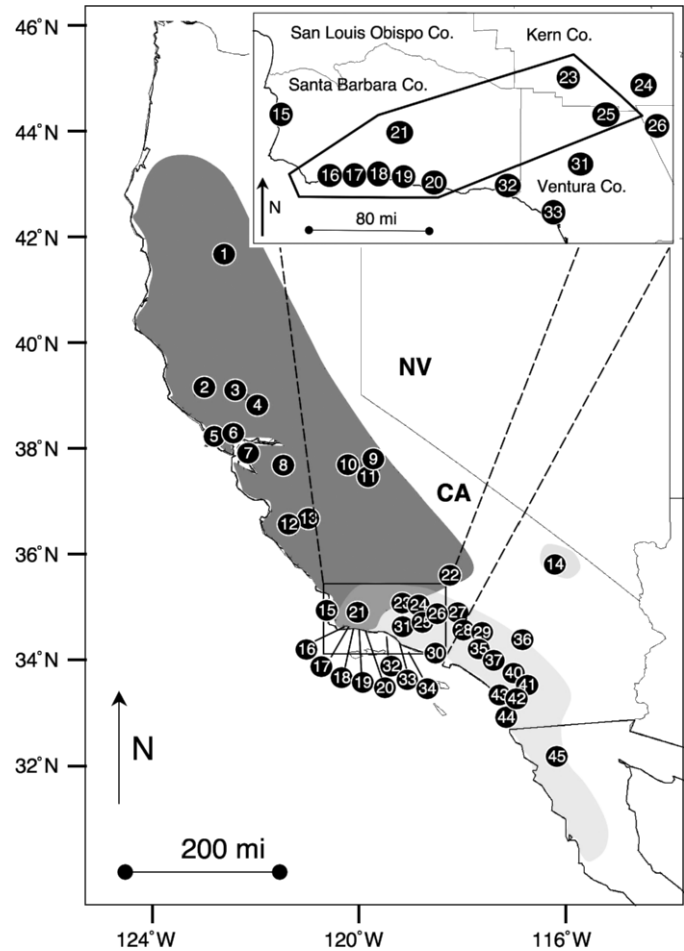


FIG. 1.—Localities sampled for *Microtus californicus* in California and Baja California. Numbers refer to localities listed in Appendix I. Shading indicates the northern clade (dark), southern clade (light), and zone of contact. Map inset shows the contact zone in Santa Barbara, Kern, and Ventura counties. Localities within the polygon show elements of both the northern and southern clades.

to increase sample size, we used 23 museum study skins as sources of DNA. We used tissues taken from an additional 19 live animals from a protected population examined in a separate study (Neuwald 2002). We acquired 14 tissue biopsies from other studies (material from M. Ball and R. Davis). We handled all specimens in accordance with guidelines of the American Society of Mammalogists (Gannon et al. 2007), and the Animal Care and Use Committee at University of California Berkeley approved the research protocol (R277).

Molecular data.—We extracted genomic DNA from 178 specimens with either a Qiagen DNeasy Tissue Kit (following the manufacturer's instructions; Qiagen, Valencia, California) or by using a modified salt extraction (Miller et al. 1988). We performed polymerase chain reaction to amplify the mitochondrial cytochrome-*b* gene (*Cytb*) and the 2nd intron for the nuclear gene acid phosphatase V (*AP5*). We used primers MVZ 05 (Smith and Patton 1993) and vole-14 (Hadly et al. 2004) to amplify the complete *Cytb* locus (1,143 base pairs [bp]). When the complete region would not amplify, we targeted partial regions with micro-06, vole-07, and vole-14 primers (Hadly

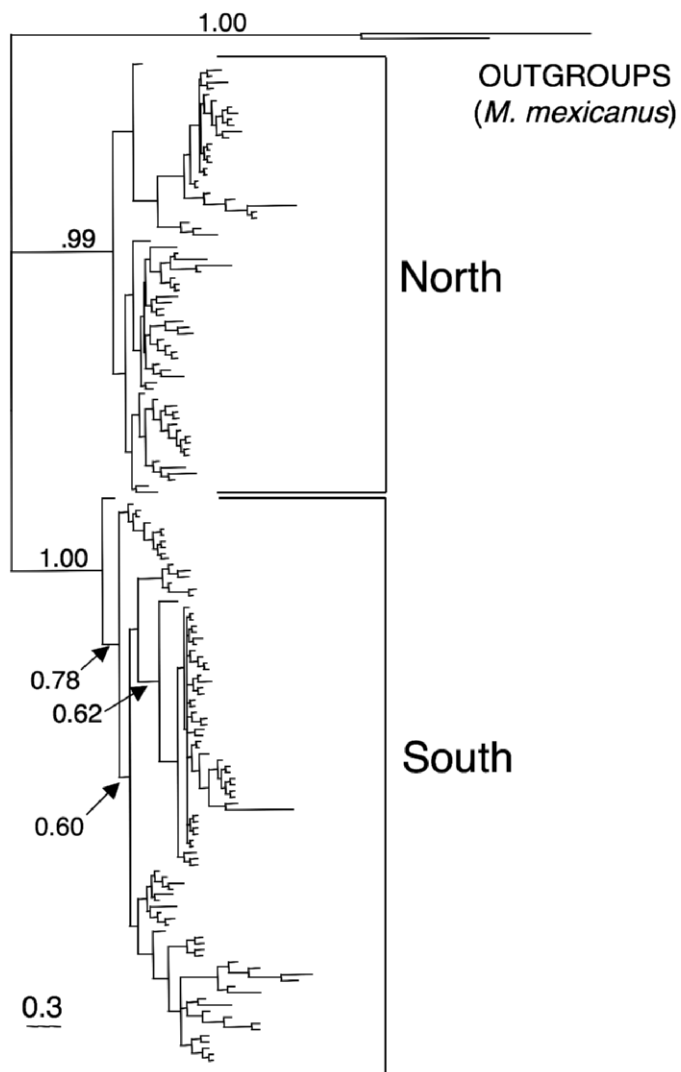


FIG. 2.—Bayes phylogram of mitochondrial DNA cytochrome-*b* sequences from 164 specimens of *Microtus californicus*. Two sequences of *Microtus mexicanus* were included as outgroups. Taxon names have been removed for clarity. Numbers on branches are posterior probabilities greater than 0.5, except near tips where they were removed for clarity. This analysis used the GTR+I+ Γ model and was partitioned by codon position (see text).

et al. 2004). We used primers MVZ 05 and MVZ 22 (5′–3′, CGTTAGCATGTAT[A/G]TATCG[G/A]AT), which target a much smaller fragment (~300 bp), to amplify DNA from museum study skins. We amplified the *AP5* intron (~450 bp) with primers *AP5* 120fwd and *AP5* 564rev as described in DeBry and Seshadri (2001). We generated *AP5* sequences for 138 specimens, yielding 26 variable nucleotides and 43 haplotypes. We cleaned amplicons with either Qiagen PCR Purification kits or with ExoSAP-IT (BioLynx Inc., Brockville, Ontario, Canada). We ran cycle sequencing reactions under standard protocols with ABI Prism 3.1 (Applied Biosystems, Foster City, California), and cleaned them with either an ethanol and magnesium chloride precipitation or with Sephadex G-50 (Sigma-Aldrich, St. Louis, Missouri) columns.

