The Auk 120(2):346–361, 2003

PHYLOGEOGRAPHY OF THE CALIFORNIA THRASHER (*TOXOSTOMA REDIVIVUM*) BASED ON NESTED-CLADE ANALYSIS OF MITOCHONDRIAL-DNA VARIATION

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ABSTRACT.—Distribution of genealogical lineages within a species is likely the result of a complicated series of ecological and historical events. Nested-clade analysis is specifically designed as an objective phylogeographic approach for inferring evolutionary processes on a spatial and temporal scale for small subclades within a larger set of intraspecific relationships. Here, we use nested-clade analysis as well as other phylogeographic methods to investigate the evolutionary history of California Thrasher (*Toxostoma redivivum*) populations. Inferences resulting from nested clade analysis suggest a history that includes past fragmentation, range expansion, and isolation-by-distance. Along with root information, those inferences enable the construction of a biogeographic scenario for this species involving general southern ancestry, an early north–south division, northward range expansion, and a southward back-expansion into an already populated southern region. Isolation-by-distance is also identified, particularly in southern California, indicating that gene flow between localities does occur but is restricted. Many conclusions drawn from this study are concordant with geologic data as well as phylogeographic scenarios drawn for other codistributed California taxa. *Received 8 April 2002, accepted 2 February 2003.*

RESUMEN.—La distribución de linajes genealógicos al interior de una especie probablemente es el resultado de una complicada serie de eventos ecológicos e históricos. El análisis de clados anidados fue diseñado específicamente como un método filogeográfico objetivo para inferir procesos evolutivos en escalas espaciales y temporales para pequeños subclados inmersos en un grupo más amplio de relaciones intraespecíficas. Aquí utilizamos el análisis de clados anidados y otros métodos filogeográficos para estudiar la historia evolutiva de las poblaciones de *Toxostoma redivivum*. Las inferencias hechas con base en el análisis de clados anidados sugieren una historia que incluye fragmentación, expansión del rango y aislamiento por distancia. Junto con información sobre la raíz del árbol, estas inferencias permiten construir un escenario biogeográfico para esta especie. Este escenario sugiere que las poblaciones del sur son ancestrales y que hubo una división norte–sur temprana, una expansión hacia el norte y una expansión secundaria hacia el sur a un área previamente ocupada. También se identifica aislamiento por distancia, particularmente en el sur de California, lo que indica que aunque hay flujo génico entre localidades, éste es restringido. Muchas de las conclusiones de este estudio concuerdan con datos geológicos y escenarios filogeográficos propuestos para otros taxa con distribuciones similares en California.

PHYLOGEOGRAPHY USES GEOGRAPHIC distributions and genealogical relationships to infer biogeographic and demographic history of an individual species or group of closely related taxa. Current distributions and relationships among lineages are likely the result of a dynamic history, making a fine-scaled phylogeographic approach necessary for adequate historical interpretation. Recent advances in population genetics, such as nested cladistic analysis, can detect geographical associations and separate current population structure from historical processes for an entire set of intraspecific relationships and smaller evolutionary subsets (clades) independently (Templeton 1998). Although nested cladistic analysis is routinely used in intraspecific studies in a wide variety of organisms, few ornithological studies have explored its utility. Because this method performs well in species with low levels of genetic divergence, it has strong potential as a method for inferring population-level history in avian species.

Vertebrates inhabiting California and surrounding areas have been the focus of many such phylogeographic studies. That region has experienced a complicated geologic history

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involving multiple climatic and topographic changes. Because of this, California holds many endemic taxa, many of which demonstrate substantial variation across their range. Although there have been a number of phylogeographic studies for California vertebrate taxa (e.g. Smith 1979, Moritz et al. 1992, Tan and Wake 1995, Rodriguez-Robles et al. 1999, Maldonado et al. 2001), very few (e.g. Cicero 1996, Barrowclough et al. 1999) have focused on birds of that region.

The California Thrasher (*Toxostoma redivivum*) is a resident bird of California, for which two subspecies have been described (American Ornithologists' Union 1957). Using primarily chaparral habitat, this species' distribution forms a more-or-less continuous ring around the Central Valley and extends into southern California and northern Baja California (Fig. 1). Ranges of the southern *T. r. redivivum* and northern *T. r. sonomae* meet along the Southern Coast Range near Monterey Bay and in the western slope of the Sierra Nevada in El Dorado County (Grinnell and Miller 1944), although morphological variation has recently been described as clinal (Cody 1998). This sedentary species shows high site-fidelity and low postnatal dispersal (Cody 1998), suggesting low gene flow among populations relative to other birds.

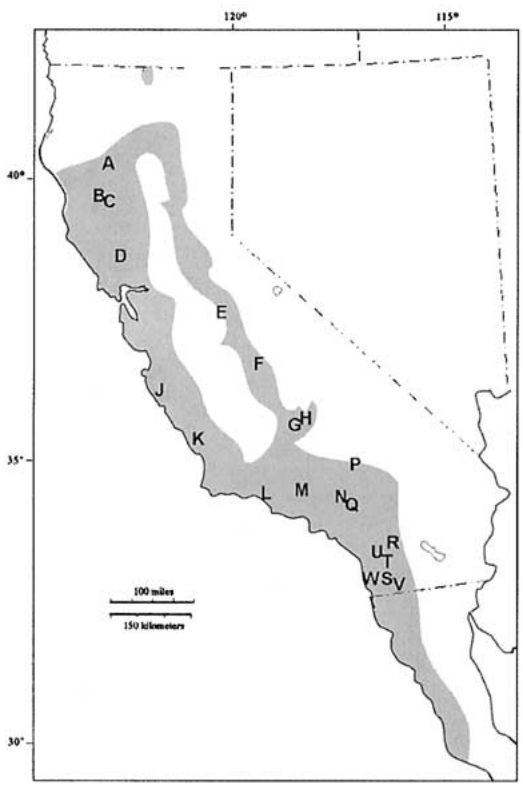

FIG. 1. Distribution of the California Thrasher (after Cody 1998), including sampling localities lettered to correspond with Table 1.

Presented here is the phylogeography of the California Thrasher, based on nested-clade analysis of mtDNA data. Here, we use data from three mitochondrial gene regions (cytochrome *b* [cyt *b*], ATP synthase 6 [ATPase6], and ATPase8) to describe patterns of geographic structure in this species. Using traditional phylogenetic approaches as well as nested cladistic analysis, we infer what evolutionary processes might be responsible for the pattern of geographic structure. Once we infer spatial and temporal organization of the inferred processes, our results are interpreted within the biogeographic context of (1) the geologic history of this region and (2) the phylogeographic patterns proposed for other codistributed taxa of similar age ("comparative phylogeography").

METHODS

Taxon sampling.—We collected 50 California Thrashers and obtained tissue samples from 14 others from museum collections (Table 1). In total, we sampled 64 individuals representing 21 localities throughout the range of the California Thrasher (Fig. 1). Breast muscle, liver, and heart tissues were taken from collected birds and preserved in 100% EtOH, frozen at –80°C, or both. Le Conte's Thrasher (*T. lecontei*, LSUMZ B-30364) and Crissal Thrasher (*T. crissale*, LSUMZ B-30563) were sequenced as outgroup taxa based on their relationship as a monophyletic sister group to the California Thrasher (Zink et al. 1999).

Molecular lab methods.—DNA was extracted from breast muscle using the QIAmp DNA MiniKit (Qiagen, Valencia, California) tissue protocol. Template DNA was amplified using avian-specific primers for fragments of cyt *b* (1,107 bp), and a continuous strand containing ATPase6 (668 bp), ATPase8 (158bp), transfer RNA-Lysine (tRNA-Lys; 70 bp), and a small portion of cytochrome oxidase subunit 2 (COII; 83 bp). Cytochrome *b* was sequenced in three overlapping segments using primer pairs H15298/L14830, H15710/L15184, and H16108/L15635 (Groth 1998). Primers A8PWL (Hunt et al. 2001) and H9906 (5'-ATT GGG ATT AGA TGT TTT CTT GGA G-3') were used for ATPase6, and primers H9481 (3'-GAG TAG GCC TAA TAG GTT GAC TAA TA-5') and CO2QL (Hunt

TABLE 1. Locality and voucher information for 64 individual California Thrashers from 21 localities (A–W). Localities labeled with "?" indicates lack of specific locality data. SDSU = Vertebrate Collections, San Diego State University; MVZ = Museum of Vertebrate Zoology, University of California (Berkeley); SDNHM = San Diego Natural History Museum; LSUMZ = Collection of Genetic Resources, Louisiana State University Museum of Natural Science.

