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Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province

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Abstract

The California Floristic Province harbours more endemic plant and animal taxa and more identifiable subspecies than any other area of comparable size in North America. We present evidence that physical historical processes have resulted in congruent patterns of genetic diversity over the past 2–10 million years. Using a molecular clock approach we show that diversification and establishment of spatial genetic structure across six taxonomic groups coincide with the putative age of California's mountain ranges and aridification in the region. Our results demonstrate the importance of geographical barriers and climatological events to species diversification and the overall geographical structure of biodiversity. These results should facilitate conservation efforts in this biodiversity hotspot for taxa whose population genetic structure is still unknown and may suggest the potential utility of this approach in regional conservation planning efforts.

Keywords: biodiversity, gene flow, hotspot, molecular clock, phylogeography

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Introduction

As the number of studies of the phylogeographical structure of individual species have increased in recent years, so have the opportunities for linking those studies with broader analyses of the genetic structure of multiple taxa across entire geographical landscapes. Recent attempts have included comparative phylogeographical analyses of interacting species (Althoff & Thompson 1999) and the search for patterns of genetic discontinuities in regional floras and faunas (Moritz & Faith 1998; Walker & Avise 1998; Avise 2000; Avise *et al*. 2000; Hewitt 2000; Brunsfeld *et al*. 2002). Such analyses should make it possible to identify pulses in the diversification of regional biota, particularly in areas rich in endemic taxa. To date, however, no study has evaluated simultaneously both the geographical structure and timing of molecular diversification for an entire biogeographical region. Here, we present a comparative analysis of phylogeographical histories for multiple species within each of six broad taxonomic groups (birds, plants, insects, mammals, reptiles and amphibians), that includes both geographical and temporal components of genetic structure among populations.

California encompasses some of the most geographically complicated patterns of genetic diversity on earth, and the California Floristic Province is considered one of the world's 25 most biologically rich and endangered terrestrial ecoregions (Myers *et al*. 1999). It harbours more plant species than the central and northeastern United States and Canada combined, and over 30% of the known insect species north of Mexico (Raven & Axelrod 1978; Arnett 1985; Raven 1988; Messick 1997). Forty-four per cent of plant and vertebrate species are endemic (restricted) to California (Myers *et al*. 1999). Widespread species in the California Floristic Province also show pronounced fine-scale genetic differences among populations, possibly resulting from the adaptation of populations to local environments (Harrison 1999) and other species (Brodie & Brodie 1990; Brown *et al*. 1997). We used the fine-scale genetic diversity of plants and animals in a preliminary evaluation of the phylogeographical structure of this entire biogeographical region. We used recent data collected for a variety of animal and plant taxa to generate a working hypothesis of the major patterns of molecular evolution and diversification. Our goal was to differentiate

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between independence and congruence in the phylogeographical histories of taxa found within this genetically diverse region.

Methods

We attempted to perform a comprehensive review of published molecular phylogeographical studies in California. In all, we reconstructed spatial distributions of genetic structure across six broad taxonomic groups from 55 studies (e.g. five birds, 24 plants, 10 insects, six mammals, eight reptiles and two amphibians). All studies in our analyses are of Californian natives, and we included all published studies that evaluated molecular differentiation among multiple populations within the California Floristic Province. The molecular tools varied among studies and included DNA sequence data, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), randomly amplified polymorphic DNA (RAPDs) or microsatellites. These data were used to determine geographical patterns of genetic structure in California. Only the DNA sequence data were used in our temporal analyses of population structure, but genetic discontinuities that were present in sequence data were congruent (i.e. separated by similar geographical barriers) with those found in the other molecular data sets. We acquired sequence data from GenBank using published accession numbers. Analyses used individuals as operational units.

Phylogenetic analysis

DNA sequences were aligned by eye into separate matrices for each study. Each data matrix was analysed using the parsimony algorithm of the software package PAUP* version 4.0d64 for Macintosh (Swofford 1998). Tree searches were conducted under the equal and unordered weights criterion (Fitch parsimony) with 1000 random sequence additions and tree bisection-reconnection (TBR) branch swapping, but saving only 10 trees per replicate. All the shortest trees collected in the 1000 replicates were then used as starting trees for another round of heuristic search. These trees were swapped to completion using TBR until more than 5000 trees were produced, at which point the number of trees was limited and swapping to completion was performed on the 5000 trees collected. We then performed 1000 bootstrap replicates using TBR swapping and saving 10 trees per replicate.

