

Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae)

JAVIER A. RODRÍGUEZ-ROBLES,* DALE F. DENARDO†‡ and RICHARD E. STAUB§

*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720-3160, USA,

†Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA, §Department of Nutritional Sciences,

44 Morgan Hall, University of California, Berkeley, CA 94720-3104, USA

Abstract

The phylogeography of the California mountain kingsnake, *Lampropeltis zonata*, was studied using mitochondrial DNA sequences from specimens belonging to the seven recognized subspecies and collected throughout the range of the species. Maximum parsimony and maximum likelihood methods identified a basal split within *L. zonata* that corresponds to southern and northern segments of its distribution. The southern clade is composed of populations from southern California (USA) and northern Baja California, Mexico. The northern clade is divided into two subclades, a 'coastal' subclade, consisting of populations from the central coast of California and the southern Sierra Nevada Mountains of eastern California, and a 'northeastern' subclade, mainly comprised of populations north of the San Francisco Bay and from the majority of the Sierra Nevada. We suggest that past inland seaways in southwestern California and the embayment of central California constituted barriers to gene flow that resulted in the two deepest divergences within *L. zonata*. Throughout its evolutionary history, the northern clade apparently has undergone instances of range contraction, isolation, differentiation, and then expansion and secondary contact. Examination of colour pattern variation in 321 living and preserved specimens indicated that the two main colour pattern characters used to define the subspecies of *L. zonata* are so variable that they cannot be reliably used to differentiate taxonomic units within this complex, which calls into question the recognition of seven geographical races of this snake.

Keywords: California, *Lampropeltis zonata*, phylogeography, snakes, subspecies

Received 7 December 1998; revision received 8 April 1999; accepted 29 June 1999

Introduction

Phylogeography is a rapidly growing subdiscipline of biogeography concerned with the principles and processes that govern the geographical distribution of genealogical lineages, especially those at the intraspecific level (Avice *et al.* 1987). The initial and still most common goal of phylogeographical studies is the use of animal mitochondrial (mt) DNA to assess the intra- and interpopulation spatial distribution of haplotypes whose phylogenetic relationships are inferred (Avice 1998), usually by cladistic methods. Among vertebrate groups, snakes have thus far attracted the least attention from phylogeographers, and con-

sequently the evolutionary relationships of conspecific populations of these secretive reptiles have rarely been studied in detail (e.g. Barker 1992; Malhotra & Thorpe 1997; Zamudio & Greene 1997).

The California mountain kingsnake, *Lampropeltis zonata*, is distributed along the mountainous regions of the Pacific Coast of North America, from southern Washington in the USA to northern Baja California, Mexico, and South Todos Santos Island, off Ensenada, along the northwestern coast of Baja California (Fig. 1). The species usually inhabits rocky outcrops and canyons associated with coniferous forests and riparian woodlands (McGurty 1988). The basic colour pattern of the body is a series of alternating black and white rings; typically, each black ring encloses a red area on each side, and the red areas may coalesce mid-dorsally to form a red ring between two black rings. The fundamental unit of colour pattern is a triad, a pair

Correspondence: J. A. Rodríguez-Robles. Fax: + 01-510-643-8238; E-mail: javier@socrates.berkeley.edu

‡Present address: Department of Biology, Arizona State University, Tempe, AZ 85287-1501, USA

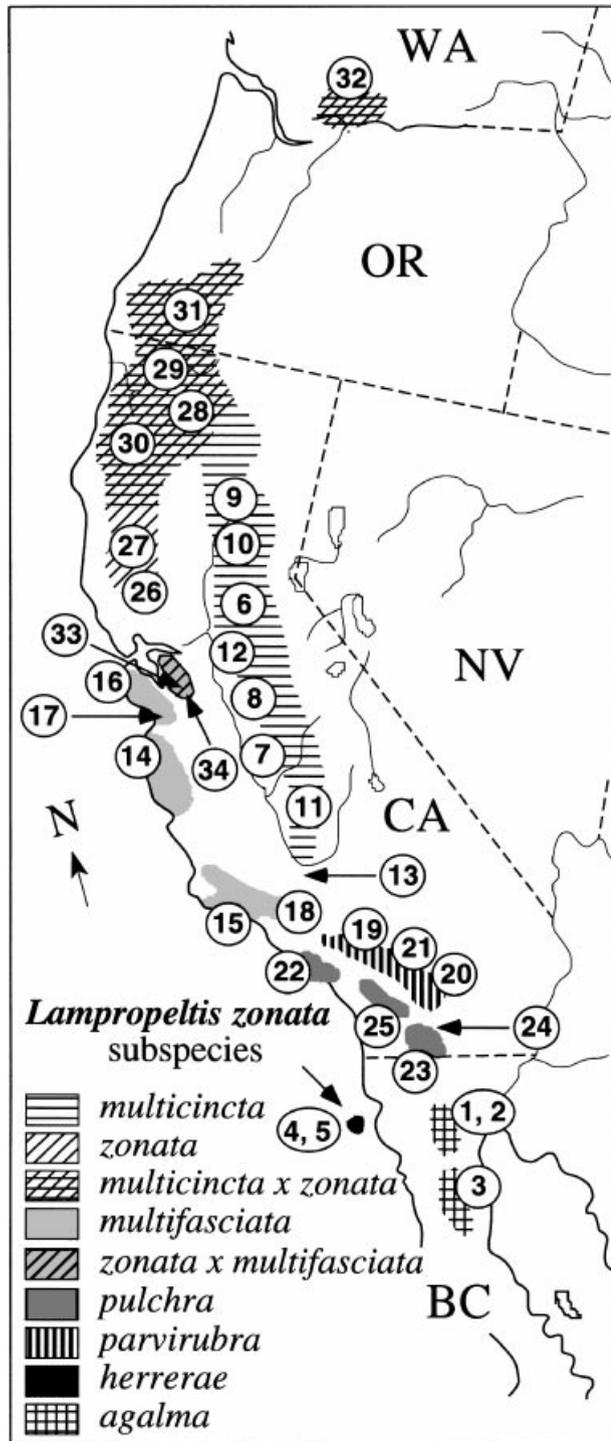


Fig. 1 Approximate distribution of the subspecies of *Lampropeltis zonata* in the USA and Mexico (after Zweifel (1974) and McGurty (1988)). Numbers indicate the localities of the specimens included in this study. The locality of sample 13 falls outside the previously known range of *L. zonata* because it corresponds to a population discovered during this study. The island of South Todos Santos (indicated with an arrow) is not drawn to scale. WA, Washington; OR, Oregon; NV, Nevada; CA, California; BC, Baja California.

of white rings together with the black or red and black areas between them. Based mainly on differences in the number of body triads and the percentage of confluent triads (where a black ring is completely interrupted by a red band dorsally), and to a lesser extent in the position of the first white ring and presence or absence of red on the snout, Zweifel (1952, 1974) recognized seven subspecies of the California mountain kingsnake (*agalma*, *herrerae*, *multicincta*, *multifasciata*, *parvirubra*, *pulchra*, and *zonata*). mtDNA sequences from specimens from throughout the range of *L. zonata* were used to infer evolutionary relationships among the recognized subspecies, and our intraspecific phylogeny was used to test previous biogeographical hypotheses (Zweifel 1952; Hayes 1975) for this snake.