Site	Ind.	County (CA)	Voucher number	Locality	Latitude	Longitude
A	1	Shasta	SDSU 2317	4.0 km south of Knob Peak	40°22.015'	122°56.170'
B	1	Lake	SDSU 2311	6.4 km south, 1.6 km east of Hull Mt.	39°27.837'	122°54.559'
C	1	Colusa	SDSU 2312	1.6 km north, 8 km east of Goat Mt.	39°17.306'	122°34.915'
	2	Colusa	SDSU 2313	1.6 km north, 8 km east of Goat Mt.	39°16.939'	122°35.161'
	3	Colusa	SDSU 2314	1.6 km north, 8 km east of Goat Mt.	39°16.939'	122°35.161'
	4	Colusa	SDSU 2315	1.6 km north, 8 km east of Goat Mt.	39°16.645'	122°35.402'
	5	Colusa	SDSU 2316	1.6 km north, 8 km east of Goat Mt.	39°16.764'	122°35.141'
D	1	Napa	LSUMZ B16576	9.9 km east of Sugarloaf Mt.	38°38.240'	122°24.500'
	2	Napa	LSUMZ B16577	9.9 km east of Sugarloaf Mt.	38°38.240'	122°24.500'
E	1	Tuolumne	SDSU 2318	6.4 km north, 6.4 km west of Coulterville	37°46.204'	120°15.501'
	2	Tuolumne	SDSU 2319	6.4 km north, 6.4 km west of Coulterville	37°45.954'	120°15.525'
	3	Mariposa	SDSU 2320	6.4 km north, 4 km west of Coulterville	37°44.708'	120°14.813'
F	1	Fresno	SDSU 2321	4.8 km south, 11.2 km west of Hume Lake	36°44.595'	119°03.625'
	2	Fresno	SDSU 2322	4.8 km south, 16 km west of Hume Lake	36°44.970'	119°05.290'
G	1	Kern	SDSU 2323	4.8 km south, 0.8 km east of Kelso Peak	35°28.324'	118°12.658'
	2	Kern	SDSU 2324	4.8 km south, 0.8 km east of Kelso Peak	35°28.324'	118°12.658'
	3	Kern	SDSU 2325	2.4 km south, 1.6 km east of Kelso Peak	35°29.668'	118°12.278'
H	4	Kern	MVZ 171846	1.2 km south of Walker Pass, Skodie Mtns.	35°39.000'	118°01.260'
J	1	Monterey	SDSU 2309	4.8 km west of Chalk Peak	35°59.281'	121°28.986'
	2	Monterey	SDSU 2310	4.8 km west of Chalk Peak	35°59.229'	121°29.202'
	3	Monterey	SDSU 2335	4.8 km west of Chalk Peak	35°59.277'	121°29.079'
	4	Monterey	SDSU 2336	4.8 km west of Chalk Peak	35°59.277'	121°29.079'
	5	Monterey	SDSU 2337	4.8 km west of Chalk Peak	35°59.295'	121°29.083'
	6	Monterey	SDSU 2338	4.8 km west of Chalk Peak	35°59.314'	121°29.087'
K	1	San Luis Obispo	SDSU 2306	9.6 km northwest of Morro Bay	35°25.143'	120°43.695'
	2	San Luis Obispo	SDSU 2307	9.6 km northwest of Morro Bay	35°25.143'	120°43.695'
	3	San Luis Obispo	SDSU 2308	9.6 km northwest of Morro Bay	35°25.272'	120°43.872'
	4	San Luis Obispo	SDSU 2339	9.6 km northwest of Morro Bay	35°25.169'	120°44.323'
	5	San Luis Obispo	SDSU 2340	9.6 km northwest of Morro Bay	35°25.172'	120°43.708'
	6	San Luis Obispo	SDSU 2341	9.6 km northwest of Morro Bay	35°25.216'	120°43.636'
L	1	Ventura	LSUMZ B16583	11.2 km north of Ojai	34°34.230'	119°14.450'
M	1	Los Angeles	SDSU 2302	San Francisquito Canyon, 6.4 km north, 11.2 km east of Castaic Lake	34°35.609'	118°27.669'
	2	Los Angeles	SDSU 2303	San Francisquito Canyon, 3.2 km north, 6.4 km east of Castaic Lake	34°33.306'	118°30.210'
	3	Los Angeles	SDSU 2304	San Francisquito Canyon, 3.2 km north, 6.4 km east of Castaic Lake	34°3.387'	118°30.136'
	4	Los Angeles	SDSU 2305	San Francisquito Canyon, 3.2 km north, 6.4 km east of Castaic Lake	34°33.306'	118°30.210'
	5	Los Angeles	SDSU 2342	San Francisquito Canyon, 3.2 km north, 6.4 km east of Castaic Lake	34°33.315'	118°30.192'
N	1	San Bernardino	SDSU 2298	4.8 km east of Mormon Rocks	34°19.182'	117°27.468'
	2	San Bernardino	SDSU 2299	4.8 km east of Mormon Rocks	34°19.264'	117°27.442'
	3	San Bernardino	SDSU 2300	2.4 km south of Mormon Rocks	34°17.657'	117°30.626'
	4	San Bernardino	SDSU 2301	2.4 km south of Mormon Rocks	34°17.657'	117°30.446'
	5	San Bernardino	SDSU 2343	2.4 km south of Mormon Rocks	34°17.731'	117°30.456'
	6	San Bernardino	SDSU 2344	2.4 km south of Mormon Rocks	34°17.741'	117°30.457'
P	1	San Bernardino	LSUMZ B19563	Harper Dry Lake, Mojave Desert	35°01.500'	117°17.210'
	2	San Bernardino	LSUMZ B19380	Harper Dry Lake, Mojave Desert	35° 01.500'	117°17.210'
	3	San Bernardino	LSUMZ B19381	Harper Dry Lake, Mojave Desert	35°01.500'	117°17.210'
	4	San Bernardino	LSUMZ B19382	Harper Dry Lake, Mojave Desert	35°01.500'	117°17.210'
Q	1	San Bernardino	LSUMZ B19373	Lytle Creek Wash, 3.2 km south of Devore	34°11.150'	117°24.000'
	2	San Bernardino	LSUMZ B19374	Lytle Creek Wash, 3.2 km south of Devore	34°11.150'	117°24.000'

TABLE 1. Continued.

Site	Ind.	County (CA)	Voucher number	Locality	Latitude	Longitude
R	1	San Diego	SDSU 2282	1.6 km south, 1.6 km east of Pine Mt.	33°18.950'	116°38.309'
	2	San Diego	SDSU 2283	1.6 km south, 1.6 km east of Pine Mt.	33°19.571'	116°38.161'
	3	San Diego	SDSU2377	1.6 km south, 1.6 km east of Pine Mt.	33°19.184'	116°38.312'
	4	San Diego	SDSU2378	1.6 km south, 1.6 km east of Pine Mt.	33°19.143'	116°38.301'
	5	San Diego	SDSU2379	1.6 km south, 1.6 km east of Pine Mt.	33°18.961'	116°38.319'
S	1	San Diego	SDSU 2281	Horsethief Canyon, 6.4 km west of Corte Madera Mt.	32°45.160'	116°39.700'
	2	San Diego	SDSU 2345	Horsethief Canyon, 6.4 km west of Corte Madera Mt.	32°44.941'	116°39.902'
	3	San Diego	SDSU 2346	Horsethief Canyon, 6.4 km west of Corte Madera Mt.	32°45.019'	116°39.860'
	4	San Diego	SDSU2375	Horsethief Canyon, 6.4 mi west of Corte Madera Mt.	32°45.160'	116°39.751'
	5	San Diego	SDSU2376	Horsethief Canyon, 6.4 km west of Corte Madera Mt.	32°45.183'	116°39.605'
T	1	San Diego	SDNHM 50268	Poway	32°57.460'	117°02.060'
U	1	San Diego	SDNHM 50470	Merriem Mts.; 8 km north of San Marcos	33°12.330'	117°08.470'
V	1	San Diego	SDNHM 50384	1.6 km east of Morena Village	32°40.500'	116°28.320'
W	1	San Diego	SDSU 2332	Ridgeview, Lemon Grove	32°44.000'	117°05.440'
?	1	San Diego	SDNHM 50383	San Diego County	N/A	N/A
	2	San Diego	SDSU 2130	San Diego County	N/A	N/A

et al. 2001) for the COII-tRNA-ATPase8 segment. An initial PCR amplification (94°C for 3 s, 43°C for 0 s, 71°C for 30 s; 40 cycles) was performed in capillary tubes in a hot-air thermocycler. The product was run through 2% agarose gel, stained with ethidium bromide, then cut from the gel, melted, and reamplified (94°C for 12 s, 52°C for 4 s, 71°C for 26 s; 41 cycles) in larger volume. Final PCR product was purified using the GeneClean Kit (Bio101, Vista, California) and cycle sequenced (96°C for 1 min, 96°C for 30 s, 50°C for 15 s, 60°C for 4 min; 28 cycles) using Big Dye (Applied Biosystems, Foster City, California) terminator reaction mix. Samples were passed through spin columns containing Sephadex beads before being sequenced on an ABI 377 DNA sequencer (Applied Biosystems). Both heavy and light strands of mtDNA fragments were sequenced for all 66 samples.