Molecular clock calibrations

Molecular clocks can be a useful guide for estimating timing of divergence or diversification events, but we use them cautiously here because it is now known that most evolving lineages are subject to rate heterogeneity (Gaunt & Miles 2002; Soltis *et al*. 2002). Molecular clocks generally seem more reliable when used for closely related taxa rather than for distantly related taxa (e.g. Caccone *et al*. 1997) and rates of evolution of a particular gene are less likely to vary in closely related taxonomic groups with similar life histories, metabolic rates and generation times (Hillis *et al*. 1996). We therefore applied individual rates of molecular evolution to each taxonomic group and to each gene. That is, we applied rates calibrated from reptiles to studies of reptiles, from insects to insects, and rates calibrated for cytochrome oxidase to studies using cytochrome oxidase, etc. Wherever possible we used internal calibrations for our estimates, using island age (Channel Islands (3 myr), inundation of the LA basin (2 myr) or inundation of the trans-California seaway at Monterey Bay (1 myr) (Fig. 1).

Most animal taxa in our analyses demonstrated reciprocal monophyly; that is, a distinct dichotomous split into two clades whose distributions were divided by some form of geographical boundary. In some cases individuals appear to have dispersed across these boundaries subsequent to the major split, but given the level of sampling in each of these studies these events appear to be rare. Plant studies rarely showed a clear geographical pattern and therefore did not exhibit reciprocal monophyly. We dated nodes between reciprocally monophyletic groups by calculating the average number of substitutions between the node of interest and all terminal taxa from that node (see example in Fig. 2). Average percentage sequence divergence was then determined and the date of the node of interest was calculated by applying the calibrated percentage sequence divergence rate.

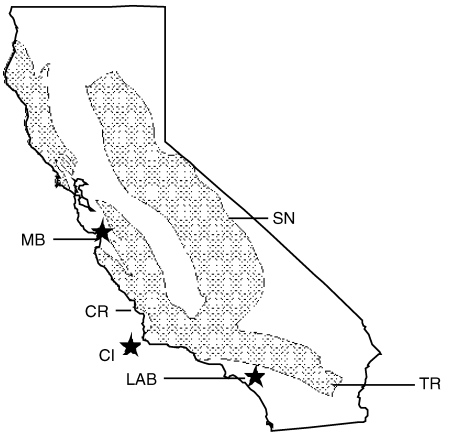

Fig. 1 The California landscape with its associated mountain ranges and geographical boundaries. TR = Transverse Ranges, CR = Coast Ranges, SN = Sierra Nevada mountains, LAB = Los Angeles basin, MB = Monterey Bay, CI = Channel islands. Geographic features used in molecular clock calibrations are indicated with a star.