We sequenced the products using an ABI 377 or an ABI 3730 DNA sequencer (Applied Biosystems). We aligned the sequences with Sequencher software, or by eye with EditView (Applied Biosystems). We identified heterozygotes from the nuclear *AP5* locus by eye as equal mixtures of 2 bases and we phased them (i.e., statistically inferred to which haplotype each nucleotide at the heterozygous site belongs [see Clark 1990]) using PHASE version 2.1.1 (Stephens and Scheet 2005; Stephens et al. 2001). All molecular data are available in GenBank (accession numbers, *M. californicus* *Cytb* EF506032–EF506197, previously published *M. californicus* AF163891, and previously published *M. mexicanus* AF163897; *AP5* EF505894–EF506031).

Phylogenetic and population genetic analyses.—We used MrBayes (version 3.1.2—Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) to construct a Bayesian tree for the *Cytb* data set. We used MODELTEST (version 3.6—Posada and Crandall 1998) to find the best nucleotide substitution model with the fewest parameters, selecting the GTR+I+ Γ model by both the likelihood ratio test and the Akaike information criterion. We also partitioned the data by codon position. The MrBayes analysis used uniform priors, 4 chains, and 2,000,000 generations. We saved every 1,000th tree for further analysis. We included 2 sequences of *M. mexicanus* as the outgroup (Conroy et al. 2001; Conroy and Cook 2000) and included both complete and partial *Cytb* sequences in the analysis. Although constructing a Bayesian tree with missing data is not ideal, our intent was only to partition sequences into either the northern or southern group. We did not use branch lengths for any subsequent analyses. Problems of missing data are therefore not especially relevant here. We plotted Bayesian scores and parameter values against generation time to determine the appropriate burn-in period (750,000 generations). We discarded pre-burn-in trees and used the remaining trees to build a consensus tree (SUMT command) with posterior probabilities greater than 60% labeled on branches, except at the tips (Fig. 2). We used the tree only to determine whether a specimen was from the northern or southern clade (e.g., to assign to groups for net and gross divergence) and did not use the branch lengths in any way.

To examine the data as a network (as opposed to a bifurcating tree), we used the software TCS version 2.0 (Clement et al. 2000) to construct gene genealogies. Unlike traditional phylogenetic approaches, this program focuses on the population level and the assumptions therein, such as commonality of ancestral polymorphisms, recombination, and low levels of variation. We used a 95% confidence interval for assigning branches in TCS and analyzed 64 sequences for which we had complete *Cytb* sequences. For the *AP5* data set, we analyzed phased sequences from 140 individuals.

To put the degree of *Cytb* divergence between northern and southern populations of *Microtus* in perspective, we examined 15 pairs of sister species of *Microtus* from Jaarola et al. (2004), excluding *M. californicus* (sister to *M. mexicanus*) and *M. agrestis* (which may be 2 species—Hellborg et al. 2005). We downloaded these data from GenBank and supplemented with unpublished sequences from CJC. We computed the average

uncorrected percent divergence using PAUP* version 4.0b10 (Swofford 2003) for each pair. We compared all complete *Cytb* sequences of *M. californicus* (28 of the northern clade and 36 of the southern clade). We computed net divergence between clades by subtracting mean within-clade distance from mean between-clade divergence (Avice and Walker 1998). We computed this divergence only for complete *Cytb* sequences. We lacked data to construct net divergence for an adequate diversity of other species.

For the intraspecific data from *M. californicus*, we used the population genetics program Arlequin (Schneider et al. 2000) to reconstruct demographic history by partitioning our *Cytb* data by clade and by testing the hypothesis of recent expansion with mismatch analysis (Schneider and Excoffier 1999). Our goal was to gauge whether the clades that are in contact are old and stable, or are undergoing expansion from a relictual population. Mismatch distribution analyses rely on the number of differences between pairs of sequences compared with a model distribution (Rogers and Harpending 1992). The distribution of pairwise differences has characteristic patterns under different models of demography. We used only sequences with complete *Cytb*. We employed 1,000 iterations and plotted both raw mismatch data for *Cytb*, as well as data modeled under an assumption of population expansion. We examined each clade to determine whether it appeared to be resident for long periods or expanding recently. We estimated levels of gene and nucleotide diversity with Arlequin and examined sources of molecular variance (analysis of molecular variance [AMOVA]) in Arlequin using 1,000 iterations. We used only pure northern and pure southern populations, excluding the overlap zone, because we were more concerned with the history of each clade as a unique unit rather than the demographic history in the overlap zone. We also examined neutrality across the species by examining Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989), using 1,000 iterations in Arlequin. We used these tests across the entire species, as well as within complete *Cytb* representatives of each major clade. These tests of selective neutrality examine the distribution of genetic variation in relation to models of neutral evolution. Therefore, when there are deviations from the model, the locus could be under selection if the population is in equilibrium. Because our tests are based on pairwise differences, they are sensitive to departures from population equilibrium. Patterns observed from these tests may therefore be a result of deviations from Hardy–Weinberg equilibrium (e.g., expansion) instead of selection.

Geographic information system modeling.—To examine the relationship between environmental variables and distribution of California voles, we acquired georeferenced localities from the Museum of Vertebrate Zoology database (<http://mvz.berkeley.edu/>) and MaNIS (<http://manisnet.org/>). We used the localities of 5,892 specimens (data available from CJC). We obtained 19 BIOCLIM climate variables (Table 1) relevant to precipitation and temperature at 2.5-min resolution from WORLDCLIM version 1.3 in DIVA-GIS (<http://www.diva-gis.org/>; Hijmans et al. 2002). We extracted climate data for all unique 2.5-min quads and investigated differences between

TABLE 1.—Eigenvalues for 19 climate variables (Hijmans et al. 2002) used in the geographic information system analysis of the distribution of *Microtus californicus* in California. Values in bold indicate the 6 highest loading variables for each principal component, as discussed in the text.

BIOCLIM no.	Variable	Eigenvectors	
		PC 1	PC 2
BIO1	Annual mean temperature	−0.227	0.235
BIO2	Mean monthly temperature range	0.238	0.205
BIO3	Isothermality	−0.195	−0.187
BIO4	Temperature seasonality	0.262	0.239
BIO5	Maximum temperature of warmest month	0.159	0.314
BIO6	Minimum temperature of coldest month	−0.321	−0.059
BIO7	Temperature annual range	0.262	0.237
BIO8	Mean temperature of wettest quarter	−0.320	0.023
BIO9	Mean temperature of driest quarter	0.029	0.334
BIO10	Mean temperature of warmest quarter	0.034	0.350
BIO11	Mean temperature of coldest quarter	−0.327	0.015
BIO12	Annual precipitation	0.149	−0.295
BIO13	Precipitation of wettest month	0.103	−0.308
BIO14	Precipitation of driest month	0.296	−0.083
BIO15	Precipitation seasonality	−0.277	−0.067
BIO16	Precipitation of wettest quarter	0.127	−0.301
BIO17	Precipitation of driest quarter	0.296	−0.134
BIO18	Precipitation of warmest quarter	0.249	−0.155
BIO19	Precipitation of coldest quarter	0.121	−0.304

clades in climate characteristics using principal component analysis in JMP (version 5.0, SAS Institute Inc., Cary, North Carolina). We plotted principal component 1 (PC 1) versus PC 2 for all representative localities to examine clustering of each clade. We also tested for spatial autocorrelation among points. We constructed predictive distribution models for the entire species, each major mtDNA clade (omitting sites where clades overlapped), and samples from the zone of overlap between the major clades.

