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Pronounced phylogeographic structure on a small spatial scale: Geomorphological evolution and lineage history in the salamander ring species *Ensatina eschscholtzii* in central coastal California

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ABSTRACT

The salamander *Ensatina eschscholtzii* is a classic example of a ring species, and has an intricate biogeographic history. Within a part of the ring distribution, earlier work using allozymes disclosed high levels of genetic structure in central coastal California, where the subspecies *oregonensis*, *xanthoptica*, and *eschscholtzii* meet. We used mitochondrial cytochrome *b* sequences to further examine patterns of divergence in this area, including data from 155 localities (309 individuals). Our focus is on the documentation of population-level haplotype lineages. We show that *oregonensis* is represented by two unrelated, phenotypically similar clades, both of which possess substantial substructure of their own. The subspecies *xanthoptica* includes two lineages that differ in phenotype, one of which has colonized the foothills of the Sierra Nevada. The subspecies *eschscholtzii* occurs mainly to the south, but some populations from a northern lineage extend into the Monterey Bay region, where they approach *xanthoptica* geographically. In sum, populations in the central coastal California region form a distributional patchwork, including three subspecies, three clades (which differ from the three subspecies), and ten haplotype lineages. We conclude that such striking levels of phylogeographic structure reflect interspersed episodes of spatial fragmentation, in part driven by the complex geomorphological evolution of the California Coast Range system.

1. Introduction

California is a globally significant biodiversity hotspot, known for its outstanding species richness and levels of endemism (Myers et al., 2000; Davis et al., 2007). Such diversity is thought to be a consequence of a confluence of many factors, including extraordinary climatic diversity, a heterogeneous topography, and dynamic geomorphological rearrangements within the last several million years. How these factors influence the diversification of organismal lineages has been the subject of much attention by phylogeographers and systematists (e.g., Patton and Smith, 1990; Jockusch and Wake, 2002; Matocq, 2002; Jacobs et al., 2004; Kuchta and Tan, 2006; Chatzimanolis and Caterino, 2007; Rich et al., 2008). A commonality uniting these studies is that a limited number of biogeographic barriers have shaped the diversification of multiple coincident taxa (Calsbeek et al., 2003; Lapointe and Rissler, 2005; Feldman and Spicer, 2006).

A particularly prominent geological feature that has impacted the evolution and distribution of diverse taxa is the central Coast Ranges of California, including the present-day San Francisco and Monterey Bay regions (Fig. 1A; e.g., Wake, 1997; Kuchta and Tan, 2005; Martínez-Solano et al., 2007; Starrett and Hedin, 2007; Stockman and Bond, 2007; Kuchta et al., in press). This is because during the geological formation of the Coast Ranges, which extend over 800 km along the coast of California, the Monterey Bay region was the final piece to be added in the formation of a continuous Coast Range system. Prior to the mid-Pliocene, the present-day Monterey Bay region functioned as the passageway to an inland sea filling much of the Central Valley of California (Hall, 2002; Wake, 2006), and was an effective dispersal barrier for many terrestrial organisms (Calsbeek et al., 2003; Lapointe and Rissler, 2005; Feldman and Spicer, 2006). Interactions between the Pacific and North American plates generated uplift of the central Coast Ranges, however, and by 2 mya the seaway was closed (Dupré, 1990; Sims, 1993; Hall, 2002). Nonetheless, the Monterey Bay region continued to function as the outlet of a massive lake that replaced the inland sea (Sarna-Wojcicki et al., 1985), and it is possible that the Monterey Bay region thereby remained an effective barrier for terrestrial organisms. Finally, 600,000 years ago, the drainage of the Central Valley shifted to the Golden Gate (just north of San Francisco), and the Monterey Barrier was removed (Sarna-Wojcicki et al., 1985).

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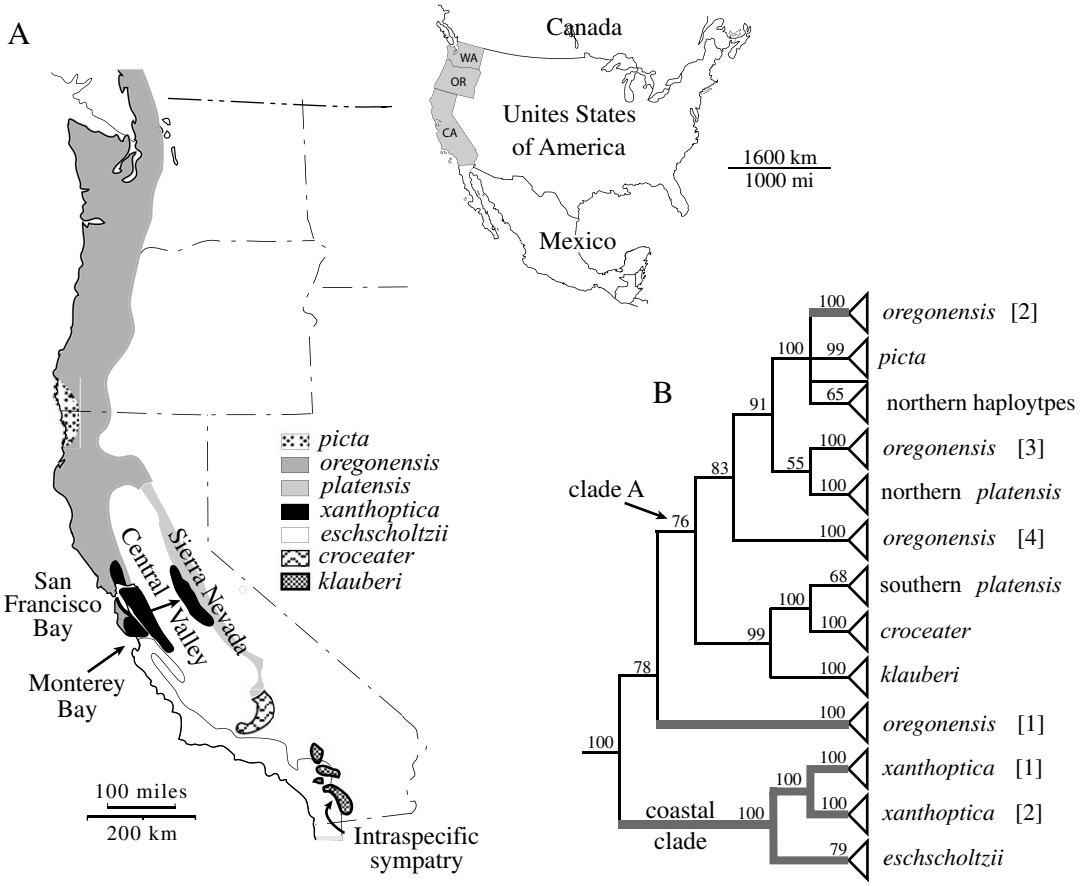