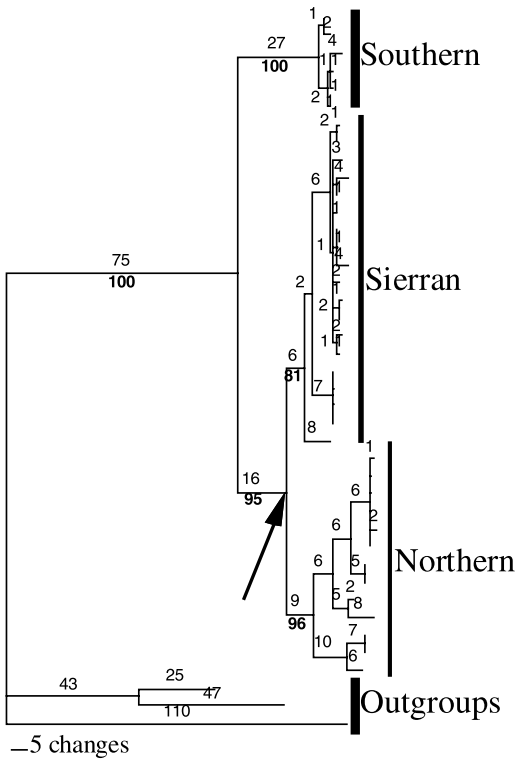

Fig. 2 Representative phylogenetic tree from rubber boas, *Charina bottae* illustrating the methods used in this study. Trees were constructed using the maximum parsimony construction criteria (see Methods). Numbers not in bold adjacent to branches in the *Charina* tree represent branch lengths. The age of the node indicated by the arrow was calculated by determining the average number of substitutions from this node to each tip, i.e. 20.63. Average percentage sequence divergence was calculated by dividing this figure by the total number of base pairs in the gene, i.e. 20.63/781 = 2.64%. A rate of 0.854% divergence per million years was then applied to this divergence giving an age of 3.09 million years for this node. Numbers in bold adjacent to tree branches indicate bootstrap support for phylogeographical breakpoints (Table 1).

Statistical analysis of congruence between genetic and geographical distance

We calculated pairwise genetic distances between individuals with geographically adjacent sampling distributions. We report results from calculations based on patristic distances. Branch lengths from parsimony trees yielded similar results. Using internally calibrated rates of molecular evolution for each taxonomic group, we estimated divergence time (millions of years) between pairs of individuals. Geographic distances between sampling locations listed in each published study were measured with the software package Adobe Illustrator version 8.0 for Macintosh. For each group, we regressed pairwise divergence dates against geographical distance. The residuals about the regression lines of time vs. distances represent divergence times holding variation among samples due to geographical distance constant. Residuals were normally distributed (Shapiro–Wilks test, $W = 0.87$). Thus, we used parametric statistics to compare the mean residual divergence dates for pairs of individuals that spanned a proposed phylogeographical boundary with pairs that did not. All tests were two-tailed. Numerator degrees of freedom in our analyses represent the proposed boundaries (e.g. Monterey Bay, Coast and Transverse ranges, Sierra Nevada mountains, no boundary) and denominator degrees of freedom represent the number of different taxa (minus 1) from which a mean divergence time was calculated among samples. We hypothesized that genetic distances (and hence divergence dates) between spatially adjacent samples should be relatively small irrespective of geographical distance compared with pairs of individuals that spanned an important phylogeographical barrier.

Results

We found strong concordance among studies in patterns of species distributions, although there were some differences among major taxa (Fig. 3). Most animal taxa had an obvious genetic split that separated northern and southern populations about the Transverse Ranges in southern California. In addition, ~70% of all animal taxa showed additional east/west differentiation within the Sierra Nevada and Coast Range mountains (Fig. 3). Hence, in taxa showing major genetic splits within California, the splits tend to be congruent. This pattern, however, did not hold for birds as well as it did for the other major taxonomic groups. This lack of population structure is due probably to the increased rates of gene flow among highly vagile and/or migratory species (e.g. Dawson *et al*. 2002; Kimura *et al*. 2002).

Although distributions of genetic break points seem to share common spatial patterns across taxa, spatial distributions alone are insufficient evidence that common historical processes shaped those distributions (Page 1994). A test of congruence must also measure the temporal component to species distributions. Therefore, using the 21 studies for which molecular sequence data were available from GenBank, we tested for a common historical framework across the floral and faunal diversity of California. Applying calibrated rates of molecular evolution (Junger & Lee Johnson 1980; Avise 2000) we located geographical features that defined major genetic splits in California (Table 1). Divergence time (genetic distances controlling for geographical distance, Fig. 4) between pairs of animal individuals sampled across the Monterey Bay, Sierra Nevada, Coast and Transverse ranges was significantly longer ($\bar{X}$ = 2.49 range