Materials and methods

Taxon sampling, DNA isolation, and sequencing

Tissue samples were obtained from two to seven individuals of the seven recognized subspecies of *Lampropeltis zonata*, *agalma* (Baja California mountain kingsnake), *herrerae* (South Todos Santos Island mountain kingsnake), *multicincta* (Sierra mountain kingsnake), *multifasciata* (coast mountain kingsnake), *parvirubra* (San Bernardino mountain kingsnake), *pulchra* (San Diego mountain kingsnake), and *zonata* (Saint Helena mountain kingsnake), and from the putative *multicincta* × *zonata* and *zonata* × *multifasciata* intergrade populations (Zweifel 1952; Table 1). Total genomic DNA was extracted from ventral scale clips or tissue samples preserved in 95% ethanol using the sodium dodecyl sulphate–proteinase K/phenol/RNase method (Sambrook *et al.* 1989). Using total cellular DNA as a template, we amplified (with the polymerase chain reaction, PCR) and used for phylogenetic analyses a 787 bp fragment of mtDNA that encompassed a 658 bp portion of the 3' end of the nicotinamide adenine dinucleotide dehydrogenase subunit 4 (*Ndh4*, or 'ND4' gene), and a 129 bp section of three transfer ribonucleic acid (tRNA) genes (tRNA^{His}, tRNA^{Ser}, tRNA^{Leu}), using primers labelled ND4 and Leu (Arévalo *et al.* 1994). PCR reactions were carried out in 25 µL volumes consisting of 12.5 µL of template DNA, 5.0 µL of primers (10 µM), 2.5 µL of 10× PCR reaction buffer (Boehringer Mannheim), 0.475 µL of deoxynucleoside triphosphates (40 mM), 0.125 µL of *Thermus aquaticus* DNA polymerase (5 U/µL), and 4.4 µL of H₂O. DNA was denatured initially at 94 °C for 5 min, then 35 cycles of amplification were carried out under the following conditions: 94 °C denaturation for 60 s, 55 °C annealing for 60 s, and 72 °C extension for 60 s, followed by a final 5 min extension at 72 °C. Four microlitres of the resulting PCR product was electrophoresed on a 1% agarose gel and stained with ethidium bromide to verify product band size.

Table 1 Sample number, GenBank Accession no., voucher number (if available), and locality of the three outgroups and 34 specimens of *Lampropeltis zonata* used in this study. (The mitochondrial DNA (mtDNA) haplotypes of *L.z. herrerae* (sample 4) and of *L.z. multincincta* (sample 10) were identical to those of *L.z. herrerae* (sample 5) and *L.z. multincincta* × *zonata* (sample 32), respectively, and therefore they share the same GenBank Accession nos.) Museum and collector abbreviations: MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; HWG, Harry W. Greene; RES, Richard E. Staub

Taxon	Sample number	GenBank Accession no., voucher number, and locality
Outgroups		
<i>Lampropeltis getula</i>	—	AF138759; HWG 1485; US: California, San Benito Co., near Pinnacles National Monument
<i>Lampropeltis mexicana</i>	—	AF138760; HWG 2650; Mexico: specific locality unknown
<i>Lampropeltis pyromelana</i>	—	AF138761; HWG 2203; US: Arizona, Cochise Co.
<i>Lampropeltis zonata</i> subspecies		
<i>agalma</i>	1	AF136189; Mexico: Baja California, Laguna Hansen
<i>agalma</i>	2	AF136190; Mexico: Baja California, Laguna Hansen
<i>agalma</i>	3	AF136191; Mexico: Baja California, Sierra San Pedro Mártir
<i>herrerae</i>	4	AF136192; Mexico: Baja California, South Todos Santos Island
<i>herrerae</i>	5	AF136192; Mexico: Baja California, South Todos Santos Island
<i>multincincta</i>	6	AF136193; MVZ 229879; US: California, El Dorado Co., Kyburz
<i>multincincta</i>	7	AF136194; US: California, Madera Co., Bass Lake
<i>multincincta</i>	8	AF136195; US: California, Mariposa Co., Greeley Hill
<i>multincincta</i>	9	AF136196; US: California, Plumas Co., near Quincy on Highway 70
<i>multincincta</i>	10	AF136217; MVZ 229910; US: California, Sierra Co., near Downieville on Highway 49
<i>multincincta</i>	11	AF136197; US: California, Tulare Co., north fork of Middle Fork Tule River
<i>multincincta</i>	12	AF136198; US: California, Tuolumne Co., Pinecrest Lake
<i>multifasciata</i>	13	AF136199; MVZ 229881; US: California, Kern Co., Tehachapi Mountains
<i>multifasciata</i>	14	AF136200; MVZ 229883; US: California, Monterey Co., Bottcher's Gap
<i>multifasciata</i>	15	AF136201; RES mf40; US: California, Santa Barbara Co., Santa Barbara
<i>multifasciata</i>	16	AF136202; MVZ 229893; US: California, Santa Cruz Co., Ben Lomond
<i>multifasciata</i>	17	AF136203; US: California, Santa Cruz Co., near Watsonville
<i>multifasciata</i>	18	AF136204; RES mf41; US: California, Ventura Co., near Frazier Park
<i>parvirubra</i>	19	AF136205; US: California, Los Angeles Co., San Gabriel Mountains, West Fork
<i>parvirubra</i>	20	AF136206; US: California, Riverside Co., San Jacinto Mountains, Black Canyon
<i>parvirubra</i>	21	AF136207; US: California, San Bernardino Co., San Bernardino Mountains, Running Springs
<i>pulchra</i>	22	AF136208; US: Los Angeles Co., Santa Monica Mountains, Decker School Road
<i>pulchra</i>	23	AF136209; MVZ 229888; US: California, San Diego Co., Mount Laguna
<i>pulchra</i>	24	AF136210; MVZ 229889; US: California, San Diego Co., Palomar Mountains
<i>pulchra</i>	25	AF136211; US: California, Orange Co., Santa Ana Mountains
<i>zonata</i>	26	AF138762; MVZ 225913; US: California, Lake Co., Mount Saint Helena, Western Mines Road
<i>zonata</i>	27	AF136212; MVZ 229882; US: California, Mendocino Co., near Hopland Grade on Highway 175
<i>multincincta</i> × <i>zonata</i>	28	AF136213; MVZ 225915; US: California, Siskiyou Co., Dunsmuir
<i>multincincta</i> × <i>zonata</i>	29	AF136214; MVZ 225917; US: California, Siskiyou Co., near Hamburg on Highway 96
<i>multincincta</i> × <i>zonata</i>	30	AF136215; US: California, Trinity Co., Forest Glen, Rattlesnake Creek
<i>multincincta</i> × <i>zonata</i>	31	AF136216; MVZ 225920; US: Oregon, Jackson Co., northeast of Ashland
<i>multincincta</i> × <i>zonata</i>	32	AF136217; MVZ 229908; US: Washington, Klickitat Co., near Bingen
<i>zonata</i> × <i>multifasciata</i>	33	AF136218; US: California, Santa Clara Co., Mount Hamilton
<i>zonata</i> × <i>multifasciata</i>	34	AF136219; MVZ 229891; US: California, Santa Clara Co., south of Mount Hamilton

The double-stranded products were cleaned with the QIAquick Spin Purification Kit (Qiagen), then cycle sequenced using fluorescent dye-labelled terminators (ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS; Perkin-Elmer). Sequencing reactions were performed in 10 µL volumes (3 µL of template DNA, 4 µL of Ready Reaction, and 3 µL of H₂O) for 25 cycles using the following conditions: 30 s at 95 °C, 15 s at 50 °C, and 4 min at 60 °C. After cycle sequencing, the DNA was ethanol precipitated, dried, and resuspended in formamide/blue

dextran (5:1) by heating at 94 °C for 2 min. All samples were run for 8 h on a 4.8% Page Plus (Amersco®) acrylamide gel using an ABI Prism™ 377 automated sequencer, and all PCR fragments were sequenced in both directions.