RESULTS

Mitochondrial DNA.—We produced *Cytb* sequences for 166 individuals, of which 64 were complete (i.e., 1,143 bases) and 102 were partial. Partial sequences ranged from 186 to 801 bases; however, more than half had 700 or more base pairs. Variation across codons was as expected for mtDNA (i.e., no insertions–deletions, most variation in 3rd positions, and least variation in 2nd positions, sensu Irwin et al. [1991]), and we detected no stop codons.

The Bayesian tree, based on all *Cytb* sequences, depicts 2 well-supported mtDNA clades (Fig. 2). The northern clade ($n = 72$) is distributed from the Oregon–California border south to Santa Barbara, Kern, and Ventura counties (Fig. 1). The southern clade ($n = 94$) overlaps the northern clade in these 3 counties and extends south throughout southern California to Baja California, Mexico. These 2 major clades are sympatric along the coast near Goleta, California, as well as in the Transverse Ranges to the west of Tejon Pass (Fig. 1, inset).

The average *Cytb* genetic distance between the 2 clades within *M. californicus* was 4.46% uncorrected p (range 3.85–5.16% \pm 0.007% SE). The mean distance between sister pairs

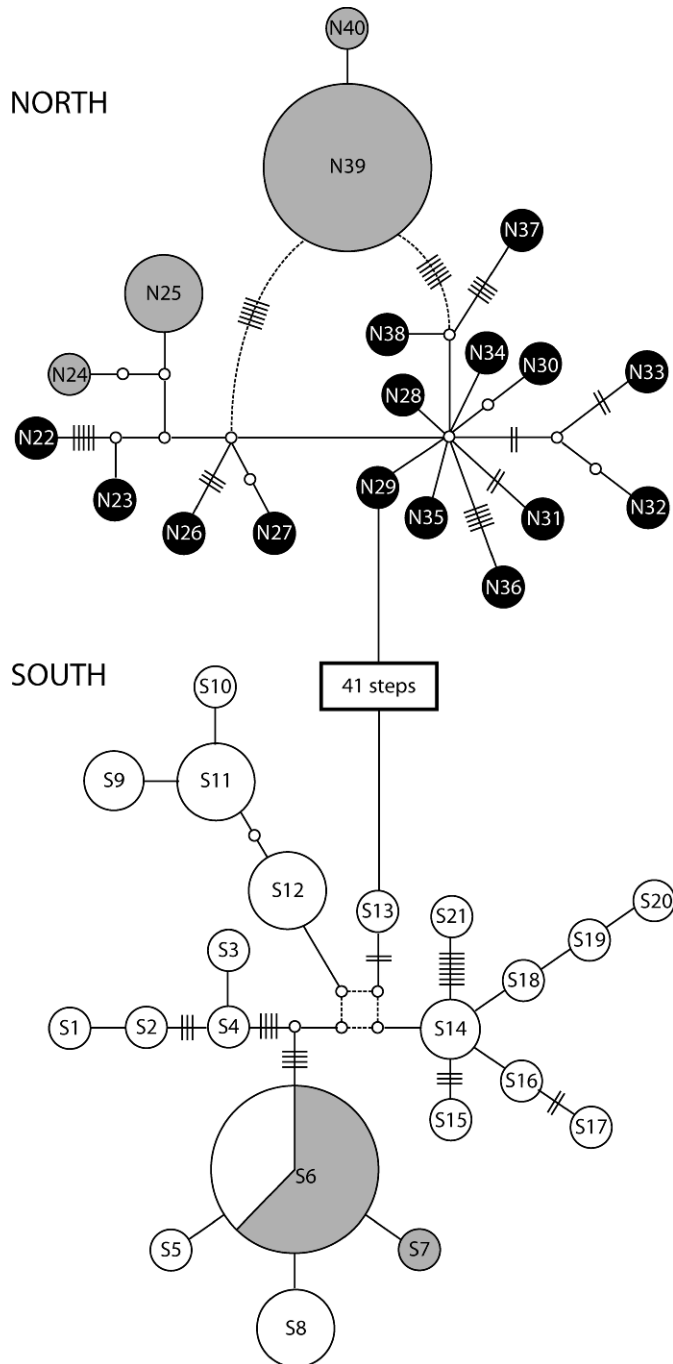


FIG. 3.—Haplotype network for complete mitochondrial cytochrome-*b* data for *Microtus californicus*. Black = northern clade; gray = area of overlap; white = southern clade. Numbers identify unique haplotypes, and small circles without numbers represent inferred haplotypes. Each branch represents 1 mutational step between haplotypes, and hash marks indicate additional mutational steps. The size of the circle indicates the frequency of that haplotype (smallest circles = 1 individual; largest circle = 8 individuals).

of *Microtus* (data from Jaarola et al. 2004) was 7.74% uncorrected *p* ($SE = 0.81\%$). Pairwise distances ranged from 0.54% ($SE = 0.314\%$) between *M. miurus* and *M. abbreviatus* to 13.35% ($SE = 0.211\%$) between *M. longicaudus* and *M.*

oregoni. Mean divergence within the northern clade was 0.66% and in the southern clade was 0.67%. Thus, the net divergence between the northern and southern clades was 3.79%.

Analyses using the TCS network, based on complete *Cytb* sequences, failed to connect the 2 major clades within the 95% confidence interval (95% *CI*) of parsimonious connections. When we adjusted the confidence intervals to force connections between haplotypes, the 2 major clades were connected by 41 steps. It is important to note that this last step only illustrates that these 2 clades are reciprocally monophyletic (as shown with our Bayesian analyses), and that the specific branch connection sites are ambiguous. We identified 40 haplotypes, 19 in the northern clade ($n = 28$) and 21 in the southern clade ($n = 36$). The northern clade had 1 loop connecting a series of terminal haplotypes to different locations near the interior of the network (Fig. 3). Most other branches radiated outward from a central unobserved haplotype. The southern clade included 1 central loop connecting most of the branches (Fig. 3).

Mismatch distributions and AMOVA.—Statistics for the mismatch distributions are summarized in Table 2. Each clade analyzed separately with complete *Cytb* sequences was indistinguishable from simulated data under an expansion model. This finding suggests that characteristics of young, expanding populations are exhibited within these clades. When we combined the clades, however, the sum of squared deviations suggested a significant difference from an expansion model ($P = 0.027$), whereas Harpending's (1994) raggedness index did not show a significant difference ($P = 0.43$). Tajima's *D* and Fu's F_S tests were significant only in the northern clade (Table 2), likely because of an expansion signal as seen in the mismatch distribution. The AMOVA indicates that most of the genetic variation in this species is found within each clade (88.64%), rather than between them (11.36%), suggesting some degree of divergence.