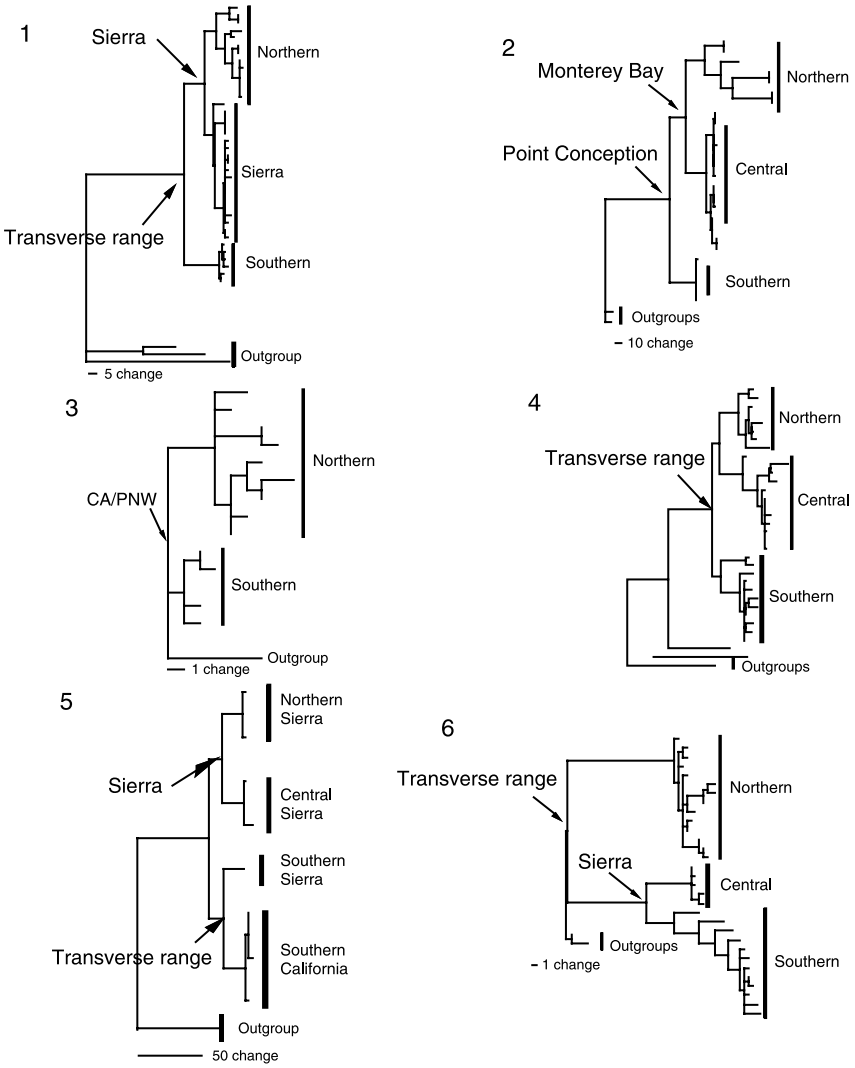

Fig. 3 Representative phylogenies reconstructed in this analysis for 1, *Charina bottae*; 2, *Tigriopus californicus*; 3, *Greya politella*; 4, *Lampropeltis zonata*; 5, *Rana muscosa*; 6, *Sorex ornatus*. Patterns of phylogeography in California animal taxa are dominated by population genetic breaks in the Sierra Nevada, Coast and Transverse Mountain Ranges. Arrows indicate nodes corresponding to major geographical boundaries as described in the text. Although the onset of genetic diversification in both plants and animals coincides with the proposed age of California's mountain ranges, geographical structure of animal taxa appears more closely related to mountain building and/or the effects of aridification than is the geographical structure of plants.

= 1.2–3.4 million years) than divergence time between pairs that did not span these features ($\bar{X}$ = 0.66 million years) (ANOVA $F_{4,33} = 4.99$, $P < 0.003$). By controlling for distance in this analysis, we ruled out the possibility that isolation by distance could explain the observed pattern. Thus, the proposed geographical boundaries appear to have had important effects on genetic divergence within animal taxa (Fig. 3; Table 1).

Molecular divergence dates for animal groups suggest that a period of molecular differentiation began in California about 7 million years BP. These dates coincide with putative ages for the uplift of the Sierra Nevada, Coast and Transverse mountain ranges (Chamberlain & Poage 2000). Pairwise genetic divergences within plant groups do not appear to have the same spatial associations with mountain ranges as reported above for animals ($\bar{X}$ = 1.35 range = 1.1–1.5 million years for taxa spanning mountain ranges vs. $\bar{X}$ = 0.986 million years for those not spanning boundaries) (ANOVA $F_{2,11} = 0.25$, $P = 0.78$). The difference in geographical structure between plants and animals was also evident in a two-factor analysis of variance (ANOVA boundary × kingdom $F_{2,26} = 3.47$, $P = 0.05$). Nevertheless, timings of diversification in plants (Table 2) roughly coincide with animal divergence. Earlier onsets of plant diversification may be attributable to Miocene era climate change in California (Raven & Axelrod 1978).