Data analysis.—DNA fragments were read, aligned, and edited in SEQUENCHER 3.1 (Gene Code Corporation, Ann Arbor, Michigan), and translated in SE-AL 1.0 (Rambaut 1995). All sequences were deposited into GenBank (Accession numbers AY239617–AY239748). Strong support for mitochondrial authenticity of sequence data comes from unambiguous alignment of overlapping sequence fragments, appropriate fit to avian sequence template, absence of inappropriate stop codons, and lack of heterozygosity during editing. Data from multiple gene regions were combined for subsequent analyses. Individuals were considered from the same population if they were collected within 20 mi of each other. Pooling nearby populations (such as G and H) did not alter our conclusions. Overall genetic structure of populations was represented in an analysis of molecular variation (AMOVA) calculation of F_{st} using pairwise distances (Excoffier et al. 1992, as calculated in ARLEQUIN 2.0 [Schneider et al. 2000]). We also used ARLEQUIN to construct plots of the distribution of pairwise differences among individuals, also known as the mismatch distribution. The shape of mismatch distributions can be used to infer whether a population has undergone a sudden population expansion (Rogers and Harpending 1992, Rogers 1995). Unimodal distributions tend to indicate a population expansion, whereas more ragged distributions indicate the population is in stable equilibrium. Agreement between the observed distributions and expected distributions under a sudden-expansion model was tested following Schneider and Excoffier (1999). In addition, we calculated the raggedness index (Harpending 1994), which has larger values for stable populations versus expanding populations. Assuming neutrality, evidence of a population expansion was also tested using Tajima's (1989a, b) D and Fu's (1997) F_s as implemented in ARLEQUIN. Significantly negative values of those statistics indicate an excess of new mutations relative to equilibrium expectations on the basis of the number of segregating sites (Tajima's D) or number of observed alleles (Fu's F_s). Nucleotide diversity (π) was estimated using ARLEQUIN for each population for which more than one individual was collected and for northern and southern samples pooled separately. Smaller nucleotide diversity values in one area may indicate that a population is expanding into that area (Zink et al. 2000a). Ingroup relationships were explored with

haplotype network construction according to the parsimony-based algorithm developed by Templeton et al. (1992), as implemented in the program TCS 1.13 (Clement et al. 2000). Inferred haplotype relationships were nested according to published nesting rules (Templeton et al. 1987, Templeton and Sing 1993, Crandall 1996) and used in conjunction with geographic locality data of each population to calculate clade distance (D_c; a measure of the geographic range of a particular n-step clade), nested-clade distances (D_n; a measure of the dispersion of an n-step clade relative to its evolutionary sister clade(s) nested in the same higher n + 1-step clade), and the difference between interior and tip clades (I–T) in both D_c and D_n values (GEODIS 2.0; Posada et al. 2000). Interior clades are defined as having connections to more than one other clade. Tip clades lie peripherally in the network and can only be connected to one other clade, an interior clade. Because tip clades are presumably derived from the interior clades to which they are connected, tip-interior status affects historical inference. The null hypothesis of no geographic association was tested separately for each clade at each nested level by comparing observed clade distance values to expected values derived from 10,000 random permutation chi-squared tests (Templeton and Sing 1993, Templeton et al. 1995, Templeton 1998). Inferences regarding evolutionary process were made according to the inference key originally provided by Templeton et al. (1995) and updated by Posada and Templeton (2001). Two birds (SDNHM 50383 and SDSU 2130) were excluded from the nested clade analysis due to insufficient locality data.

Rooting.—Root placement was inferred according to an analysis based on neutral coalescent theory, as well as outgroup criteria. Using coalescent theory-based results, root probabilities were assigned to haplotypes based on haplotype connectedness (number of other haplotypes the one in question is directly linked to) and frequency (number of sampled individuals represented by the haplotype) (implemented in TCS; Castelloe and Templeton 1994). For outgroup analysis, tree topologies were constrained such that Crissal and Le Conte's thrashers formed a monophyletic sister group. The placement of that pair relative to the ingroup was estimated using maximum-likelihood analysis as implemented in PAUP* 4.0b8 (Swofford 1998). The substitution model and parameters best fit for the data set (including outgroups) were determined using a hierarchical likelihood ratio test performed using MODELTEST (Posada and Crandall 1998). Initial model and parameters were determined on the basis of a starting parsimony tree (one of four most parsimonious trees from a heuristic search) and then used in maximum-likelihood analysis with 10 random addition replicates. The resulting maximum-likelihood topology was entered into MODELTEST as a starting tree and the process was repeated until the same model and parameters were consistently drawn. To assess support for different nodes in the topology, the maximum-likelihood tree was bootstrapped for 100 replicates. A Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) using RELL optimization and 100,000 bootstrap replicates was performed in PAUP* under maximum-likelihood criteria to test for significant difference between the outgroup tree versus a tree constrained to agree with coalescence-theory based rooting.

RESULTS

Ingroup sequence variation.—Of the total 2,086 base pairs, there are 49 variable sites, of which 29 are parsimony informative. Thirty-seven unique haplotypes were identified within the ingroup. For the 1,107 bp of cyt *b*, variation included 22 transitions and 2 transversion. Transition–transversion ratios for each position were as follows: first position, 4:0; second position, 0:0; and third position, 18:2. For 668 bp of ATPase6, variation included 20 transitions and 3 transversions (first position, 4:2; second position, 3:0; third position, 13:1). For the 158 bp of ATPase8, one transition was identified at the second-base position and two transitions at the third-base position. The 83 bp fragment of COII showed no variation. The 70 bp tRNA-Lys is non-protein-coding and showed one variable site. Average uncorrected pairwise sequence distance between individuals (*p*-distance) was 0.34% (cyt *b*, 0.35%; ATPase6, 0.37%; ATPase8, 0.41%; COII, 0.0%; tRNA-Lys, 0.17%) with a range of 0.00–0.77%.

Overall geographic structure among localities was supported by an AMOVA in which populations were not categorized into subgroups (F_{st} = 0.46; Table 2). Because the haplotype network and phylogeny (see below) indicate a general split between northern sites (A–J) and southern sites (K–W), we performed an additional AMOVA in which populations were divided into northern and southern groups. Results of that AMOVA (Table 2) indicate that much of observed structure may be explained by variation between the northern and southern groups. A mismatch distribution of all individuals (Fig. 2) was not significantly different for the expected distribution of a growing population (P = 0.219, raggedness index = 0.018). Although negative, Tajima's D did not differ significantly from zero (D = –1.0008; P = 0.1663). However, we obtained a significantly negative value for Fu's

TABLE 2. Analysis of molecular variance of California Thrashers. Results are shown first for an analysis in which populations were not divided into groups and then for an analysis in which populations were divided into northern (populations A–J) and southern (populations K–W).

Groups	Source of variation	Percent variation	*F*-statistic	*P*
None specified	Among populations within groups	45.9	0.46	< 0.0001
	Within populations	54.1		
North vs. south	Among groups	49.6	0.50	< 0.0001
	Among populations within groups	9.5	0.19	0.0059
	Within populations	40.9	0.59	< 0.0001

F_s (F_s = –11.27, P = 0.002), indicating a possible recent expansion in population size. Nucleotide diversity values are similar among populations (Table 3); thus, they do not indicate directionality to a population expansion. Nucleotide diversity of northern populations pooled together (0.0027) was also similar to that of southern populations pooled together (0.0022).