AP5 intron.—Most *AP5* haplotypes were represented by 1 individual or were localized geographically. Other haplotypes had broader distributions, such as haplotype 1 in the north, which is distributed from the very north of the sampled range (site 1) south to site 19. Twelve haplotypes (1–12), representing 34 specimens, were identified in northern California and 31 haplotypes (13–43), representing 104 specimens, were found in southern California. The A–T transversion at site 260 was diagnostic for the north–south split, and individuals with a “T” in this position are considered “northern type,” and those with “A” are “southern type.” In this way they match the mitochondrial clade designations, except in the contact area (haplotype designations available from the authors). Heterozygotes among *AP5* haplotypes were found throughout California. However, only intraclade mixing was observed. That is, heterozygotes were either “north–north” or “south–south.” There were no observed heterozygotes with an A–T mix at position 260.

The software TCS identified a haplotype network in which haplotypes from the north were distinct from those in the south. Because there were no interclade heterozygotes, the figure presented herein has heterozygote individuals removed for easier visualization of the pattern (Fig. 4; total network available upon request). The northern clade has 1 main

TABLE 2.—Summary statistics of mismatch distribution modeling for samples with complete cytochrome-*b* sequences. Raggedness is Harpending’s (1994) raggedness index, followed by probability of deviation from an expansion model. SSD is the sum of squared deviations, followed by the probability of deviation from expansion model. F_S is Fu’s F_S and D is Tajima’s D , each followed by P -values.

Group	<i>n</i>	Haplotypes	Polymorphic sites	Gene diversity	Nucleotide diversity	Mismatch mean (variance)	Raggedness (P)	SSD (P)	F_S (P)	D (P)
Northern clade	28	20	56	0.924 ± 0.041	0.007 ± 0.004	8.005 (14.474)	0.025 (0.209)	0.013 (0.162)	−5.127 (0.028)	−1.5620 (0.040)
Southern clade	36	21	45	0.938 ± 0.027	0.007 ± 0.004	8.308 (3.940)	0.0208 (0.216)	0.015 (0.163)	−4.676 (0.057)	−0.8404 (0.201)
Clades combined	64	41	126	0.966 ± 0.012	0.026 ± 0.013	29.661 (470.258)	0.007 (0.429)	0.032 (0.027)	−2.552 (0.248)	0.4805 (0.760)

haplotype (1), which is interior to the others. The southern clade has 4 common haplotypes (13, 19, 35, and 36), the first 3 of which are interior and have wide geographic distribution. Although northern and southern haplotypes are found in sympatry in some areas, no back connections were found between any northern and southern haplotypes.

Area of contact between clades.—The contact zone identified by the nuclear and mitochondrial data stretches for 52 km along the coastline west of Santa Barbara and from there, northeastward for 130 km (Fig. 1, inset). Populations in which *Cytb* and *AP5* haplotypes were either all northern or all southern were found several kilometers to the west and east of this region (e.g., sites 15, 32, 24, and 26).

Within this contact area, north and south *AP5* haplotypes were not always concordant with the mitochondrial clade, suggesting limited gene flow between northern and southern clades. There are 4 distinct patterns for haplotype arrangement within the contact zone (Table 3). Overall, there were 6 individuals with both northern mitochondrial and nuclear haplotypes, 29 individuals with northern mitochondrial and southern nuclear haplotypes, 4 individuals with southern mitochondrial and northern nuclear haplotypes, and 21 individuals with both southern mitochondrial and nuclear haplotypes.

Along the coastal edge of the contact zone, the frequency of *Cytb* haplotypes changes gradually from north to south and from west to east (Fig. 5). The *AP5* data demonstrate a more abrupt change from northern to southern haplotypes along these same axes. The inland, eastern portion of the contact zone has only 2 sites (23 and 25), both of which exhibit a predominance of northern mtDNA and southern nuclear haplotypes. However, nearby sites outside of this zone are southern at both markers.

Environmental differences between clades.—The localities in this study showed spatial autocorrelation under both Geary and Moran statistics (not shown). This might lead to the data being less evenly distributed than one would like. However, in the context of this study, spatial autocorrelation does not present a problem. First, climate data often are spatially autocorrelated at relatively large spatial scales (Koenig 2002) and do not affect the ability to construct predictive distribution models. Second, the present analysis is largely descriptive in nature, and we do not attempt to assign significance levels to the observed differences. Third, the distribution of voles across their range is naturally clumped into suitable habitat types, thus some spatial autocorrelation is expected.

Principal component analysis was used to compare the geographic information system data between northern and southern clades (Table 1). Given the resolution of localities and the scale of the climate layers (2.5 min), 470 unique 2.5-min grids were used. The first 2 principal components explain 44.8% and 35.5% of the variation, respectively, for the entire species.

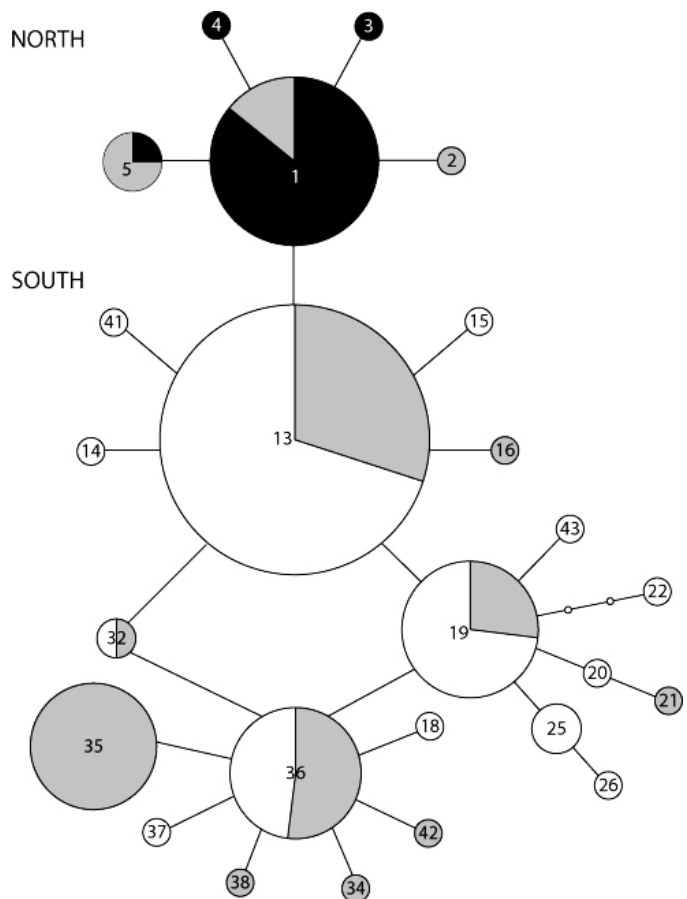


FIG. 4.—Haplotype network for the nuclear acid phosphatase V phased data for *Microtus californicus*, excluding heterozygous individuals (see text for explanation). Black = northern clade; gray = area of overlap; white = southern clade. Numbers identify unique haplotypes, and small circles without numbers represent inferred haplotypes. Each branch represents 1 mutational step between haplotypes. The size of the circle indicates the frequency of that haplotype (smallest circles = 2 individuals; largest circle = 46 individuals).

TABLE 3.—Frequency of northern and southern haplotypes in contact zone (see inset in Fig. 1). Only those individuals and sites with both cytochrome-*b* (*Cytb*) and acid phosphatase V (*AP5*) data are shown ($n = 60$).