Discussion

Our analyses suggest, as a working hypothesis, that animal taxa in California show striking patterns of continuity in both the timing and geographical structure of molecular diversification. Together, the molecular data provide a signature of the common history of diversification by vicariance in California's floral and faunal diversity (Riddle *et al*. 2000; Zink *et al*. 2000b; Arbogast & Kenagy

Table 1 Summary table of phylogeographical data collected for animal taxa in this study that utilized DNA sequences. 'Major split/barrier' refers to the proposed boundaries within California (Sierra Nevada, Coast and Transverse Mountain Ranges, Monterey Bay and the Los Angeles basin). Bootstrap support for nodes that represent a dichotomy and for clades on either side of this dichotomy are indicated (*phylogenetic breaks for which only one individual was sampled and hence no bootstrap support could be calculated). Internal calibrations were estimated from lineage divergences following species isolation on California's Channel islands (3 mya, N. Pinter, pers.comm.). Sources for other rates are indicated in the table. Each rate is given as an average rate of change with the estimated range of rates in parentheses. Applying taxon specific rates to each tree, we estimated the timing of divergence across the proposed geographical barriers. We report estimates of divergence time as the mean time with estimated ranges in parentheses. Pairs of animal taxa showed significantly greater genetic divergences across the proposed phylogeographical barriers than did pairs of taxa not spanning these boundaries (see text)

Group	Taxon	Major split/ barrier	Bootstrap percentage	Genes	Rate (%divergence /mya)	Source	Timing of split (mya)
Arthropoda	*Aptostichus simus*	LA basin	76 (95 and 100)	16 s RNA	0.95	Santa Rosa island divergence (3 mya)	6.84
Reptilia	*Lampropeltis zonata*	LA basin	96 (99 and 76)	NDH4/ND4	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	3.19 (1.52–5.79)
Arthropoda	*Tigriopus californicus* (point conception)	LA basin	< 50 (100 and 100)	cytochrome oxidase	2.4	crustacean fossils described in (Edmands 2001)	5.28
Reptilia	*Charina bottae*	LA basin	100 (100 and 95)	NDH4/ND4	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	5.34 (2.55–9.72)
Arthropoda	*Timema*	Monterey Bay	85 (100 and *)	cytochrome oxidase	2	(Sandoval *et al*. 1998)	4.4
Reptilia	*Anniella pulchra*	Monterey Bay	100 (95 and 55)	cytochrome B	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	1.54 (0.73–2.78)
Mammalia	*Neotoma fuscipes*	Monterey Bay	100 (73 and 99)	mitochondrial DNA	2.185	(Maldonado *et al*. 2001)	2.22
Mammalia	*Sorex ornatus*	Monterey Bay	100 (93 and 96)	mitochondrial DNA	2.185	(Maldonado *et al*. 2001)	1.87
Arthropoda	*Greya politella*	Northern CA/PCNW	98 (81 and 54)	cytochrome oxidase	2.15 (2–2.3)	(Brown *et al*. 1997)	0.78 (0.73-0.84)
Amphibia	*Rana muscosa*	Sierran	88 (100)	cytochrome oxidase/tRNAs	1.3	4 geological events; (Macey *et al*. 2001)	1.33
Amphibia	*Taricha torosa*	Sierran	< 50	cytochrome B	0.85	(Tan & Wake 1995)	1.19
Reptilia	*Charina bottae*	Sierran	95 (81 and 96)	NDH4/ND4	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	3.09 (1.47–5.62)
Reptilia	*Crotalus viridis*	Sierran	98 (100 and 57)	cytochrome B	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	2.46 (1.18–4.47)
Reptilia	*Pituophis catenifer/ruthvenii*	Sierran	< 50	NDH4/ND4	0.52	Santa Cruz island divergence (3 mya)	3.75
Reptilia	*Crotalus viridis*	Transverse	98 (57 and < 50)	cytochrome B	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	1.59 (0.76–2.89)
Reptilia	*Pituophis*	Transverse	< 50	NDH4/ND4	0.52	Santa Cruz island divergence (3 mya)	4.8
Reptilia	*Sauromalus obesus*	Transverse	99 (*)	cytochrome B	0.854 (0.47–1.79)	(Zamudio *et al*. 1997)	0.9 (0.43–1.63)
Amphibia	*Rana muscosa*	Transverse	92 (100 and *)	cytochrome oxidase/tRNAs	1.3	4 geological events; (Macey *et al*. 2001)	0.68
Arthropoda	*Tegeticula maculata*	Transverse	100 (93 and 87)	cytochrome oxidase	2.15 (2–2.3)	(Brown *et al*. 1997)	0.36 (0.34–0.39)
Mammalia	*Thomomys townsendii*	Transverse	100 (100 and < 50)	cytochrome B	2.185	(Maldonado *et al*. 2001)	5.33