Nested-clade analysis.—Haplotype network construction resulted in a single network (Fig. 3), in which all connections fall within a 95% plausible set of relationships. Minor ambiguities were resolved using rules of parsimony. That is, in comparing alternate mutational paths in a network, the path providing the shortest overall connection between all haplotypes is preferred. This haplotype network did not disagree with the maximum-likelihood tree in the monophyly or placement of any clade at any nesting level (Fig. 4). The final network, showing relationships among 35 sampled haplotypes, was subdivided into one-step, two-step, and three-step clades. The entire network was contained at the four-step level (Fig. 3). Chi-squared analysis identified three clades with significant overall association between clades and location (Table 4). Although that nested contingency analysis does identify associations between clades and localities, it does not incorporate geographic distance information (Templeton et al. 1995). For a clade to be taken through the inference key, the clade must contain both genetic and geographic variation and show a statistically significant relationship in at least one D_c or D_n value. Four such clades (Fig. 5) were identified in this study. Those four clades include the three clades identified in the nested contingency analysis (Table 4) as well as one additional clade (3-3).

Rooting.—Alternate rooting methods did not agree on root placement of the gene tree. Coalescence-based calculations weighted the most frequent (shared by 11 individuals), most geographically widespread (represented by six localities) haplotype K_1-K_3-K_4-K_6-L_1-M_4-N_1-N_4-N_5-P_3-Q_1 as having the highest probability of ancestry (outgroup weight calculated using the coalescent-based methods = 0.14).

Under maximum-likelihood criteria, the like-

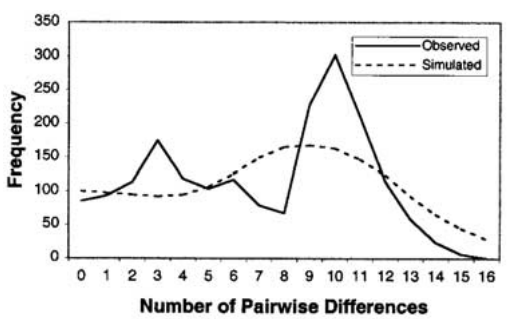

FIG. 2. Mismatch distribution of haplotypes of California Thrasher. Solid line indicates observed distribution and dashed line indicates expected distribution under a model of sudden expansion.

TABLE 3. Estimates of within-site variability for California Thrasher for sites in which more than one individual was collected. See Table 1 for locations of sites.

Site	*n*	Number of haplotypes	Nucleotide diversity
C	5	5	0.0027
D	2	2	0.0045
E	3	3	0.0013
F	2	2	0.0005
G	3	1	0.0000
J	6	2	0.0021
K	6	3	0.0018
M	5	5	0.0020
N	6	4	0.0021
P	4	4	0.0014
Q	2	2	0.0040
R	5	5	0.0034
S	5	4	0.0013

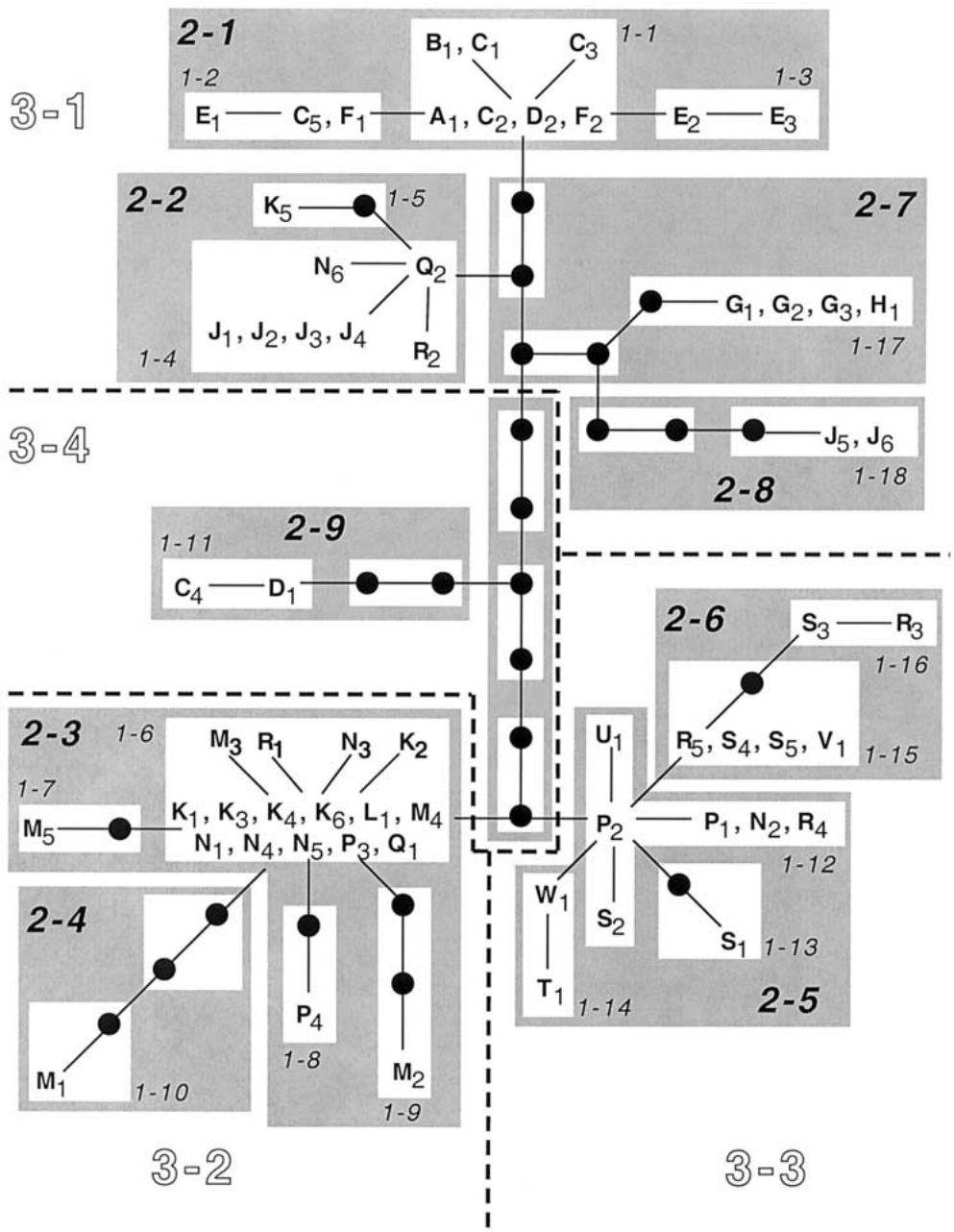

FIG. 3. Nested haplotype network. Each branch represents a single base pair mutation. Nodes between branches represent sampled haplotypes. Black circular nodes represent unsampled haplotypes; lettered nodes represent sampled haplotypes. Each letter with subscript is one individual from its corresponding locality (Fig. 1, Table 1). Therefore, haplotypes shared by more than one individual are represented by multiple letters. One-step clades are contained within white boxes, two-step clades are contained within larger shaded boxes, and three-step clades are delineated by dashed lines. The overall cladogram is a single four-step clade.