Site	<i>Cytb</i>		<i>AP5</i>	
	North	South	North	South
17	3	1	4	—
18	1	—	—	1
19	3	2	5	—
20	5	12	1	16
21	1	7	—	8
23	1	—	—	1
25	21	3	—	24
Total	35	25	10	50

The 3 highest positive loadings on PC 1 were precipitation of driest month, precipitation of driest quarter, and temperature annual range. The 3 most negative loadings on PC 1 were mean temperature of coldest quarter, minimum temperature of coldest month, and mean temperature of wettest quarter. The 3 highest positive loadings on PC 2 were maximum temperature of warmest month, mean temperature of driest quarter, and mean temperature of warmest quarter. The 3 most negative loadings on PC 2 were precipitation of wettest month, precipitation of coldest quarter, and precipitation of wettest quarter.

When PC 1 was plotted against PC 2 (Fig. 6), northern clade points were spread across both axes. However, southern clade points were restricted largely to the negative end of the PC 1 axis at the periphery of the northern clade points, suggesting that the 2 clades occur in environments with a different combination of climate parameters. Some sites extended onto the positive side of the x-axis, but only in the upper portion of the PC 2 axis. Analysis of variance results indicated significant differences between the distribution of the northern and southern points along each axis ($P \leq 0.005$).

When we predicted the potential distribution of the entire species using 19 climate variables, DIVA-GIS gave a close approximation of the known distribution of the species with no high predictions where there are no known records or unlikely habitats (Fig. 7). When we predicted the potential distribution of the northern clade alone, minus any mixed samples in the contact zone, we, again, found that DIVA-GIS accurately predicted the distribution of the entire species (Fig. 7B). However, when we did the reciprocal experiment for the southern clade, we found good prediction for the species' known distribution in southern California, but only patchy prediction into northern California (Fig. 7C). A prediction using only the points in the overlap zone (Fig. 7D) predicted well around the zone of overlap and patches in the coastal hills in southern California, but also predicted areas on the eastern side of the San Francisco Bay area (e.g., Alameda and Contra Costa counties). This latter experiment relied on very few points ($n = 17$).

DISCUSSION

Species can be identified as distinct if they form independent evolutionary lineages (Simpson 1961). However, what drives

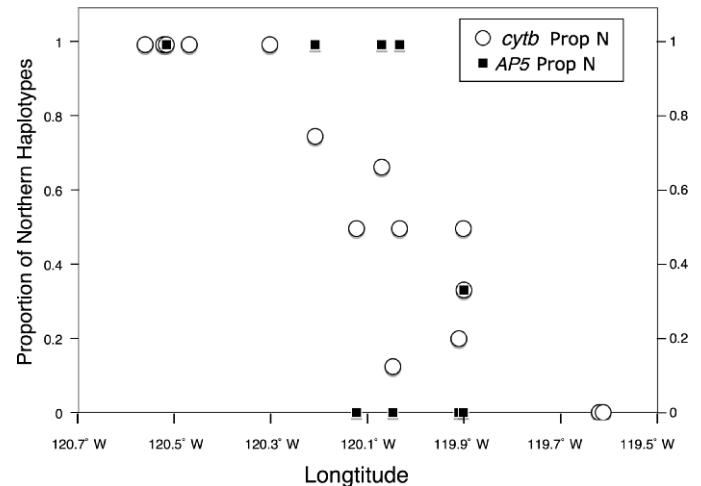


FIG. 5.—Proportion of northern mitochondrial DNA and acid phosphatase V haplotypes across a contact zone between northern and southern clades of *Microtus californicus* near Santa Barbara, California. Points on west are near Vandenburg Air Force Base and points on east are near Carpinteria, California.

incipient speciation is more difficult to discern. Environmental, behavioral, morphological, or genetic factors all can contribute to the process of speciation. More likely, species differentiation is influenced by a combination of these factors, and not only by any particular one. In this study we examine speciation in a species using both molecular and environmental data sets. Examination of the data presented here suggests that *M. californicus* shows early stages of speciation on several levels: both the northern and southern mitochondrial and nuclear clades are relatively well differentiated with little apparent gene flow; the north-south pattern is consistent with geographic barriers, such as the Transverse Ranges; and there are differences in the climate experienced by the northern and southern clades. Here, we discuss these factors as well as the dynamics of the contact between the 2 clades and place them in the context of phylogeography of other Californian vertebrates.

Phylogenetic differentiation.—Recent work on systematics of *Microtus* has made available comparable molecular data for multiple species (Conroy et al. 2001; Conroy and Cook 2000; Jaarola et al. 2004). The average distance between the 2 clades of *M. californicus* (4.46%) is within the range of interspecific distances (0.54–13.35%) for the species of *Microtus* studied by Jaarola et al. (2004). Although this alone does not address whether the 2 clades investigated here are incipient species, it provides a context in which we can examine their genetic distance. Comparable distances are found between species such as *M. townsendii* and *M. canicaudus* (5.074%, $n = 6$ pairs averaged), and a smaller distance exists between *M. miurus* and *M. abbreviatus* (0.54%, $n = 4$ pairs averaged). These 2 pairs are somewhat relevant to the process of speciation. *M. townsendii* and *M. canicaudus* have a fixed chromosomal difference (Hsu and Johnson 1970). *M. abbreviatus* and *M. miurus*, although they do not have a karyotypic difference, likely diverged because of isolation of 1 species, *M. abbreviatus*, on islands in the Bering Sea at the end of the Pleistocene.

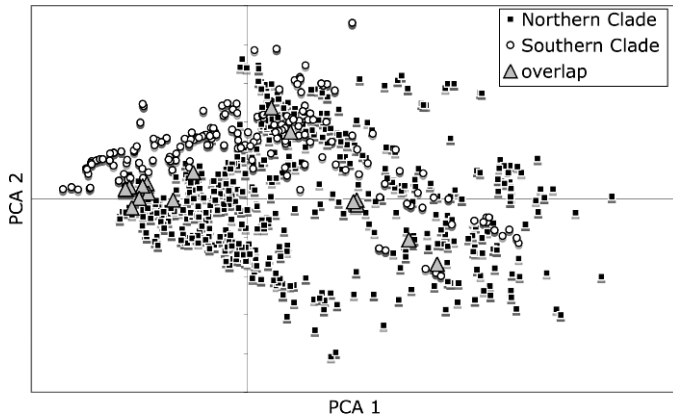


FIG. 6.—Principal component analysis plot of axes 1 and 2 from analysis of 19 BIOCLIM climate variables for the northern and southern clades of *Microtus californicus*. Each point represents an individual specimen. Axis 1 explained 44.8% and axis 2 explained 35.5% of overall variation in the data.

To gauge the relative depth of divergence between the clades of *M. californicus*, we estimated the depth of divergence between *M. mexicanus* and *M. californicus*. This divergence had a mean of 10.19% ($SE = 0.079\%$) uncorrected p . Given the interclade divergence of 4.46%, a very rough examination would suggest that these clades have been separated for nearly half the time they have been separated from *M. mexicanus*, and each clade has shown limited introgression into the range of the other. Unfortunately, given the lack of precision about the historical movement of *M. californicus*, we cannot infer much about the geography of its diversification. Further refinements of this analysis will be explored elsewhere.