Phylogenetic analyses

Sequences from the light and heavy DNA strands were input into the SEQUENCE NAVIGATOR (version 1.0.1) program and aligned to each other. Pairwise comparisons of observed proportional sequence divergence (*p*-distance)

and corrected sequence divergences, and number of transitions and transversions by codon position were obtained using the computer program PAUP* (Swofford 1999). To estimate the phylogenetic information content of the mtDNA character matrix, the *g*-test (Huelsenbeck 1991; Hillis & Huelsenbeck 1992) was used to assess the skewness of the tree length distribution of 100 000 trees randomly generated with PAUP*. The probability of the phylogenetic structure was assessed using the values provided by Hillis & Huelsenbeck (1992).

Two methods of phylogenetic reconstruction were used: maximum parsimony (MP; Camin & Sokal 1965; Swofford *et al.* 1996) and maximum likelihood (ML; Felsenstein 1981; Huelsenbeck & Crandall 1997), as implemented by PAUP*. MP was used in combination with two character weighting schemes: equal weighting, where all nucleotide substitutions were weighted equally regardless of type or codon position, and differential codon position weighting, where we down-weighted third position transitions (see below). Sites with insertion or deletion events were removed from the analyses. Each base position was treated as an unordered character with four alternative states. Ancestral character states were determined via outgroup comparison (Watrous & Wheeler 1981; Farris 1982; Maddison *et al.* 1984). *L. getula* (common kingsnake), *L. mexicana* (Mexican kingsnake), and *L. pyromelana* (Sonoran mountain kingsnake) were used as outgroups because previous morphological and molecular systematic studies (Keogh 1996; Rodríguez-Robles & De Jesús-Escobar 1999) identified these taxa as close relatives of *L. zonata*. Unique mtDNA haplotypes were used for our analyses, and therefore those of *herreriae* (sample 5) and *multicincta* (sample 10) were omitted, as they were identical to the haplotypes of *herreriae* (sample 4) and *multicincta* × *zonata* (sample 32), respectively.

Because the number of terminal taxa ($n = 35$) in our data set was too large to permit evaluating all trees or employing the branch-and-bound algorithm (Hendy & Penny 1982), heuristic search strategies were used for each tree-building methodology. One hundred repeated randomized input orders of taxa were used for all MP analyses to minimize the effects of entry sequence on the topology of the resulting cladogram(s). MP analyses were conducted without the steepest descent option, and with accelerated character transformation (ACCTRAN) optimization, tree bisection–reconnection (TBR) branch swapping, save all minimal trees (MULPARS), and zero-length branches collapsed to yield polytomies settings in place. Nonparametric bootstrapping (100 pseudoreplicates, 10 addition-sequence replicates for MP, 50% majority rule) was used to assess the stability of the internal branches in the cladograms (Felsenstein 1985; Felsenstein & Kishino 1993; Sanderson 1995; Berry & Gascuel 1996). Nonparametric bootstrap values generally are a conservative meas-

ure of the probability that a recovered group represents a true clade (Zharkikh & Li 1992; Hillis & Bull 1993; Li 1997).

For ML analyses, one of the trees found during the MP searches was randomly selected as the starting tree. Using empirical nucleotide frequencies and five rate categories, we fixed the probabilities of the six possible nucleotide transformations ($A \leftrightarrow C$, $A \leftrightarrow G$, $A \leftrightarrow T$, $C \leftrightarrow G$, $C \leftrightarrow T$, $G \leftrightarrow T$), the proportion of invariable sites θ , and the α 'shape' parameter of the gamma distribution of rate heterogeneity across nucleotide positions (Yang 1996a) to the empirical values calculated from the starting tree in a search for a better ML tree (a tree with a higher log-likelihood value) under the general time-reversible model of nucleotide substitution (Yang 1994; Gu *et al.* 1995; Swofford *et al.* 1996); that is, the most parameter-rich model available was used to search for ML trees. When a tree of higher likelihood was found, the parameters were re-optimized and fixed for a subsequent ML search (Swofford *et al.* 1996). This procedure was repeated until the same tree was found in successive iterations.

Because *ND4* is a protein-coding gene, *p*-distance (y) was plotted against corrected (with the Tamura–Nei model; Tamura & Nei 1993) estimates of proportional sequence divergence (x) for first, second, and third codon positions and for transitions and transversions separately to test for the possibility that some types of nucleotide substitutions have become saturated. Points that fall along the $y = x$ line have the same observed and estimated numbers of changes and thus have not been subjected to multiple hits. Points that fall below the $y = x$ line indicate that multiple hits have occurred; saturation is reached when observed sequence divergence does not continue to increase, despite the fact that corrected estimates do. Conventional statistical tests of the relationship between estimated and observed sequence divergence are not appropriate because of nonindependence of the data points due to the inclusion of each point in more than one pairwise comparison. Therefore, the plots were used as heuristic devices to help identify classes of changes occurring at different rates, which should be weighted differently in phylogenetic analyses.

Colour pattern characters

Colour pattern variation was examined on 245 wild-caught (most of which were released) and 76 preserved specimens of the seven subspecies of *L. zonata* in the California Academy of Sciences, San Francisco (CAS) and the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ). The number of colour body triads was determined by counting the number of white bands, beginning with the first band on the neck and continuing to the vent. A triad was found to be confluent

if the red band was continuous across the dorsal midline (Zweifel 1952). Scales that contained any red coloration were deemed red scales, and consequently a red band had to be interrupted by a complete row of black scales to be considered nonconfluent. The percentage of confluent triads was calculated by dividing the number of confluent red bands by the total number of body triads.

Results

Sequence variation

There were 189 variable and 105 potential phylogenetically informative characters (sites with at least two shared differences among all taxa) in the 787 bp mtDNA data matrix. Of the informative characters, 22 were at first codon positions, six at second positions, 63 at third positions, and 14 at noncoding positions. Within *Lampropeltis zonata* there were 14, five, 40, and 10 informative characters at first, second, third, and noncoding positions, respectively. Significant phylogenetic signal was present in the data set ($g_1 = -0.4787$, $P \lll 0.01$; mean \pm standard deviation (SD) tree length = 703.6 ± 27.6 , range 552–774), therefore inferring cladograms was justified.

Scatter plots (not shown) of observed vs. estimated sequence divergences indicated that first and second posi-

tion transitions and transversions, and third position transversions were linear. Third position transitions deviated greatly from a linear pattern, suggesting that these mutations are becoming saturated. To estimate the transition-to-transversion bias for third position transitions, a least-squares regression line, forced through the origin, was fitted to the part of the curve that was approximately linear. The slope of the regression line, 0.825, is an estimate of the transition-to-transversion ratio (Lara *et al.* 1996; Moore & DeFilippis 1997). Therefore, third codon transitional changes were down-weighted by a factor of eight using a 1:1:0.125 codon position weighting (first, second, and third codon position, respectively) to correct for the biased substitution rates at this position.