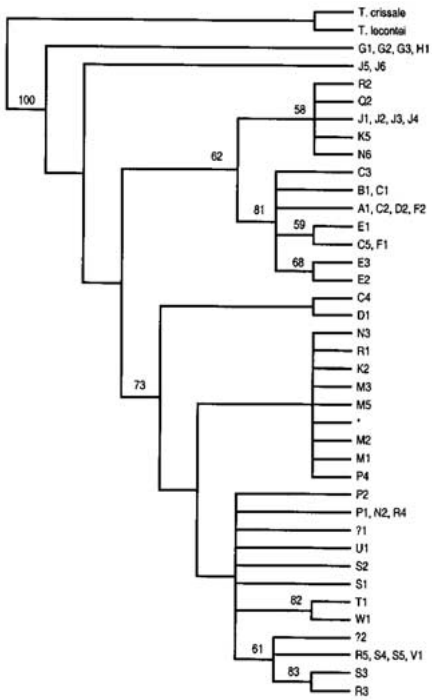

FIG. 4. Maximum-likelihood tree (–ln likelihood = 4338.5583) under GTR + I + Γ. Terminal haplotypes are coded by individual according to Table 1. Asterisk indicates haplotype K_1-K_3-K_4-K_6-L_1-M_4-N_1-N_4-N_5-P_3-Q_1. For illustrative purposes, branch lengths are not proportional. Numbers at nodes indicate clades retained by >50% of bootstrap replicates.

lihood-ratio test converged on the GTR + I + Γ model of molecular evolution as most consistent with the data. Haplotype G_1-G_2-G_3-H_1 (outgroup weight = 0.02), represented by four birds from two localities, is identified as basal (Fig. 4). The Shimodaira-Hasagawa test found no significant difference between the best tree (–ln likelihood = 4338.5583) and a tree constrained to be rooted at haplotype K_1-K_3-K_4-K_6-L_1-M_4-N_1-N_4-N_5-P_3-Q_1 (–ln likelihood = 4241.4320; P = 0.087).

DISCUSSION

The haplotype network, phylogeny, and results of AMOVA indicate significant geographic structure, mostly divided between northern (A–J) and southern (K–W) populations. However, the division between northern and southern populations is not absolute. That is, some individuals in southern populations are closely related to some individuals in the north. Results of the mismatch distribution and Fu's F_s test suggest that the population is expanding. However, comparison of nucleotide diversity values gives no indication of the direction the expansion might be occurring. If diversity values had been lower in a particular part of the distribution, that may have indicated that populations were expanding into that area. However, the estimation of accurate nucleotide diversity values was probably hindered by the small population samples of this study.

TABLE 4. Chi-square statistics and probability values for all clades with both geographic and genetic variation. Significant values (P < 0.05) are indicated in bold.

Clade	χ^2	Probability
1–1	5.8333	1.0000
1–2	3.0000	0.6549
1–4	**21.0000**	**0.0264**
1–6	26.4545	0.3837
1–12	14.0000	0.6491
1–14	2.0000	1.0000
1–16	2.0000	1.0000
1–19	2.0000	1.0000
2–1	10.1905	0.6082
2–2	8.0000	0.5026
2–3	16.2000	0.4901
2–5	12.7500	0.5549
2–6	0.7500	1.0000
3–1	**56.3333**	**0.0055**
3–2	2.9556	1.0000
3–3	7.2222	0.6186
Cladogram	**108.4124**	**0.0013**

In general, the nested-clade analysis complemented these approaches and provides additional insight into the details of the population history of the California Thrasher. Nested-clade analysis was developed to distinguish specific evolutionary population processes more explicitly and objectively than traditional phylogeographic methods by providing greater spatial and temporal resolution. Spatially, each inference considers a specific geographic area represented by the members of a particular genetic clade, not just the overall species and its range. Temporally, the nesting level of each clade can indicate the order of historical events (past fragmentation, past range expansion, long-distance colonization) or current population conditions (isolation-by-distance). Because birds are highly vagile organisms and typically have lower levels of among-population genetic differentiation than other vertebrates (Ditchfield

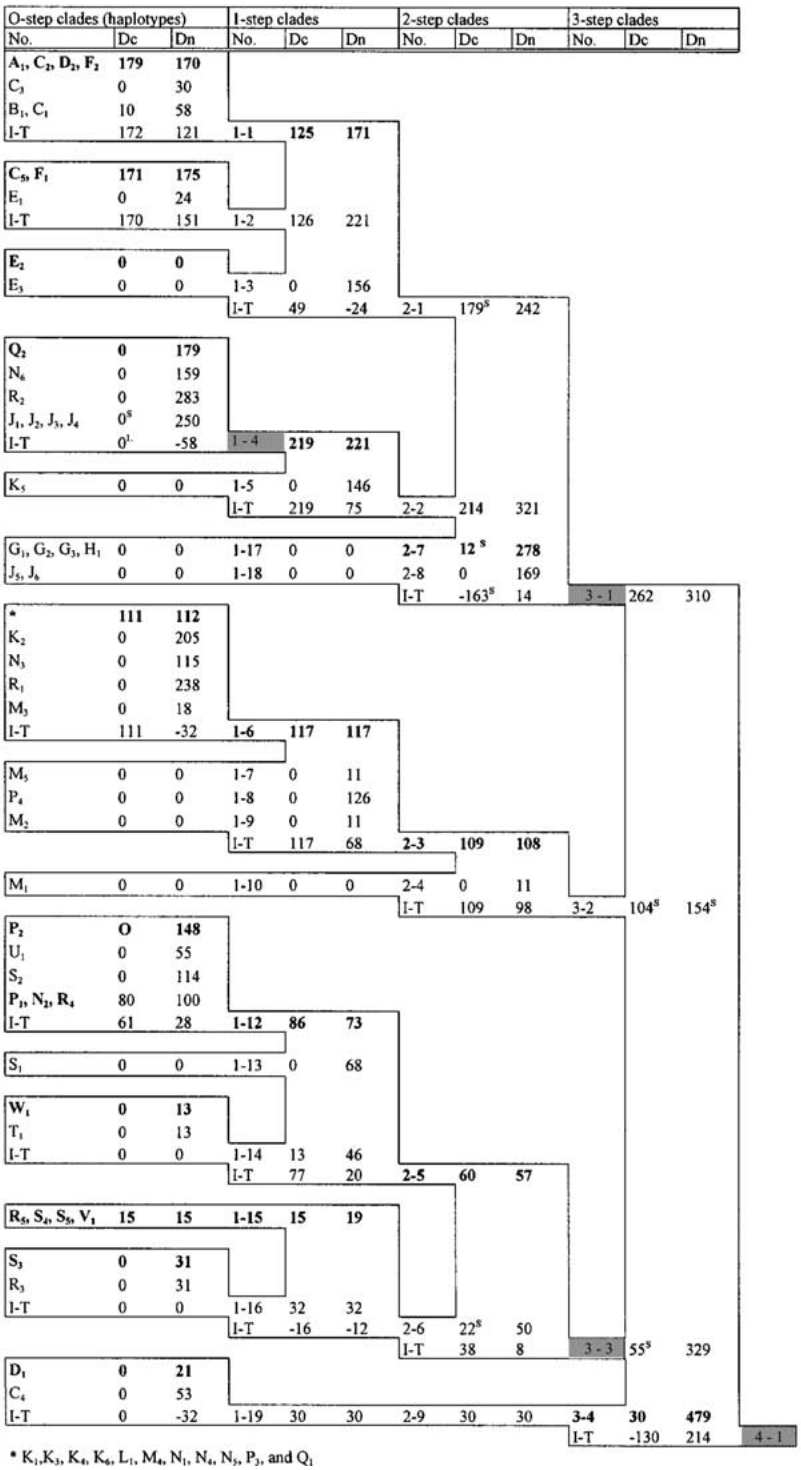

FIG. 5. Results of nested geographic analysis. D_c and D_n values for all clades and I–T comparisons at all clade levels are shown. Interior clades are highlighted in bold type. Superscript "S" and "L" indicate significantly small and large values respectively ($P < 0.05$). Clades for which inferences were drawn are shaded.

and Burns 1998), it is often difficult to infer such historical events. However, despite the fact that the California Thrasher has a more-or-less continuous distribution, little morphological variation, and low levels of molecular variation, geographic structure is clearly evident. Nested clade analysis is able to reject the null hypothesis of no geographic structure for four clades within this species, inferring past fragmentation, contiguous range expansion, and isolation-by-distance. The strength of each of these inferences is evaluated below.

Clade 1–4: Past fragmentation.—The haplotype J_1-J_2-J_3-J_4 is shared by four individuals, all restricted geographically to a specific region of the central California coast known as Big Sur (Fig. 6A). That restricted haplotype is geographically distant from the combined range of the other three localities represented in this clade (N, Q, and R). Nested-clade analysis infers past fragmentation. Although that inference may accurately reflect history, it seems reasonable from visual inspection of the geographic distribution of those haplotypes that this pattern might also be the result of a long-distance colonization event. For long-distance colonization to be inferred using the inference key, some differences in the relative geographic distance of each subclade from the clade's center (D_n values) must be detectable statistically. Because the number of birds representing haplotype J_1-J_2-J_3-J_4 (4) is far greater than any of the other haplotypes in clade 1–4 (one each), the geographic center of all seven individuals within the clade is very different from the geographic center of the four localities represented (J, N, Q, and R). Therefore, because of unequal representation of localities within that clade, D_n values are very similar among haplotypes. In addition, each of the four haplotypes is restricted to a single locality. But because three of the four haplotypes are represented by just a single bird, only haplotype J_1-J_2-J_3-J_4 can show a significantly small D_c value, which drives the inference of past fragmentation.