It is difficult to ascertain a direction of movement by either clade from analyses of our markers. Currently, the contact zone may be described largely as a bimodal hybrid zone (Harrison and Bogdanowicz 1997). That is, there is overlap with few intermediates (e.g., heterozygotes) in our *AP5* marker. The mismatch analysis suggested that each clade is characterized by an expansion, but combined they suggest long-term stability. Further evidence for within-clade patterns of expansion were seen with the northern clade's significantly negative Fu's F_S and Tajima's D values. This might suggest that northern populations of voles have been able to expand more rapidly over continuous habitat, in contrast with the more stable southern populations, which are restricted to habitat patches.

Across the entire contact area, there is a discrepancy between the 2 markers (Table 3). The preponderance of northern mtDNA haplotypes but southern *AP5* haplotypes suggests that gene flow here is not random. Given that 1 marker is maternally inherited and the other biparentally inherited, plus the shape of the clines (Fig. 5), patterns could reflect sex-biased differences in gene flow. More autosomal and sex-linked markers and denser sampling may provide better resolution.

It is possible that voles have moved passively and their geographic range has shifted as their respective habitats fluctuated in size and shape. Given the differences in habitat distribution in northern and southern California, this could have

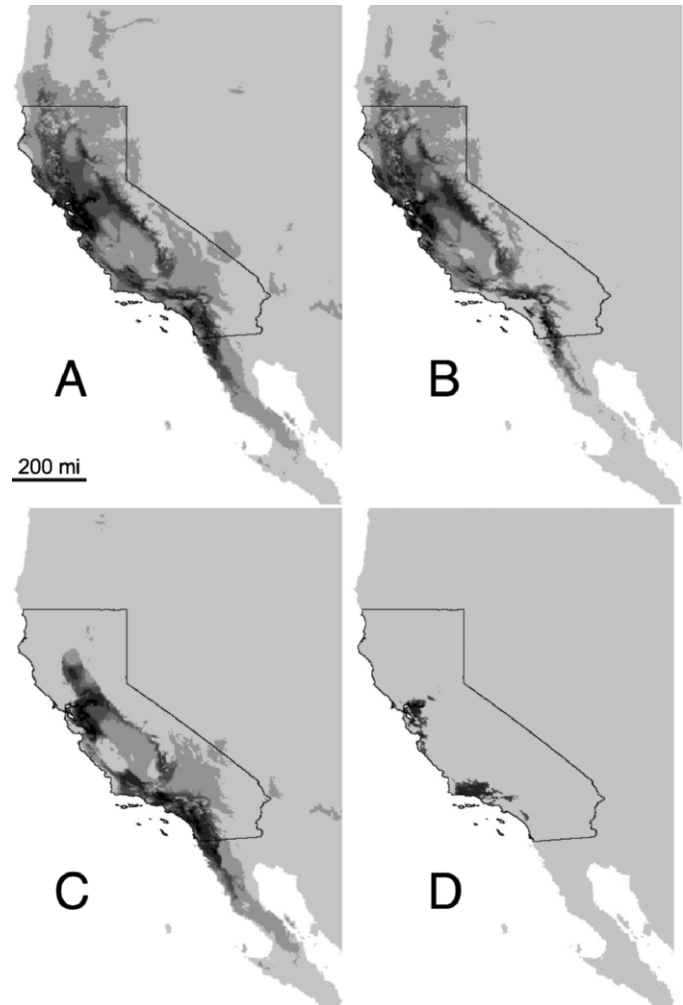


FIG. 7.—DIVA-GIS predictive map of distribution of *Microtus californicus* based on 19 BIOCLIM climate variables. A) Map based on localities for all individuals. B) Map based only on northern individuals. C) Map based only on southern individuals. D) Map based only on individuals in or near the zone of overlap. Darker shades on maps indicate areas with higher probability of occurrence.

immediate effects on genetic diversity. There is the possibility that long-distance dispersers venture through inhospitable habitat to reach potential new sites. However, in the longer term, voles probably have moved more often within riparian zones and less frequently between drainages (Neuwald 2002). Identifying such trends would require analysis of more rapidly evolving markers (e.g., microsatellites—Neuwald 2002), as well as larger sample sizes. This would help us determine whether these contact zones are better characterized as tension zones (Key 1968) or are caused by abutting environmental biomes, both likely scenarios.

Comparative phylogeography.—Calsbeek et al. (2003), Feldman and Spicer (2006), and Sgariglia and Burns (2003) note that many vertebrate taxa in California have significant phylogeographic structure. In several cases, the divergence involves differentiation on different mountain ranges, such as the Transverse Ranges. Chatzimanolis and Caterino (2007) have examined this area in more detail using Brooks parsimony

analysis and suggest that the Transverse Ranges might be better described as having multiple units (i.e., eastern, central, and western regions). Although it is clear that our data are consistent with a break between the central and western regions, we also have overlap of clades well within the western region. Although no other vertebrate is known to have a phylogeographic break perfectly coincident with *M. californicus*, it is clear that many species have diverged roughly by north–south segregation at a larger scale across California. In birds, for example, Sgariglia and Burns (2003) found that the California thrasher exhibited a similar break at the Transverse Ranges. Similar patterns are seen in reptiles as well (Feldman and Spicer 2006). Although other mammals exhibit geographic genetic structure across California (e.g., woodrats—Matocq 2002), it is not coincident with the break in *M. californicus*. As Chatzimanolis and Caterino (2007) cautioned, we should be concerned about pseudocongruence because of the disparity in timing of divergence of these various taxa.

The Transverse Ranges suggested to be important in within-species differentiation may be primarily barriers to secondary contact, although sometimes leaky ones. It would be valuable to identify where these clades arose, perhaps in centers of glacial refugia. Future comparative work should focus attention on zones of contact of various ecological communities, as well as decipher the origins of those unique communities. Southern California, with its steep terrain, may hold many examples of secondary contact of recently diverged populations or species. Indeed, Davis et al. (2007) found that the Transverse Ranges are hotspots of mammalian diversity, likely because of the confluence of several different communities. The mismatch analysis suggested that both clades of *M. californicus* may be expanding and therefore are still in the process of secondary contact. Other taxa also may be in this stage.

We did not focus on subspecific taxonomy, largely because we lacked sufficient sample sizes within each subspecies to make valid comparisons. However, it is worth noting briefly that genetic structure in the 2 clades was not partitioned along subspecific boundaries. More-detailed analyses that include samples from all subspecies will be required before we can assess whether the distinctions among subspecies exist within the more shallow clades (Fig. 2). It is noteworthy that Kellogg (1918) identified just as much morphological divergence between other subspecies outside the region of north–south genetic divergence identified herein. Clearly, mtDNA and nDNA are recovering somewhat different structure than the pelage and skull shape characters Kellogg used in his study. Other studies have noted the discrepancy between morphology-based taxonomy and mitochondrial phylogeography (e.g., *M. longicaudus*—Conroy and Cook 2000); thus it is not surprising to find this discrepancy. Further work should include morphological variation in context with these molecular results.

Climatic differences between clades.—Our use of geographic information system analysis as a descriptive method in conjunction with molecular data suggests that the 2 clades of voles studied here experience somewhat different climates. Specifically, the southern clade occupies drier and warmer habitats than the northern clade, and the northern clade inhabits

a broader array of habitats than does the southern clade. Southern California is generally warmer and drier than northern California, therefore, the 1st observation is concordant with physiography. However, we need to examine habitat selection in more detail to ascertain what differentiates the habitats of the 2 clades. They may have many more similarities in habitat selection than suggested by this analysis, such as particular kinds of grass for forage or soil types for burrowing.