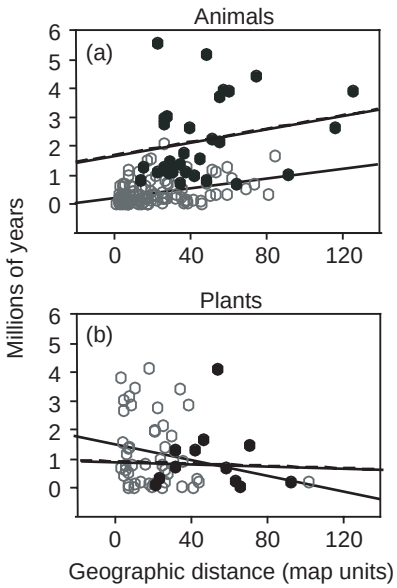

Fig. 4 Regressions of divergence times (based on percentage sequence divergence, see Methods) vs. geographical distance for (a) animals and (b) plants. Light symbols represent genetic distances between pairs of taxa that do not span one of the proposed boundaries. Dark circles represent genetic distances between pairs of taxa that span either the Sierra Nevada, Coast or Transverse Ranges, or which occur near the inundation of Monterey Bay. The best fit regression on light symbols is shown as a solid line and the best fit regression on dark circles is shown as the hatched line. We computed the residuals about each regression line and considered these residuals to estimate average molecular divergence correcting for geographical distance between pairs of samples. Note that the regression for pairs of animal taxa that span boundaries always lies above the regression for pairs of animal taxa that do not span a boundary, indicating longer divergence times for animal taxa that span boundaries. The same is not true of plants.

2001). None the less, we interpret these results cautiously. Although our analysis used multiple species across major groups, only a few species have yet been analysed in detail within any group. In addition, many other major groups have not yet been studied at all for patterns of molecular diversification within California. In addition, some of the intraspecific variation in tree depths presented in this study may have arisen without barriers to gene flow (e.g. limited dispersal distances, Irwin 2002). The patterns we have identified, however, provide a comparative basis for additional studies of molecular diversification, which will resolve the extent to which the patterns hold within and across taxa.

Birds will be a particularly important taxonomic group for future studies, because they are highly mobile and therefore some species may provide a potential contrast to other animal groups. The few phylogeographical data that are available for birds in California suggest patterns of population structure different from that found in most

Table 2 Summary table of data collected for plant taxa that utilized DNA sequences in this study. Bootstrap support for nodes that represent diversification events are indicated. The date for *Ceanothus* was calculated using the rate from *Phylica*, a genus in the same family that has a similar generation time. For taxa for which there was no internal calibration, the range and average rates from those reported in (Richardson *et al.* 2001) were applied, but using only rates from taxa with similar generation times. In these cases each rate is given as an average rate of change with the estimated range of rates in parentheses