Phylogenetic relationships

The MP analyses using equally weighted characters resulted in 14 040 most-parsimonious trees 308 steps in length (L), a consistency index (CI) of 0.68 and a retention index (RI) of 0.83. Adjusting for the third position transitional bias in the coding region of the fragment of the *ND4* gene sequenced resulted in 104 most-parsimonious trees ($L = 479$, $CI = 0.75$, $RI = 0.87$). The bootstrap consensus tree for the two weighting schemes used is shown in Fig. 2. The log-likelihood score for the two ML trees

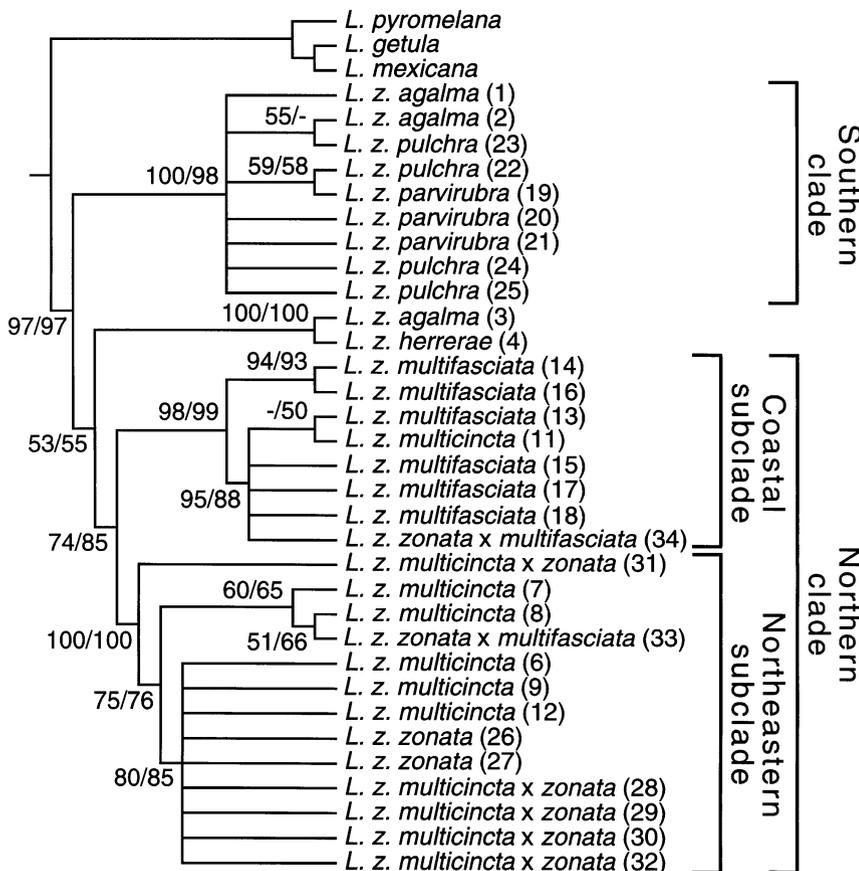


Fig. 2 Maximum parsimony bootstrap consensus phylogeny for 32 mitochondrial DNA (mtDNA) haplotypes of *Lampropeltis zonata*. Numbers on the tree indicate percentage of nonparametric bootstrap support for nodes retained by more than 50% of bootstrap replicates; bootstrap values obtained with all characters weighted equally/ bootstrap values obtained with third position transitions down-weighted by a factor of 8:1.

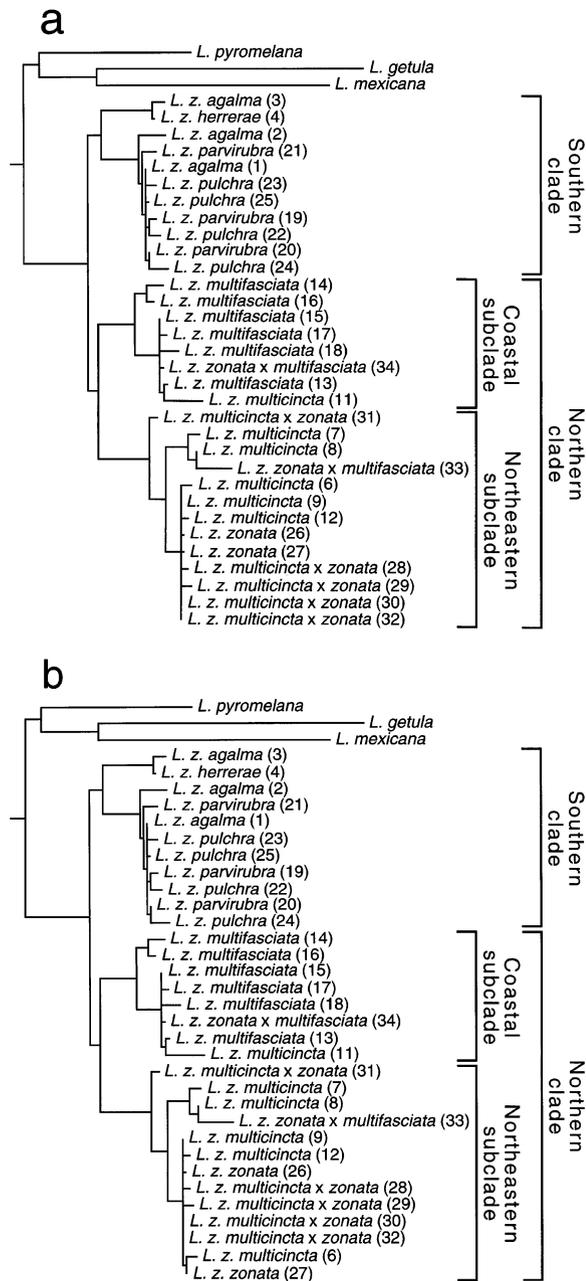


Fig. 3 Two alternative maximum likelihood trees for 32 mitochondrial DNA (mtDNA) haplotypes of *Lampropeltis zonata*. Branches are drawn proportional to branch lengths (expected amount of character change) estimated by the maximum likelihood algorithm. The two trees only differ in the inferred relationships of *L.z. multicincta* (sample 6) and *L.z. zonata* (sample 27).

obtained (Fig. 3) is $\text{LnL} = -2699.53407$. MP and ML methods recovered almost exactly the same three major nodes, which are supported by high bootstrap values in the two weighting schemes used in MP (Fig. 2). Together, these findings suggest that the groupings represent true clades. The most basal split within *L. zonata* corresponds to