Although these 2 clades have similar levels of genetic diversity at the *Cytb* locus (Table 2), they differ in habitat diversity. The northern clade has much more climatic variability than the southern clade, as seen in the principal component analysis plots and modeled distributions, even though the southern clade exists over more topographic diversity (e.g., from sea level to over 7,000 feet). This contradicts a pattern observed by Guralnick (2006), who showed that some western North American mammals (not including *M. californicus*) display less variability in temperature among habitats in the northern part of their ranges. It will be necessary to examine all habitats, occupied and not occupied, within the range of this species before drawing any conclusions about the evolution of differences in climatic preferences.

Identifying climatic habitat requirements may allow us to determine the location of past refugia, how animals have responded to recent climate change, and where vole populations may go given predictions of climate change in California (e.g., Ruegg et al. 2006). What would be most intriguing would be predictions of the movement of the hybrid zone given a variety of climate change scenarios and the effects on gene flow. Hopefully, the apparent differences in the climate experienced by each clade will aid in modeling its future distribution.

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APPENDIX I

Specimens examined.—The 178 specimens for which molecular data were produced or examined in this study are listed here by locality number (as depicted in Fig. 1) and by museum voucher number (or by other identifying number if lacking a voucher), followed by GenBank number(s) in parentheses. A "V" prefix is a laboratory number for specimens with no voucher; MVZ refers to Museum of Vertebrate Zoology, University of California, Berkeley, California; CPP refers to Cal Poly Pomona, Pomona, California; M prefix is for the San Bernardino County Museum of Natural History, Redlands, California; SBMNH refers to the Santa Barbara Museum of Natural History; UAM refers to the University of Alaska Museum; JRD refers to John R. Demboski. Latitude and longitude of all specimens, as well as *AP5* or *Cytb* clade, or both, assignment and phase can be supplied by the authors.

Locality 1: Shasta Valley Wildlife Area, Siskiyou County, California—MVZ 199493 (EF506189), MVZ 199494 (EF506082, EF505907), MVZ 199495 (EF506083, EF505906).

Locality 2: Hopland Research Station, Mendocino County, California—MVZ 216784 (EF506052, EF506027).

Locality 3: 2.5 km S junction CA-20 and CA-16, Bear Creek, Colusa County, California—MVZ 206896 (EF506033, EF505898), MVZ 206897 (EF506034, EF505897).

Locality 4: 3 km ENE junction I-505 and CA-16, Cache Creek, Yolo County, California—MVZ 206898 (EF506032, EF505924).

Locality 5: Point Reyes National Seashore, Marin County, California—MVZ 191734 (EF506074, EF506031), MVZ 196008 (EF506075, EF505978).

Locality 6: China Camp, Marin County, California—MVZ 216786 (EF506106, EF506026).

Locality 7 (localities grouped on map): Briones Regional Park, Contra Costa County, California—MVZ 198292 (EF506096, EF505908); Brooks Island, Contra Costa County, California—MVZ 167262 (AF163891, EF505909), MVZ 167263 (EF506071, EF505910).

Locality 8: Corral Hollow Creek, San Joaquin County, California—MVZ 199154 (EF506188).

Locality 9: Merced Grove, Yosemite National Park, Mariposa County, California—MVZ 201675 (EF506180, EF505905).

Locality 10 (localities grouped on map): Black's Creek, 1.9 miles W Coulterville via Highway 132, Mariposa County, California—MVZ 207434 (EF506178, EF505901); Horseshoe Bend Recreation Area, Lake McClure, Mariposa County, California—MVZ 207440 (EF506177, EF505900); Kelsey Ranch, 1 km S Kelsey Reservoir, Merced County, California—MVZ 207431 (EF506179, EF505902);

La Grange, Stanislaus County, California—MVZ 207427 (EF506098, EF505903), MVZ 207428 (EF506095), MVZ 207425 (EF506097, EF505904).

Locality 11: Sweetwater Mine, Mariposa County, California—MVZ 216595 (EF506105, EF506012), MVZ 216596 (EF506104).

Locality 12: Hastings Natural History Reservation, Monterey County, California—MVZ 149763 (EF506141), MVZ 198787 (EF506079, EF505966), MVZ 198788 (EF506080, EF505965).

Locality 13: Bear Gulch, Pinnacles National Monument, San Benito County, California—SBMNH 2374 (EF506187).

Locality 14: Tecopa, Inyo County, California—no voucher (EF506078).

Locality 15: Vandenburg Air Force Base, Santa Barbara County, California—SBMNH 2682 (EF506185), SBMNH 2679 (EF506186), V11.7 (EF506085), V14.10 (EF506194, EF505970), V14.11 (EF506195), V14.12 (EF506091, EF505976), V14.13 (EF506092, EF505969).

Locality 16: Balito Creek, 7 miles W Gaviota, Santa Barbara County, California—MVZ 85151 (EF506173).

Locality 17: Gaviota State Beach, Santa Barbara County, California—MVZ 216066 (EF506127, EF505950), MVZ 216067 (EF506124, EF505956), MVZ 216068 (EF506116, EF505911), MVZ 216113 (EF506114, EF505939).

Locality 18: Tajiguas landfill, Santa Barbara County, California—V14.4 (EF506190), V14.5 (EF506089, EF505974).

Locality 19 (localities grouped on map): El Capitan State Beach, Santa Barbara County, California—MVZ 216065 (EF506129, EF505952), MVZ 216115 (EF506112, EF505940); Refugio State Beach, Santa Barbara County, California—MVZ 216069 (EF506128, EF505947), MVZ 216070 (EF506137, EF505951), MVZ 216116 (EF506113, EF505913).

Locality 20 (localities grouped on map): Bishop Ranch, Goleta, Santa Barbara County, California—V14.6 (EF506090, EF505973), V14.7 (EF506191, EF505972), V14.8 (EF506192, EF505971), V14.9 (EF506193, EF505967); Coal Oil Pt. Reserve, Santa Barbara County, California—MVZ 215976 (EF506111, EF505916), MVZ 216058 (EF506133, EF505920), MVZ 216059 (EF506132, EF505919), MVZ 216060 (EF506135, EF505918), MVZ 216061 (EF506134, EF505954), MVZ 216063 (EF506120, EF505914), MVZ 216064 (EF506121, EF505943), MVZ 216100 (EF506136, EF505949), MVZ 216103 (EF506123, EF505946), MVZ 216108 (EF506118, EF505938); Del Sol Open Space, Goleta, Santa Barbara County, California—V14.1 (EF506086, EF505968), V14.2 (EF506087, EF505977), V14.3 (EF506088, EF505975).

Locality 21: Sedgwick Reserve, Santa Barbara County, California—MVZ 216071 (EF506139, EF505953), MVZ 216072 (EF506126, EF505917), MVZ 216073 (EF506107, EF505937), MVZ 216074 (EF506108, EF505912), MVZ 216076 (EF506047, EF505929), MVZ 216077 (EF506048, EF505928), MVZ 216078 (EF506049, EF505927), MVZ 216079 (EF506050, EF505926).

Locality 22: French Meadows, Piute Mountains, Kern County, California—MVZ 60289 (EF506176).

Locality 23: Wind Wolves Preserve, Kern County, California—MVZ 200047 (EF506065, EF505961).