Family	Genus	No. of species in genus (no. of Californian species in parentheses)	Bootstrap support for node at which diversification began	Gene	Rate (substitutions/site/year)	Source of rate estimate	Timing of diversification (myr)
Asteraceae	*Calycadenia*	11 (11)	58	ITS	4.25 (1.72–7.83)	Summarized in Richardson *et al.* (2001)	7.64 (4.1–18.9)
Polemoniaceae	*Linanthus* (*Leptosiphon* clade)	23 (23)	100	ITS	4.25 (1.72–7.83)	Summarized in Richardson *et al.* (2001)	17.58 (9.5–43.4)
Polemoniaceae	*Gilia*	57 (39)	98	ITS	4.25 (1.72–7.83)	Summarized in Richardson *et al.* (2001)	12.11 (6.5–29.9)
Rhamnaceae	*Ceanothus*	53 (44)	100	ITS	2.44	*Phylica* (Richardson *et al.* 2001)	13.9
Saxifragaceae	*Lithophragma*	10 (10)	100	ITS	7.18	San Clemente island divergence (3 mya)	1.47
Liliaceae	*Calochortus*	60 (38)	100	*rbcL*/*ndhF*	See reference	(Patterson & Givnish 2002)	7.3

other animal taxa analysed so far. At least some birds seem to exhibit relatively little population structure within California (Zink 1991) and in some cases California populations remain less well resolved due to extensive polytomies (Barrowclough *et al*. 1999; Zink *et al*. 2000a). Gene flow between geographically isolated populations may prevent the formation of population specific haplotype diversity in some species (Kimura *et al*. 2002).

Although our phylogeographical analyses are restricted to studies that incorporated DNA sequence data, a wide variety of other taxa that have been analysed using other molecular techniques show similar patterns of population genetic structure within California. For example, previous studies of population structure have revealed similar genetic breaks across the California landscape for a large number of taxa that includes *Batrachoceps* (Jockusch *et al*. 2001), *Bufo canorus* (Shaffer *et al*. 2000), *Passerella iliaca* (Zink 1994) and *Diadophis punctatus* (Feldman 2000). Explanations of the historical processes that have shaped these genetic patterns remain controversial and the relative importance of inland waterways and mountain building has been debated extensively [see contrasting opinions in Macey *et al*. (2001) and Maldonado *et al*. (2001)]. Nevertheless, it is increasingly evident that a combination of geological and climatological events has had important influences on the genetic structure of populations in California.

Genetic discontinuities have therefore arisen on the template of California's rich geological past and sharp climatic gradients. According to some reports (Chamberlain & Poage 2000) orogeny of the Sierra Nevada, Coast and Transverse ranges may have occurred between 2 and 5 million years ago, and this defined the great Central Valley that spans California's interior (Fig. 1) (Huber 1981; Unruh 1991). Although there is some controversy surrounding the age of these ranges, our data support current (Chamberlain & Poage 2000) hypotheses that significant environmental changes associated with the Sierra region occurred 2–5 million years ago. Contemporaneous changes in Pacific Ocean currents led to aridification across much of California (Ravelo *et al*. 1997) beginning about 2.8 million years ago. The combination of California's complex geological history, sharp climatic gradients across its landscapes and climatic fluctuations generated by changes in ocean currents (Herbert *et al*. 2001) have created a physical template that has driven ongoing genetic diversification over millions of years.

Our results add insight into the importance of vicariance and climate change, long presumed to play a critical role in generating biodiversity (Fisher 1930; Mayr 1942). Our results are particularly important given the mounting interest in establishing manageable reserves for the conservation of biological diversity (Mittermeier *et al*. 1999; Myers *et al*. 1999; Sechrest *et al*. 2002). Previous studies have demonstrated phylogeographical congruence on very large spatial scales such as Western Europe (Hewitt 2000) and Southeastern United States (Walker & Avise 1998), northern North America (Arbogast & Kenagy 2001) and the Pacific coast of North America (Soltis *et al*. 1997; Zink *et al*. 2001). Such studies suggest the importance of comparative phylogeographical analyses in developing our understanding of the historical structure of species assemblages (Avise 2000). Results from our study suggest that at a much smaller spatial scale, diverse groups of animal taxa may share a similar response to shifts in climate or habitat change, at least within some well-defined floristic and faunistic regions like the California Floristic Province. This congruence in population structure suggests that regional scale conservation efforts can be a useful planning tool for protecting biological diversity. Increased use of these kinds of analyses to identify geographical patterns of genetic discontinuity should facilitate conservation efforts in biodiversity hotspots worldwide (Moritz & Faith 1998; Smith *et al*. 2000).

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