This is a good example of the difficulties in inference confidence at low clade levels, despite statistical significance. Regardless of overall sampling efforts for a study, patterns identified within low-level clades are more weakly represented by samples due simply to the nature of the hierarchical nesting process. According to the inference key, inferences for high-level clades can be further supported by patterns seen in the lower-level subclades nested within. However, that aspect of additional support can not be found for low-level clade inferences because a low-level clade has little or no subclade patterns to investigate. Therefore, if locality representation within a low-level clade is largely inconsistent with a trend seen in higher-level clades (e.g. clade 1–4), the inference for the low-level clade, though numerically significant, is not as strongly supported.

Clade 3–1: Contiguous range expansion.—The four subclades that form clade 3–1 collectively cover a large proportion of the entire sampling distribution (Fig. 6B). Together, the three tip clades include all members from two Sierra Nevada populations (E, F), all but two individuals from the five northernmost coastal populations (A, B, C, D, and J), and representatives from five other populations. Alternatively, the interior subclade (2–7) is restricted to a small area at Kern County (G, H) in the southern Sierra. The pattern of geographically widespread tip-clades (specifically 2–1 and 2–2) descended from a more restricted, centrally located interior clade (2–7) is consistent with a model of range expansion (Cann et al. 1987, Templeton et al. 1995). Contiguous range expansion results from an accumulation of short-distance movements in a particular direction. Descendents of the ancestral Kern region may have dispersed to newly inhabitable or newly accessible adjacent areas. Clade 2–1 may have been the result of a northward range expansion during the Holocene, when changes in temperature, precipitation, and vegetation promoted northward expansion for many California organisms previously confined to glacial-age refugia further south (Cicero 1996). Clade 2–2 represents a southward expansion from the same southern Sierran ancestor. A mismatch distribution of just the individuals of clade 3–1 agrees with the inference of contiguous range expansion. The shape (not shown) does not differ from the expected distribution of an expanding population ($P = 0.41$). The raggedness index (0.02) is also similar to that observed for expanding populations (Harpending 1994).

Clade 3-3: Isolation-by-distance.—The range of clade 3–3 extends over the far southern portion of the species' California distribution and includes all members of the five southernmost localities in this study (Fig. 6C). The tip clade

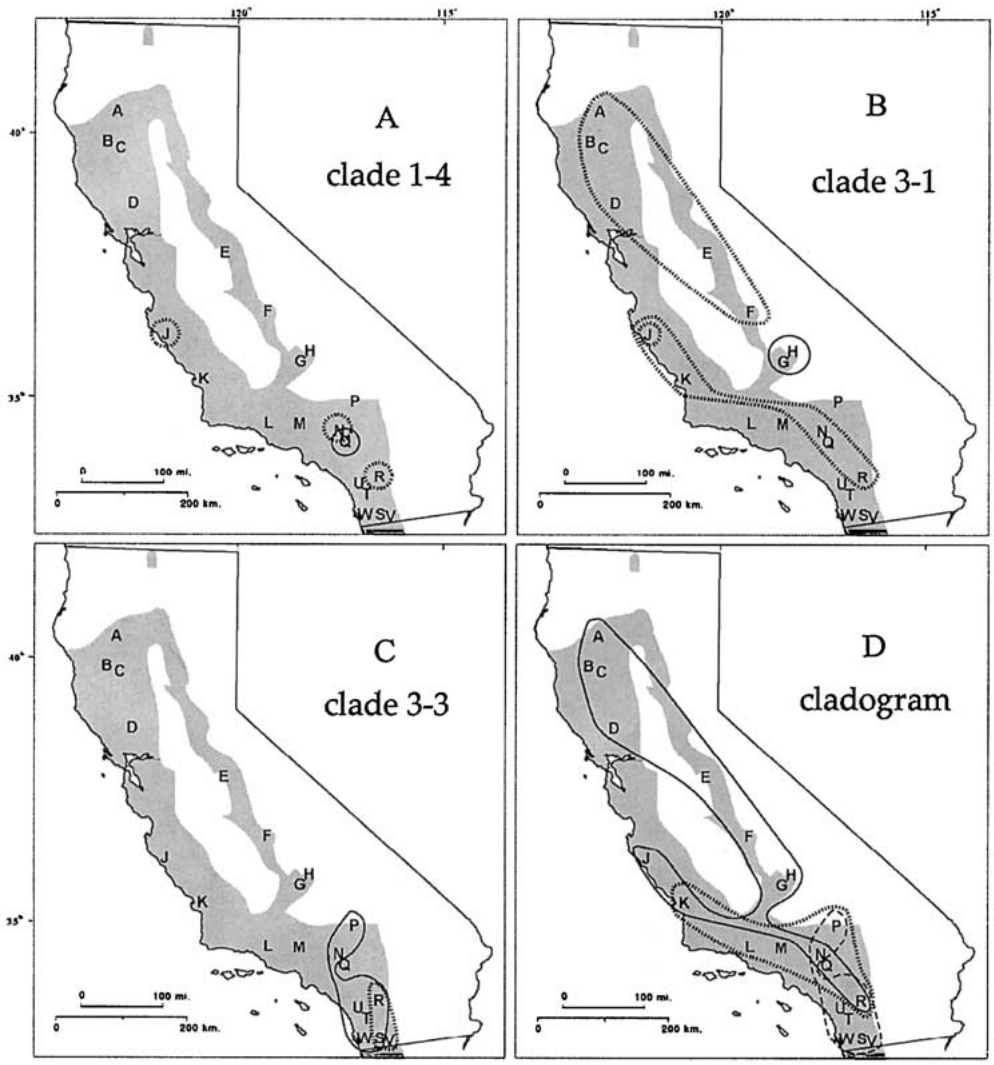

FIG. 6. Geographic representation of each of the four inferences. For (A–C), interior subclades are outlined with a solid line; tip clades are outlined with a dotted line. (A) Clade 1–4; past fragmentation (B), clade 3–1; contiguous range expansion (C), clade 3–3; isolation-by-distance (D), entire cladogram; clade 3–1 is delineated with a solid line, clade 3–2 with a dotted line, and 3–3 with a dashed line.

(2–6) spans three eastern localities within that region (R, S, and V), whereas the interior clade (2–5) is more widespread (N, P, R, S, U, T, and W). The geographic overlap of a restricted tip clade with a more widespread interior clade suggests an absence of any absolute barrier to gene flow. Therefore, the observed pattern of genetic variation can be explained by restricted gene flow due to isolation-by-distance, in which geographic distance and short dispersal ability serve to restrict free genetic exchange. A mismatch analysis of just the individuals of clade 3–3 conflicts somewhat with that conclusion. Although the mismatch distribution (not shown) does not differ statistically from the expected distribution of a growing population, the significance level was low ($P = 0.09$). In addition, the raggedness index (0.11) is similar to that observed in stable populations (Harpending 1994). If the number of individuals sampled in

clade 3–3 had been greater, the mismatch distribution may have been able to reject the idea of an expanding population. Nevertheless, the pattern of isolation-by-distance for clade 3–3 is supported by the nested clade analysis and the raggedness index.

Clade 4–1 (overall cladogram): Isolation-by-distance.—Nested-clade analysis reveals four subclades within the overall network and infers restricted gene flow via isolation-by-distance as responsible for their differentiation (Fig. 6D). Clade 3-1 is the most geographically widespread of the subclades and covers nearly the entire northern two-thirds of the range. Clade 3-3 spans the far southern region of the sampling distribution. Clade 3-2 lies geographically intermediate, containing overlap with clade 3-1 (K, N, Q, and R) and with clade 3–3 (N, M, P, Q, and R) to the south. This large amount of overlap drives the inference of isolation-by-distance for the overall cladogram. However, the overlapping area from clade 3–1 was identified separately as a more recent southern range expansion of subclade 2–2 (see above), possibly obscuring inference of the process primarily responsible for the pattern of differentiation between the three-step clades. It is possible to explore events prior to that range expansion by removing it (2–2) and all other events of similar age (all other two-step tip clades). Applying the inference key under those conditions, isolation-by-distance is still demonstrated among southern clades (between 2–3 and 2–5), but past fragmentation separates them from the southern Sierran ancestral clade (2–8).