Locality 24: Castac Valley, on Digier Road off Lebec Road, Kern County, California—M 2985 (EF506039, EF506020), M 2986 (EF506040, EF506019).

Locality 25 (localities grouped on map): 1.3 miles SE (by road) Mt. Pinos Ranger Station, Ventura County, California—MVZ 208672 (EF506146, EF506003), MVZ 208673 (EF506147, EF506008); Chuchupate Campground, Ventura County, California—SBMNH 3251 (EF506182), SBMNH 3252 (EF506181), MVZ 200048 (EF506057, EF505963), MVZ 200049 (EF506058, EF505962), MVZ

200050 (EF506197, EF505935), MVZ 200051 (EF506053, EF505934), MVZ 200052 (EF506054, EF505932), MVZ 200053 (EF506055, EF505925), MVZ 200054 (EF506056), MVZ 200055 (EF506093, EF505933), MVZ 200056 (EF506196, EF505899), MVZ 200057 (EF506094, EF505936), MVZ 208663 (EF506142, EF506000), MVZ 208664 (EF506143, EF505894), MVZ 208665 (EF506144, EF506002), MVZ 208666 (EF506145, EF505996), MVZ 208675 (EF506148, EF505995), MVZ 208676 (EF506149, EF506006), MVZ 208677 (EF506150, EF506005), MVZ 208678 (EF506151), MVZ 208679 (EF506152, EF505998), MVZ 208680 (EF506153, EF506001), MVZ 208681 (EF506154, EF506007), MVZ 208698 (EF506155, EF505999), MVZ 208699 (EF506156, EF505994).

Locality 26 (localities grouped on map): 2 miles SE Three Points, Los Angeles County, California—MVZ 198395 (EF506081, EF505964); Pyramid Lake off of Smokey Bear exit, Los Angeles County, California—M 2987 (EF506041, EF506018), M 2988 (EF506042, EF506017), M 2989 (EF506043, EF506016), M 2990 (EF506044, EF506015).

Locality 27: Edwards AFB, 5 miles S, 3 miles E Rosamond, Los Angeles County, California—MVZ 182976 (EF506073, EF505979).

Locality 28: Littlerock Dam, Los Angeles County, California—M 2983 (EF506037, EF506022), M2984 (EF506038, EF506021).

Locality 29: Jackson Lake, Los Angeles County, California—M 2991 (EF506045, EF506014), M 2992 (EF506046, EF506013).

Locality 30: Malibu Mountains, above Topanga Canyon, Los Angeles County, California—CPP 00454 (EF506160).

Locality 31 (localities grouped on map): Bear Creek, Ventura County, California—MVZ 200871 (EF506063, EF505959); Lower Rose Lake, Ventura County, California—MVZ 200874 (EF506059, EF505957), MVZ 200875 (EF506061, EF505931), MVZ 200876 (EF506064, EF505960); Wheeler Gorge Campground, Ventura County, California—MVZ 200872 (EF506060, EF505958), MVZ 200873 (EF506062, EF505930).

Locality 32 (localities grouped on map): 1 mile W Carpinteria, Santa Barbara County, California—MVZ 84837 (EF506172); Carpinteria marsh, E side Sandyland Cove Road, Santa Barbara County, California—SBMNH 3227 (EF506183); Carpinteria Saltmarsh Reserve, Santa Barbara County, California—MVZ 216052 (EF506140, EF505923), MVZ 216053 (EF506138, EF505922), MVZ 216054 (EF506131, EF505948), MVZ 216055 (EF506109, EF505915), MVZ 216056 (EF506119, EF505945), MVZ 216057 (EF506110, EF505944), MVZ 216097 (EF506130, EF505921), MVZ 216098 (EF506125, EF505955), MVZ 216102 (EF506122), MVZ 216106 (EF506115, EF505941), MVZ 216107 (EF506117, EF505942).

Locality 33: Ventura, Ventura County, California—MVZ 84923 (EF506174), MVZ 84926 (EF506175).

Locality 34: Pt. Mugu, Ventura County, California—SBMNH 2667 (EF506184).

Locality 35 (localities grouped on map): San Antonio Dam, Los Angeles County, California—CPP 00723 (EF506169); Cal Poly, Kellogg Campus, Pomona, Los Angeles County, California—CPP 00460 (EF506168), CPP 00457 (EF506162); Cal Poly, Voorhis Campus, San Dimas, Los Angeles County, California—CPP 00453 (EF506159); County Fair Grounds, Pomona, Los Angeles County, California—CPP 00458 (EF506163); Pomona, Los Angeles County, California—CPP 00455 (EF506161); San Gabriel Mountains (Experimental Forest), Los Angeles County, California—CPP 00937 (EF506170); San Gabriel Mountains, Big Dalton Canyon, Los Angeles County, California—JRD 275 (EF506167, EF506004); Banning Canyon, north of Banning, Riverside County, California—M 2980 (EF506035, EF506025), M 2981 (EF506036, EF506024), M 2982 (EF506023); Corona, Riverside County, California—CPP 00459

(EF506164); Horse Meadows, San Bernardino Mountains, San Bernardino County, California—CPP 00450 (EF506157); Prado Basin, Chino, San Bernardino County, California—CPP 00854 (EF506166); Upland, San Bernardino County, California—CPP 00485 (EF506165).

Locality 36 (localities grouped on map): Bluff Lake, San Bernardino County, California—MVZ 198769 (EF506070, EF506009), MVZ 198782 (EF506068, EF506010); 1/2 way up Mt. Baldy Road, Mt. Baldy, San Bernardino County, California—CPP 00451 (EF506158); 4.8 miles W Forest Service Station on Highway 38, San Bernardino County, California—MVZ 198784 (EF506069, EF506029); Cushenbury Spring, S of Lucerne Valley, San Bernardino County, California—UAM 67125 (EF506072); Forest Service Station, Fawnskin, San Bernardino County, California—MVZ 198775 (EF506066, EF506030); Metcalf Meadow, San Bernardino Mountains, San Bernardino County, California—MVZ 198779 (EF506067, EF506011).

Locality 37: Starr Ranch, Orange County, California—V20.14 (EF506103, EF505991), V20.15 (EF505992), V20.16 (EF505993).

Locality 40 (localities grouped on map): Lake Skinner, Riverside County, California—V20.9 (EF506101, EF505987), V20.10 (EF505988), V20.11 (EF505895), V20.12 (EF506102, EF505989); Rawson Canyon, Riverside County, California—V20.13 (EF505990).

Locality 41: Tule Creek, intersection highways 79 and 371, Riverside County, California—M2977 (EF506051, EF506028).

Locality 42: Santa Margarita Ecological Reserve, Riverside County, California—V20.5 (EF505984), V20.6 (EF505896), V20.7 (EF505985), V20.8 (EF505986).

Locality 43: Camp Pendleton, San Diego County, California—V20.1 (EF505980), V20.2 (EF506099, EF505981), V20.3 (EF505982), V20.4 (EF506100, EF505983).

Locality 44: La Jolla, San Diego County, California—no laboratory number (EF506076); San Diego, San Diego County, California—no laboratory number (EF506077).

Locality 45: 0.5 mile S La Mision Arroyo Martinez, Baja California del Norte, Mexico—MVZ 148251 (EF506171).