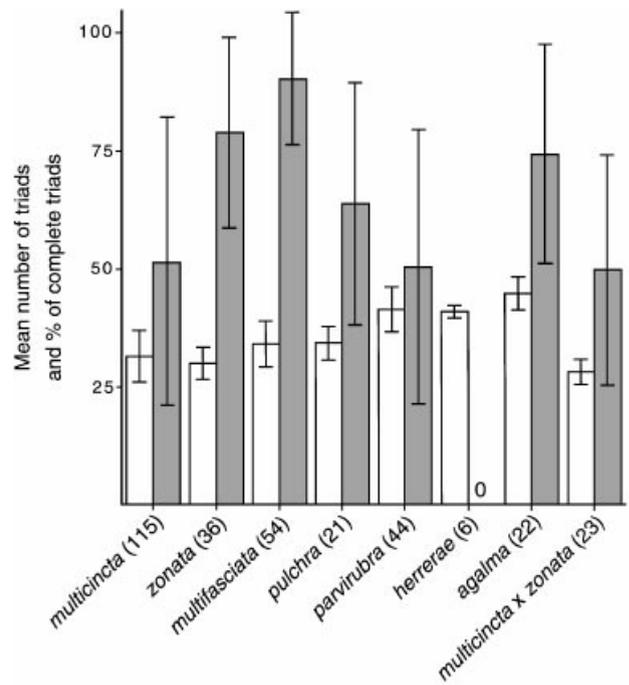


Fig. 4 Mean number of body triads (empty bars) and percentage of confluent triads (grey bars) for the seven subspecies of *Lampropeltis zonata* and the *multicincta* × *zonata* intergrades. Bars represent the mean ± 1 standard deviation (SD), and numbers in parentheses indicate sample sizes.

southern and northern segments of the distribution of the species. The northern clade is in turn composed of two subclades, a ‘coastal’ subclade, consisting of populations from the central coast of California and the southern Sierra Nevada Mountains of eastern California, and a ‘northeastern’ subclade, mainly comprised of populations north of the San Francisco Bay and from the majority of the Sierra Nevada. The relationships within the southern, coastal, and northeastern groupings were poorly resolved in the MP and ML trees, which indicates that there is a relatively high degree of gene flow among these populations, or alternatively, that the time since their divergence has been short, in which case the observed pattern probably represents the retention of ancestral, shared polymorphisms. Because ML methods generally outperform MP when inferring phylogenetic relationships using DNA sequence data (e.g. Kuhner & Felsenstein 1994; Huelsenbeck 1995; Yang 1996b; Cunningham *et al.* 1998), our discussion of phylogenetic and biogeographical patterns within *L. zonata* is based on the ML tree.

Colour pattern characters

The mean number of colour body triads and percentage of confluent triads per subspecies of *L. zonata* and for the *multicincta* × *zonata* intergrades are illustrated in Fig. 4.

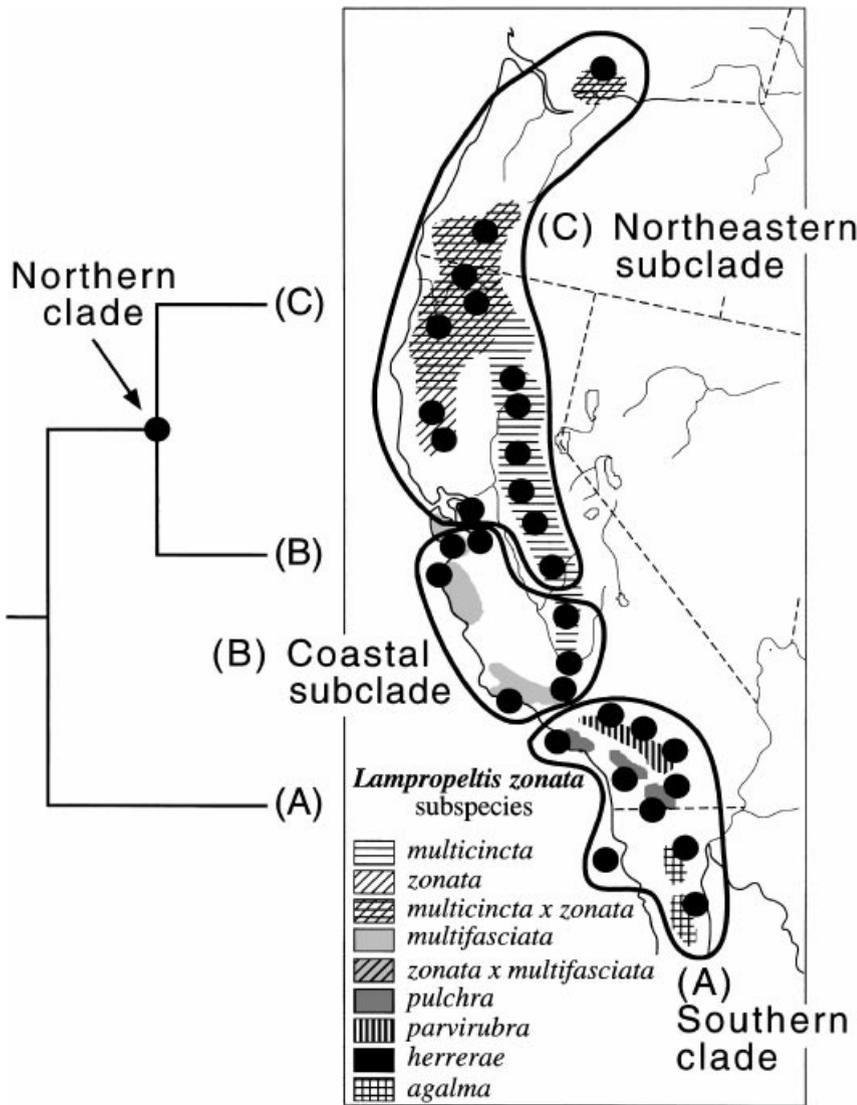


Fig. 5 Simplified interpretation of geographical structuring of mitochondrial DNA (mtDNA) haplotypes of *Lampropeltis zonata*, based on the maximum likelihood trees (Fig. 3). A phylogenetic hypothesis indicates the relationship among the three major groupings of this species complex.

The degree of overlap in these two characters among the seven subspecies is notable, which suggests that their usefulness to diagnose the subspecies of *L. zonata* is questionable.

Discussion

Phylogeography

Two previous biogeographical hypotheses have been proposed for *Lampropeltis zonata*. Zweifel (1952) suggested that *multicincta* was the most primitive form of the *L. zonata* complex, and that the mountain kingsnakes from southern California (*parvirubra*, *pulchra*) and northern Baja California (*agalma*) are derivatives primarily of coastal forms (*multifasciata*) which invaded these areas by

dispersing across the northern end of California and then southwards from a foothold in the Sierra Nevada. He further hypothesized that the northern Californian and southern Oregonian populations of *L. zonata* represent a recent northward extension of the range of the species.

Alternatively, Hayes (1975) suggested that two divergent segments of *L. zonata* evolved from an ancestral area in southcentral California, one associated with the southern Sierra Nevada and the other with the San Gabriel Mountains, south of the Sierra Nevada (location of sample 19). The Sierran stock then dispersed around the northern section of the Central Valley, south to the Santa Cruz and Santa Lucia Mountains on the western side of the valley, south of the San Francisco Peninsula. Meanwhile, the San Gabriel population expanded first southwards and then westwards and northwards, eventually

intermingling with the southern branch of the Sierran-derived populations.