Nested-clade analysis conclusions.—As exemplified by discussion of clade 1–4 and the overall cladogram, finding a truly objective phylogeographic approach to interpret dynamic population histories remains a challenge. Nested-clade analysis is an invaluable tool in its capacity to hypothesize and locate process of diversification in both a spatial and temporal framework, while concurrently promoting deep investigation of all intraspecific relationships. However, rather than using the inference key strictly as an "answer key," each inference must still be considered against the numerical factors driving each inference, including the effects from other inferences in the same nested series and the effects of sampling, especially at low clade levels.

Taxonomy.—The north–south division among the haplotypes in this study (localities A–J vs. K–W) is not congruent with the proposed north–south division between previously described subspecies of the California Thrasher. If haplotypes had sorted according to subspecies, a division should have been observed between populations A–D and populations E–W. Morphological differentiation among subspecies, detectable in some plumage, tail, and tarsus measurements, is minor and considered to be clinal from north to south (Cody 1998). If a historic north–south division were in fact responsible for the acquisition of unique morphological characters, a southward expansion of northern traits (as indicated in the mitochondrial genes) might slowly obscure the distinction between these different morphs at the areas of interface, causing morphological variation to appear clinal.

Rooting.—For purposes of biogeographic construction, both methods of rooting suggest general southern ancestry for the California Thrasher. Southern ancestry is further supported by the distribution of the sister taxa, Crissal and Le Conte's thrashers, which are both southwestern aridland species. The haplotype identified by coalescent rooting is much more geographically widespread and interior within the network than the haplotype identified using outgroups. In fact, outgroup methods often have difficulty confidently placing outgroup taxa onto intraspecific networks of minimally differentiated haplotypes (Castelloe and Templeton 1994), such as this one. However, coalescence based rooting assumes population panmixia and can be influenced by sampling efforts due to the fact that assigned root probability values consider haplotype frequency. Although the two methods differed in the exact placement of the root, the Shimodaira-Hasegawa test identified no significant difference between the best maximum-likelihood tree and the tree constrained to agree with coalescence-based rooting. In addition, biogeographic conclusions are not affected by which of those two rootings is preferred.

Biogeographic history.—Based on DNA data (Zink et al. 2000b), the California Thrasher is believed to have diverged from other thrashers in the late-Pliocene or early-Pleistocene, roughly 3–5 mya. Assignment of a 1.6% (Fleischer et al. 1998) to 2% per million years (Shields and Wilson 1987) molecular clock dates the most recent com-

mon ancestor of the sampled haplotypes in this study to roughly 385,000–480,000 years ago, during the Pleistocene (maximum sequence divergence between California Thrasher individuals = 0.77%). A proposed biogeographic scenario is depicted diagrammatically in Figure 7.

The ancestral population of California Thrashers was most likely separated from its Sonoran counterparts and confined to the Californian refugia during desertification of the Great Basin during the late-Pliocene (Hubbard 1973). Restricted from the north due to cool temperatures, populations at this time probably ranged from somewhere along the south-central California coast to Baja California. That general region of ancestry does not conflict with the conclusions drawn from either of the root placements presented herein. Inland at that time, the uplift of the Transverse Range could have served as a substantial barrier to gene flow between populations to the north (localities A–J) and south (localities K–W). Similarly along the coast, internal seaways connecting the Central Valley with the Pacific Ocean through the uplifting Coastal Range may have also played a role in the north–south division of the ancestral range. That might explain the large genetic divergence between adjacent coastal localities, Big Sur (J) and Morro Bay (K). The enduring role of those geologic features as a substantial barrier is noticeable in the long branch that divides clade 3–1 from clades 3–2 and 3–3. Although past fragmentation is not inferred statistically from strict implementation of the inference key, the pattern is apparent when the key is used to analyze older, interior relationships in the nested network (as discussed in the previous section).

During postglacial warming, northward range expansion was probably simpler for populations north of the aforementioned barriers. Because many coastal land connections were still unstable, inland birds were in better position to colonize northern California by extending up the western slope of the Sierra and into far northern regions of the current distribution. That can explain why the Kern clade 2–7 is interior within clade 3–1. That range expansion could have occurred very rapidly; resulting in the genetically similar haplotypes from as far apart as Fresno (F) and Colusa (C) counties. Also at that time, a range expansion over the Transverse Ranges into the already inhabited

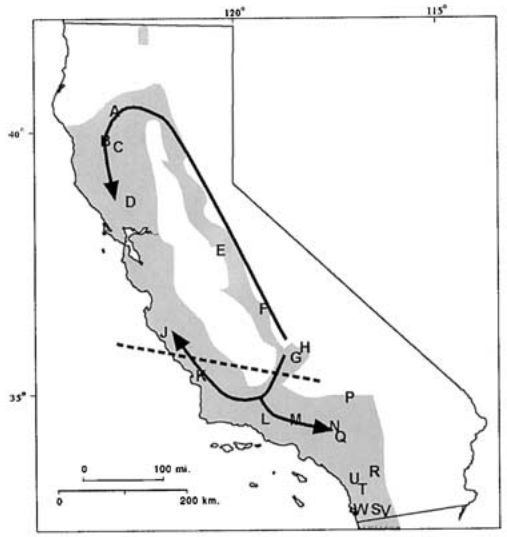

FIG. 7. Diagrammatic representation of biogeographic conclusions. Scenario begins with a general southern ancestor. Dashed line reflects a fragmentation event. Arrows represent direction of a range expansion.

southern region would explain the presence of a southern subclade (2–2) within an otherwise northern-dominated clade (3–1). Expansion of that southern clade (2–2) extends up the coast to Morro Bay (K) and Big Sur (J), two localities that were apparently disconnected earlier (based on relationship of other K and J individuals). Restricted gene flow in the relative long-term association of southern populations has resulted in an isolation-by-distance situation in the south.

Comparative phylogeography.—Concordance between inferences for the California Thrasher and codistributed taxa suggests that multiple sympatric organisms responded similarly to topographic and climatic changes. Incongruencies reveal the possibility of unique dispersal events, differential responses to barriers, or lack of long-term sympatry of those taxa altogether. The following phylogeographic studies were used for comparison: *Ensatina* salamander complex (Wake 1997), California mountain kingsnake (*Lampropeltis zonata*; Rodriguez-Robles et al. 1999), California newt (*Taricha torosa*; Tan and Wake 1995), *Eumeces skiltonianus* species complex (Richmond and Reeder 2002), rubber boa (*Charina bottae*; Rodriguez-Robles et al. 2001), California mouse (*Peromyscus califor-*

nicus; Smith 1979), ornate shrew (*Sorex ornatus*; Maldonado et al. 2001), dusky-footed woodrat (*Neotoma fuscipes*; Matocq 2002) and the Oak Titmouse (*Baelophus inornatus*; Cicero 1996). Based on molecular clock estimates, the timing of diversification within the Oak Titmouse most closely matches that of the California Thrasher, whereas *Ensatina* appears to be much older than the above mentioned taxa. However, regardless of time frame, diversification within any of those species might be expected to show some concordance with the California Thrasher because all of those species have coexisted for at least some period of time.

Like the California Thrasher, general southern ancestry was proposed for *L. zonata*, *T. torosa*, *E. skiltonianus*, *B. inornatus*, *P. californicus*, and *S. ornatus*, suggesting that the Pleistocene southern retreat affected a wide variety of organisms. An inland north-south split between southern populations and southern Sierran populations is apparent within *E. eschscholtzii*, *E. skiltonianus*, *T. torosa*, and *C. bottae*, while *P. californicus*, *B. inornatus*, *L. zonata*, and *S. ornatus* show a north–south division between both inland and coastal populations. Vicariance events related to the uplift of the Transverse Range and inland seaways are likely responsible for that fragmentation, as suggested earlier. The identity of a strictly southern Sierran clade similar to the Kern population of California Thrashers (G, H) is identified in *L. zonata*, *T. torosa*, and *E. eschscholtzii.* That suggests the presence of some current or historical northern barrier to these southern Sierran populations, although none have been identified by any of the authors. The close association of a southern Sierran clade and a central coastal clade, similar to the relationship between the Kern (G and H) and Big Sur (J) in California Thrashers, was identified in *L. zonata*, *E. skiltonianus*, and *S. ornatus*. It is possible that individuals north of the transverse barrier were not restricted by any east–west barriers and, therefore, were able to expand from the coast to the Sierran foothills. However, the continued desertification of the Central Valley would eventually serve to divide much of central California. The proposed northward range expansion from a Sierran ancestor as described for the California Thrasher is also proposed for *L. zonata* and *N. fuscipes*. A similarly distributed northern clade also appears within *T. torosa*.