To the extent that our gene tree is an accurate representation of the evolutionary history of *L. zonata* (see Moore (1995, 1997) and Brower *et al.* (1996)), we found little support for the above scenarios. The two basal clades in the ML tree correspond to southern and northern segments of the distribution of California mountain kingsnakes (Figs 3,5). Consequently, our data did not confirm the suggestion that *multicincta* is the most primitive form of the *L. zonata* complex, a question that remains unanswered. Furthermore, all *multifasciata* nested in the coastal subclade of the northern clade of *L. zonata*, contradicting Zweifel's and Hayes' view that the southern clade forms, *parvirubra*, *pulchra*, and *agalma*, are derivatives of *multifasciata*. Within the southern clade, the haplotypes of the specimens from Baja California are basal, suggesting that the ancestors of southern Californian populations dispersed north from Baja California, which again refutes Hayes' suggestion that Mexican *L. zonata* evolved from Californian populations. On the other hand, the phylogenetic position of the specimen from the northern extreme of the distribution of *L. zonata* in Washington (sample 32) supports Zweifel's hypothesis that the precursors to these populations spread northwards. The Wisconsin glacial interval began about 70 000 years ago, with the last glacial maxima occurring about 20 000 years ago. During this period ice sheets covered parts of southern Washington and Oregon (Barnosky *et al.* 1987), and nearby regions that remained exposed experienced an arctic climate, being extremely cold, dry, and devoid of most vegetation (Pielou 1991). It is thus likely that extant populations of *L. zonata* in southern Oregon and Washington are the result of a recent invasion from northern California. (The disjunct status of the Washington population of *L. zonata* may be artefactual, as there is suitable, but remote habitat for this species between Washington and southern Oregon that has not been adequately searched for California mountain kingsnakes.)

In the absence of an appropriate fossil record for *L. zonata*, we rely on molecular dating to gain an idea of the age of this taxon. Estimates of mtDNA sequence divergence for reptile species for which branching events have been confidently dated using fossil records or geological events range from 0.47 to 1.32% per million years (Zamudio & Greene 1997). The smallest (uncorrected) percentage sequence divergence between *L. zonata* and the outgroup (*L. pyromelana*) is 6.9%, which implies that the ancestor of California mountain kingsnakes evolved 14.6–5.2 million years ago (Mya; middle to late Miocene), during a time of increasingly cool climatic conditions over much of North America (Behrensmeyer *et al.* 1992).

The separation of the southern and northern clades represents the deepest divergence within *L. zonata*, and

our data suggest that this split occurred 5.7–2.0 Mya. Present-day southwestern California was separated from regions to the north by extensive, shallow inland seaways that mostly did not recede until the Pliocene (5–1.6 Mya; Peabody & Savage 1958; Oakeshott 1978; Norris & Webb 1990; Dupré *et al.* 1991). We propose that these marine barriers probably account for the most basal split within *L. zonata*. Within the southern *L. zonata* clade, molecular data suggest that the population of *L. zonata* from South Todos Santos Island diverged from its closest relatives on the mainland 1.1–0.4 Mya, which coincides with the period when this continental island became isolated from modern Baja California by rising sea levels following Pleistocene glacial recessions (cf. Wilcox 1980; Rohling *et al.* 1998).

The second major split in *L. zonata* occurred in the northern clade, between the coastal (mostly *multifasciata*) and northeastern (*multicincta*–*zonata*) subclades. The smallest percentage sequence divergence between representatives of these two clusters is 2.7%, which using Zamudio & Greene's (1997) figures of reptilian mtDNA rate evolution translates into 5.7–2.0 Mya. This period coincides with the continuing embayment of central California (i.e. the formation of the San Pablo and Suisun Bays, northeast of San Francisco; Oakeshott 1978; Dupré *et al.* 1991), a process which we suggest probably interrupted gene flow between specimens from the southern and the northern extremes of the ranges of modern *zonata* and *multifasciata*, respectively, allowing them to diverge. (Although the orogeny of the Sierra Nevada has been postulated to have played a role in the biogeographical history of some lineages (e.g. Riddle 1995), recent analyses suggest that this mountainous range is 50–60 million years older than generally thought (House *et al.* 1998), and therefore too ancient to have affected the diversification of California mountain kingsnakes.)

The distinct genetic break between specimens from the coastal and northeastern subclades occurs in the absence of any apparent geographical barrier, and we propose the following historical scenario to account for this phylogeographical pattern. Originally the northern clade of *L. zonata* possibly consisted of a chain of largely continuous populations distributed around California's Central Valley. The geological events described above, perhaps aided by associated changing climatic conditions, separated the populations along the central coast (*multifasciata*) from those to the north (*zonata*) and to the east (*multicincta*), and differentiation proceeded during this period of isolation. Subsequently the coastal and northeastern subclades expanded and came into secondary contact in the southern Sierra Nevada. (Because mtDNA is maternally inherited, the fact that a specimen that phenotypically is a *multicincta* (sample 11) nested within the *multifasciata* clade suggests that dispersal south from the central Sierra Nevada has been male biased.) The designated *zonata* ×

multifasciata population in central California (Zweifel 1952) suggests the possible existence of an intergrade zone between the coastal and northeastern subclades, but examination of additional specimens from this population is necessary to test this hypothesis.

The distribution patterns of some other vertebrate taxa parallel that of *L. zonata*. mtDNA haplotypes of *Pituophis catenifer* (gopher snake) indicate that specimens from southern California and most individuals from northern California nest phylogenetically in separate, sister clades (Rodríguez-Robles & De Jesús-Escobar in press). Genetic and morphological data indicate that the distribution of the California mouse, *Peromyscus californicus*, also consists of southern and northern segments (Smith 1979), whereas the ornate shrew, *Sorex ornatus*, is phylogeographically structured into three genotype clades representing southern, central, and northern localities (J. E. Maldonado *et al.*, unpublished) whose boundaries coincide those of the three major clusters of *L. zonata*. Thus, perhaps the distributions of gopher snakes, California mice, ornate shrews, and California mountain kingsnakes were affected by the same geological events.

On the other hand, although the ensatina salamander, *Ensatina eschscholtzii*, and the California newt, *Taricha torosa*, have distributions similar to that of *L. zonata* around California's Central Valley, in each case a somewhat different general biogeographical scenario from the one postulated here for California mountain kingsnakes has been proposed. *Ensatina* possibly evolved in present-day northwestern California and southwestern Oregon and spread south down the Pacific coast and along the Sierra Nevada (as well as north to southwestern Canada), and these coastal and inland populations represent independent radiations (Moritz *et al.* 1992; Wake 1997). *T. torosa* probably originated in southern California and dispersed northwards along the coast and the Sierra Nevada, with these two lineages also remaining largely independent (Tan & Wake 1995). Interestingly, southern Californian seaways and the embayment of central California do not appear to have been major distributional barriers for these salamanders, in contrast to the suggested biogeographical scenario for *P. catenifer*, *P. californicus*, and *L. zonata*. It will be of interest to conduct studies of additional reptilian taxa with distributions around California's Central Valley (e.g. *Elgaria multicarinata* (southern alligator lizard), *Diadophis punctatus* (ringneck snake), *Masticophis lateralis* (California whipsnake)) to determine whether they exhibit a similar phylogeographical pattern to that of *L. zonata*.

Phylogenetic and conservation implications of mtDNA variation

Our analyses suggest that some populations of *L. zonata*

are more closely related to geographically closer populations of different subspecies than to more distant, consubspecific populations (Figs 2 and 3). Nevertheless, except in the case of geographically isolated races (e.g. those on islands), one does not necessarily expect subspecies to be reciprocally monophyletic. To the contrary, taxa are recognized as subspecies, not full species, precisely because they intergrade with neighbours. Gene flow will blur the boundaries of subspecies, and keep them from attaining reciprocal monophyly at the mtDNA level (cf. Patton & Smith 1994). Therefore, the lack of reciprocal monophyly does not invalidate the subspecies of *L. zonata*.

Because these races were defined strictly by characters of body coloration, our analyses of the colour pattern data are much more pertinent to the issue of the validity of the recognized subspecies. After examining a larger number of specimens than was available to earlier authors (Klauber 1943; Zweifel 1952, 1974), we found that the two main colour traits used to diagnose the various subspecies are so variable that they cannot be reliably used to differentiate taxonomic units within *L. zonata*. Although we acknowledge that conducting these analyses on a much finer geographical scale might reveal areas of relatively homogenous colour patterns that then grade into other similar geographical areas (as it would be expected for subspecies that are relatively continuously distributed), we believe that this will not be the case, and therefore question whether the recognition of seven races within *L. zonata* is warranted.

Relying on a noncladistic analysis of morphological data, Hayes (1975) proposed elevating the South Todos Santos Island mountain kingsnake (*herreriae*) to full species rank, and recognized only two subspecies within the rest of the *L. zonata* complex, *L.z. zonata* (including *multicincta*) and *L.z. multifasciata* (including *agalma*, *parvirubra*, and *pulchra*). (Hayes actually placed *zonata* in the synonymy of *multicincta*, but because it is incorrect to exclude the nominal subspecies from the classification of a polytypic species, the proper name for Hayes' *L.z. multicincta* is *L.z. zonata*.) Our results indicate that *herreriae* is more closely related to the southernmost population of *agalma* from Baja California, but the relevance of this finding for the taxonomic position of the mountain kingsnakes from South Todos Santos Island is uncertain. The ML trees also indicate that mainland populations of *L. zonata* belong to either the southern or the northern clade, but these basal genetic clusters do not correspond to Hayes' subspecific designations because *multifasciata* is part of the northern clade, which also includes *multicincta* and *zonata*. Our data suggest as well that the southern clade of *L. zonata*, whose populations are allopatric to those that nest phylogenetically within the northern clade, may have attained the status of independent evolutionary lineage. Nevertheless,

we hesitate to propose nomenclatural changes using mtDNA patterns as the sole criterion for determining species boundaries, and thus await the completion of morphological and other studies before determining which taxonomic arrangement better reflects evolutionary relationships within the *L. zonata* complex.

Populations of *L. zonata* in Oregon and Washington, and of *L.z. pulchra* in southern California are considered 'taxa of special concern' and receive protection by the respective state agencies (Levell 1997). Our data on colour patterns establish that these populations cannot be reliably distinguished from neighbouring, more widespread populations, and in light of these findings the protected status conceded to these populations perhaps needs to be re-evaluated. *L. zonata* is widespread, with much of its range in public lands, and we believe that it is not in need of protection at the species level. Nevertheless, local populations are clearly being impacted by legal and illegal collecting and urban encroachment (personal observation). Because assessments of population genetic structure and diversity can provide valuable information for making conservation decisions (e.g. Avise & Hamrick 1996; Smith & Wayne 1996; Gibbs *et al.* 1997; Prior *et al.* 1997), more detailed genetic studies are needed before determinations regarding the protection of specific populations of *L. zonata* can be appropriately reached.

Acknowledgements

We thank the Oregon and Washington Departments of Fish and Wildlife, the California Department of Fish and Game, the California Department of Parks and Recreation, and the East Bay Regional Park District for issuing the necessary permits to conduct this study; the Nashville Zoo, Chaffee Zoological Garden, Sacramento Zoo, and the numerous individuals who provided animals or access to private land, without whose help this study would not have been possible; L. García, K. Zamudio, E. Taylor, G. Merker, L. Grismer, R. Lovich, and B. McGurty for their assistance; J. Patton and D. Wake for patiently explaining to J.A.R. the intricacies of population-level genetic evolutionary dynamics; and H. Greene, J. Patton, D. Wake, and M. Mahoney for constructive criticisms on the manuscript. This study was partly supported by an Annie M. Alexander Fellowship from the Museum of Vertebrate Zoology, University of California, Berkeley, to J.A.R.

References

Arévalo E, Davis SK, Sites JW Jr (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, **43**, 387–418.

Avise JC (1998) The history and purview of phylogeography: a personal reflection. *Molecular Ecology*, **7**, 371–379.

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population

genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Avise JC, Hamrick JL, eds (1996) *Conservation Genetics: Case Histories from Nature*. Chapman & Hall, New York.

Barker DG (1992) Variation, infraspecific relationships and biogeography of the ridgenose rattlesnake, *Crotalus willardi*. In: (eds Campbell JA, Brodie ED Jr) *Biology of the Pitvipers*, pp. 89–105. Selva, Tyler.

Barnosky CW, Anderson PM, Bartlein PJ (1987) The northwestern U.S. during deglaciation; vegetational history and paleoclimatic implications. In: (eds Ruddiman WF, Wright HE Jr) *The Geology of North America. North America and Adjacent Oceans During the Last Deglaciation*, Vol. K-3, pp. 289–321. Geological Society of America, Boulder.

Behrensmeier AK, Damuth JD, DiMichele WA *et al.*, eds (1992) *Terrestrial Ecosystems Through Time: Evolutionary Paleocology of Terrestrial Plants and Animals*. University of Chicago Press, Chicago.

Berry V, Gascuel O (1996) On the interpretation of bootstrap trees: appropriate threshold of clade selection and induced gain. *Molecular Biology and Evolution*, **13**, 999–1011.

Brower AVZ, DeSalle R, Vogler A (1996) Gene trees, species trees, and systematics: a cladistic perspective. *Annual Review of Ecology and Systematics*, **27**, 423–450.

Camin JH, Sokal RR (1965) A method for deducing branching sequences in phylogeny. *Evolution*, **19**, 311–326.

Cunningham CW, Zhu H, Hillis DM (1998) Best-fit maximum-likelihood models for phylogenetic inference: empirical tests with known phylogenies. *Evolution*, **52**, 978–987.

Dupré WR, Morrison RB, Clifton HE *et al.* (1991) Quaternary geology of the Pacific margin. In: (ed. Morrison RB) *The Geology of North America. Quaternary Nonglacial Geology: Conterminous U.S.*, Vol. K-2, pp. 141–214. Geological Society of America, Boulder.

Farris JS (1982) Outgroups and parsimony. *Systematic Zoology*, **31**, 328–334.

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17**, 368–376.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Felsenstein J, Kishino H (1993) Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. *Systematic Biology*, **42**, 193–200.

Gibbs HL, Prior KA, Weatherhead PJ, Johnson G (1997) Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus c. catenatus*: evidence from microsatellite DNA markers. *Molecular Ecology*, **6**, 1123–1132.

Gu X, Fu Y-X, Li W-H (1995) Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution*, **12**, 546–557.

Hayes MP (1975) The taxonomy and evolution of *Lampropeltis zonata*. MA Thesis, California State University, Chico.

Hendy MD, Penny D (1982) Branch and bound algorithms to determine minimal evolutionary trees. *Mathematical Biosciences*, **59**, 277–290.

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, **42**, 182–192.