Comparative phylogeography identified congruencies for many of the historical processes inferred for the California Thrasher: southern faunal retreat, the early north–south division, and subsequent northern range expansion. Whereas coastal seaway barriers disappeared, the Central Valley and the Transverse Ranges continue to serve as barriers dividing east from west and north from south, resulting in a ringlike distribution for many terrestrial organisms. Phylogeographic analysis of other similarly distributed avian taxa, such as Lawrence's Goldfinch (*Carduelis lawrencei*), Wrentit (*Chamaea fasciata*), Rufous-crowned Sparrow (*Aimophila ruficeps*), Nuttall's Woodpecker (*Picoides nuttallii*), and California Towhee (*Pipilo crissalis*), would contribute greatly to these comparisons and possibly elucidate unique avian biogeographic patterns in this geologically dynamic region of the world.

Acknowledgments

Funds for this project were provided by the Frank M. Chapman fund of the American Museum of Natural History and the San Diego State University Foundation. We thank the California Department of Fish and Game for providing permits. For their tissue contributions, we thank the Museum of Vertebrate Zoology at University of California-Berkeley (N. Johnson and C. Cicero), the Collection of Genetic Resources at Louisiana State University (F. Sheldon and D. Dittman), and the San Diego Natural History Museum (P. Unitt). For helpful discussions and comments on the manuscript, we thank M. Hedin, D. Wood, and M. Lowder. We also thank D. Barhoum for primer development and other lab assistance, as well as K. Klitz for help with map construction.

Literature Cited

American Ornithologists' Union. 1957. Checklist of North American Birds, 5th ed. American Ornithologists' Union, Washington, D.C.

Barrowclough, G. F., R. J. Gutíerrez, and J. G. Groth. 1999. Phylogeography of Spotted Owl (*Strix occidentalis*) populations based on mitochondrial DNA sequences, gene flow, genetic structure, and a novel biogeographic pattern. Evolution 53:919–931.

Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 325:31–36.

Castelloe, J., and A. R. Templeton. 1994. Root probabilities for intraspecific gene trees under neutral coalescent theory. Molecular Phylogenetics and Evolution 3:102–113.

CICERO, C. 1996. Sibling species of titmice in the *Parus inornatus* complex. University of California Publications in Zoology, no. 128

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

CODY, M. L. 1998. California Thrasher (*Toxostoma redivivum*). *In* The birds of North America, no. 323 (A. Poole and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

CRANDALL, K. A. 1996. Multiple interspecies transmissions of human and simian T-cell leukemia/lymphoma virus type I sequences. Molecular Biology and Evolution 13:115–131.

DITCHFIELD, A. D., AND K. J. BURNS. 1998. DNA sequences reveal phylogeographic similarities of Neotropical bats and birds. Journal of Comparative Biology 3:165–170.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491.

FLEISCHER, R. C., C. E. MCINTOSH, AND C. L. TARR. 1998. Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K–Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 7:533–545.

FU, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

GRINNELL, J., AND A. H. MILLER. 1944. The Distribution of Birds of California. Pacific Coast Avifauna, no. 27.

GROTH, J. G. 1998. Molecular phylogenetics of finches and sparrows: Consequences of character state removal in cytochrome *b* sequences. Molecular Phylogenetics and Evolution 10: 377–390.

HARPENDING, H. C. 1994. Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Human Biology 66:591–600.

HUBBARD, J. P. 1973. Avian evolution in the aridlands of North America. Living Bird 12: 155–196.

HUNT, J. S., E. BERMINGHAM, AND R. E. RICKLEFS. 2001. Molecular systematics and biogeography of Antillean thrashers, tremblers, and mockingbirds (Aves*:* Mimidae). Auk 118:35–55.

MALDONADO, J. E., C. VILÀ, AND R. K. WAYNE. 2001. Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). Molecular Ecology 10: 127–147.

MATOCQ, M. 2002. Phylogeographic structure and regional history of the dusky-footed woodrat, *Neotoma fuscipes*. Molecular Ecology 11: 229–242.

MORITZ, C., C. J. SCHNEIDER, AND D. B. WAKE. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Biology 41:273–291.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

POSADA, D., K. A. CRANDALL, AND A. R. TEMPLETON. 2000. GEODIS: A program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology 9:487–488.

POSADA, D., AND A. R. TEMPLETON. 2001. Inference key for the nested haplotype tree analysis of geographical distances. [Online.] Available at bioag.byu.edu/zoology/crandall_lab/dposada/documents/NCA-key(24Oct01).pdf.

RAMBAUT, A. 1995. SE-AL version 1 d. 1: Sequence Alignment Program. Department of Zoology, University of Oxford, Oxford.

RICHMOND, J. Q., AND T. W. REEDER. 2002. Evidence for parallel ecological speciation in scincid lizards of the *Eumeces skiltonianus* species group (*Squamata:Scincidae*). Evolution 56:1498–1513.

RODRIGUEZ-ROBLES, J. A., D. F. DENARDO, AND R. E. STAUB. 1999. Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). Molecular Ecology 8:1923–1934.

RODRIGUEZ-ROBLES, J. A., G. R. STEWART, AND T. J. PAPENFUSS. 2001. Mitochondrial DNA-based phylogeny of North American rubber boas, *Charina bottae* (Serpentes*:* Boidae). Molecular Phylogenetics and Evolution 18:227–237.

ROGERS, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:606–615.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

SCHNEIDER, S., AND L. EXOFFIER. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. Genetics 152:1079–1089.

SCHNEIDER, S., D. ROESSLI, AND L. ESCOFFIER 2000. ARLEQUIN version 2.0: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SHIELDS, G. F., AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution 24:212–217.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log-likelihoods with applica-

tions to phylogenetic inference. Molecular Biology and Evolution 16:1114–1116.

SMITH, M. F. 1979. Geographic variation in genic and morphological characters in *Peromyscus californicus*. Journal of Mammalogy 60: 705–722.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0b8. Sinaur Associates, Sunderland, Massachusetts.

TAJIMA, F. 1989a. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–596.

TAJIMA, F. 1989b. The effect of change in population size on DNA polymorphism. Genetics 123: 597–601.

TAN, A. M., AND D. B. WAKE. 1995 Mitochondrial DNA phylogeny of the California Newt *Taricha torosa* (Caudata, Salamandridae). Molecular Phylogenetics and Evolution 4:83–394.

TEMPLETON, A. R. 1998. Nested clade analysis of phylogeographic data: Testing hypotheses about gene flow and population history. Molecular Ecology 7:381–397.

TEMPLETON, A. R., E. BOERWINKLE, AND C. F. SING. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. Genetics 117:343–351.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

TEMPLETON, A. R., E. ROUTMAN, AND C. A. PHILLIPS. 1995. Separating population structure from population history: A cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767–782.

TEMPLETON, A. R., AND C. F. SING. 1993. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134: 659–669.

WAKE, D. B. 1997. Incipient species formation in salamanders of the *Ensatina* complex. Proceedings of the National Academy of Sciences USA 94:7761–7767.

ZINK, R. M., G. F. BARROWCLOUGH, J. L. ATWOOD, AND R. C. BLACKWELL-RAGO. 2000a. Genetics, taxomony, and conservation of the threatened California Gnatcatcher. Conservation Biology 14:1394–1405.

ZINK, R. M., R. C. BLACKWELL-RAGO, AND F. RONQUIST. 2000b. The shifting roles of dispersal and vicariance in biogeography. Proceedings of the Royal Society of London, Series B 267:497–503.

ZINK, R. M., D. L. DITTMANN, J. KLICKA, AND R. C. BLACKWELL-RAGO. 1999. Evolutionary patterns of morphometrics, allozymes, and mitochondrial DNA in thrashers (Genus *Toxostoma*). Auk 116:1021–1038.

Associate Editor: S. J. Hackett