Hillis DM, Huelsenbeck JP (1992) Signal, noise, and reliability in molecular phylogenetic analysis. *Journal of Heredity*, **83**, 189–195.

- House MA, Wernicke BP, Farley KA (1998) Dating topography of the Sierra Nevada, California, using apatite (U-Th)/He ages. *Nature*, **396**, 66–69.
- Huelsenbeck JP (1991) Tree-length distribution skewness: an indicator of phylogenetic information. *Systematic Zoology*, **40**, 257–270.
- Huelsenbeck JP (1995) Performance of phylogenetic methods in simulation. *Systematic Biology*, **44**, 17–48.
- Huelsenbeck JP, Crandall KA (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics*, **28**, 437–466.
- Keogh JS (1996) Evolution of the colubrid snake tribe Lampropeltini: a morphological perspective. *Herpetologica*, **52**, 406–416.
- Klauber LM (1943) The coral king snakes of the Pacific Coast. *Transactions of the San Diego Society of Natural History*, **10**, 75–82.
- Kuhner MK, Felsenstein J (1994) A simulation comparison of phylogeny algorithms under equal evolutionary rates. *Molecular Biology and Evolution*, **11**, 459–468.
- Lara MC, Patton JL, Da Silva MNF (1996) The simultaneous diversification of South American echimyid rodents (Hystricognathi) based on complete cytochrome b sequences. *Molecular Phylogenetics and Evolution*, **5**, 403–413.
- Levell JP (1997) *A Field Guide to Reptiles and the Law*, 2nd rev edn. Serpent's Tale, Lanesboro.
- Li W-H (1997) *Molecular Evolution*. Sinauer Associates, Sunderland.
- Maddison WP, Donoghue MJ, Maddison DR (1984) Outgroup analysis and parsimony. *Systematic Zoology*, **33**, 83–103.
- Malhotra A, Thorpe RS (1997) New perspectives on the evolution of south-east Asian pitvipers (genus *Trimeresurus*) from molecular studies. In: (eds Thorpe RS, Wüster W, Malhotra A) *Venomous Snakes: Ecology, Evolution and Snakebite*, pp. 115–128. Clarendon Press, Oxford.
- McGurty BM (1988) Natural history of the California mountain kingsnake *Lampropeltis zonata*. In: (eds De Lisle HF, Brown PR, Kaufman B, McGurty BM) *Proceedings of the Conference on California Herpetology*, pp. 73–88. Southwestern Herpetologists Society, Van Nuys.
- Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718–726.
- Moore WS (1997) Mitochondrial-gene trees versus nuclear-gene trees, a reply to Hoelzer. *Evolution*, **51**, 627–629.
- Moore WS, DeFilippis VR (1997) The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome b. In: (ed. Mindell DP) *Avian Molecular Evolution and Systematics*, pp. 83–119. Academic Press, San Diego.
- Moritz C, Schneider CJ, Wake DB (1992) Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology*, **41**, 273–291.
- Norris RM, Webb RW (1990) *Geology of California*, 2nd edn. John Wiley & Sons, New York.
- Oakeshott GB (1978) *California's Changing Landscapes: a Guide to the Geology of the State*, 2nd edn. McGraw-Hill, New York.
- Patton JL, Smith MF (1994) Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Systematic Biology*, **43**, 11–26.
- Peabody FE, Savage JM (1958) Evolution of a coast range corridor in California and its effect on the origin and dispersal of living amphibians and reptiles. In: (ed. Hubbs CL) *Zoogeography*, pp. 159–186. American Association for the Advancement of Science, Washington, D.C.
- Pielou EC (1991) *After the Ice Age: the Return of Life to Glaciated America*. University of Chicago Press, Chicago.
- Prior KA, Gibbs HL, Weatherhead PJ (1997) Population genetic structure in the black rat snake: implications for management. *Conservation Biology*, **11**, 1147–1158.
- Riddle BR (1995) Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshopper mice (*Onychomys*): the late Cenozoic development of a North American aridlands rodent guild. *Journal of Mammalogy*, **76**, 283–301.
- Rodríguez-Robles JA, De Jesús-Escobar JM (1999) Molecular systematics of New World lampropeltine snakes: implications for biogeography and evolution of food habits. *Biological Journal of the Linnean Society*, in press.
- Rodríguez-Robles JA, De Jesús-Escobar JM (in press) Molecular systematics of New World gopher, bull, and pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex. *Molecular Phylogenetics and Evolution*, in press.
- Rohling EJ, Fenton M, Jorissen FJ *et al.* (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature*, **394**, 162–165.
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.
- Sanderson MJ (1995) Objections to bootstrapping phylogenies: a critique. *Systematic Biology*, **44**, 299–320.
- Smith MF (1979) Geographic variation in genic and morphological characters in *Peromyscus californicus*. *Journal of Mammalogy*, **60**, 705–722.
- Smith TB, Wayne RK, eds (1996) *Molecular Genetic Approaches in Conservation*. Oxford University Press, Oxford.
- Swofford DL (1999) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland.
- Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference. In: (eds Hillis DM, Moritz C, Mable BK) *Molecular Systematics*, 2nd edn, pp. 407–514. Sinauer Associates, Sunderland.
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.
- Tan A-M, Wake DB (1995) MtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution*, **4**, 383–394.
- Wake DB (1997) Incipient species formation in salamanders of the *Ensatina* complex. *Proceedings of the National Academy of Sciences, USA*, **94**, 7761–7767.
- Watrous LE, Wheeler QD (1981) The out-group comparison method of character analysis. *Systematic Zoology*, **30**, 1–11.
- Wilcox BA (1980) Species number, stability, and equilibrium status of reptile faunas on the California Islands. In: (ed. Power DM) *The California Islands: Proceedings of a Multidisciplinary Symposium*, pp. 551–564. Santa Barbara Museum of Natural History, Santa Barbara.
- Yang Z (1994) Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution*, **39**, 105–111.
- Yang Z (1996a) Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution*, **11**, 367–372.
- Yang Z (1996b) Phylogenetic analysis using parsimony and

- likelihood methods. *Journal of Molecular Evolution*, **42**, 294–307.
- Zamudio KR, Greene HW (1997) Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. *Biological Journal of the Linnean Society*, **62**, 421–442.
- Zharkikh A, Li W-H (1992) Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences. I. Four taxa with a molecular clock. *Molecular Biology and Evolution*, **9**, 1119–1147.
- Zweifel RG (1952) Pattern variation and evolution of the mountain kingsnake, *Lampropeltis zonata*. *Copeia*, **1952**, 152–168.
- Zweifel RG (1974) *Lampropeltis zonata*. *Catalogue of American Amphibians and Reptiles*, 174.1–174.4. Society for the Study of Amphibians and Reptiles, Athens, Ohio.

The research interests of Javier Rodríguez-Robles include molecular systematics and evolutionary and behavioural ecology of reptiles and amphibians from western North America and the West Indies. Dale DeNardo is an Assistant Professor in the Department of Biology with a co-appointment as the University Veterinarian at Arizona State University. His research focuses primarily on environmental endocrinology, particularly the effects of temperature and social interactions on reproduction. Richard Staub is a postdoctoral researcher at the Department of Nutritional Sciences, University of California, Berkeley, where he is currently studying the metabolism of indole-3-carbinol, an anticarcinogenic chemical found in dietary vegetables.