Fig. 1. (A) Map showing the distribution of subspecies of *Ensatina eschscholtzii* in western North America. In the map of the USA, the states of California, Oregon, and Washington are shown in grey. Subspecies, which circumscribe patterns of phenotypic variation (Stebbins, 1949), are differentially shaded. In southern California, the subspecies *eschscholtzii* and *klauberi* are locally sympatric in places, where they interact as distinct biological species. In addition, in central California, *xanthoptica* has colonized the foothills of the Sierra Nevada, where it forms a narrow hybrid zone with *platensis*. Note the location of San Francisco Bay, Monterey Bay, the Central Valley, and the Sierra Nevada, as these are frequently referred to in the text. (B) Topology summarizing the major features of a Bayesian phylogenetic analysis of 385 mtDNA sequences from 224 populations throughout the range of *Ensatina eschscholtzii* (Kuchta et al., in press). Numbers above branches are the posterior probabilities of clades. Clade names are used throughout the paper, and the thick grey branches identify clades that are the focus of the current study.

One species that exhibits striking phylogeographic differentiation in central coastal California is the plethodontid salamander *Ensatina eschscholtzii* (Wake, 1997). This species is a classic example of a ring species, or a species with a circular arrangement of populations with reproductively isolated elements overlapping at one point in the ring, yet phenotypic and genetic intergradation elsewhere (Mayr, 1942, 1963; Fig. 1A). Ring species originate when populations disperse around a central barrier to form a secondary contact, and interact there as separate species despite their potential linkage through a chain of interbreeding populations (Irwin et al., 2001; Irwin and Irwin, 2002). Stebbins (1949) was the first to propose that *E. eschscholtzii* was a ring species, including a specific biogeographic scenario. He postulated that the ancestor of the *Ensatina* complex originated in northern California, then dispersed southward down the Coast Ranges and the inland ranges (i.e., Sierra Nevada) as two distinct distributional arms. Populations along the two arms diverged in ecology, phenotype, and genetic structure as they spread, and in southern California the terminal ends of the two arms, represented by the subspecies *eschscholtzii* and *klauberi*, came together. There they were reported to interact as sympatric, reproductively isolated entities. The species thereby formed a ring around the arid, inhospitable Central Valley of California, with intergradation around the ring save the point of terminal overlap in southern California (Fig. 1A). (A smaller ring, midway down California, where the subspecies *xanthoptica* comes into contact with *platensis* to form a narrow hybrid zone, was also recognized.) Stebbins (1949) was not explicit with regard to the timing of this biogeographic scenario, but as the hypothesis predates the development of tectonic theory, it necessarily excludes consideration of landmass displacement along the San Andreas Fault.

The evolutionary and biogeographic predictions generated by the *Ensatina* ring species hypothesis (Stebbins, 1949) has received much analysis, including studies of allozymic differentiation (Wake and Yanev, 1986; Jackman and Wake, 1994; Wake, 1997), mtDNA phylogeography (Moritz et al., 1992; Kuchta et al., in press), hybrid zone dynamics (Brown, 1974; Wake et al., 1986, 1989; Alexandrino et al., 2005), and the ecological consequences of phenotypic variation (Kuchta, 2005; Kuchta et al., 2008). The results are complex (Wake and Schneider, 1998), but support the major tenets of the ring species scenario in that *Ensatina* possesses a ring-like distribution with separately evolved coastal and inland distributional arms. Phenotypic and genetic intergradation characterize contacts around the ring, while species-level interactions are present where lineages on either side of the ring meet, including sympatry between subspecies in southern California (summarized in Wake, 2006). Weak links in the ring include a distributional gap between the subspecies *croceater* and *klauberi* in southern California, and limited evidence of intergradation between *platensis* and *oregonensis* in northern California (likely an outcome of recent volcanic activity in the area; Jackman and Wake, 1994).

Previous studies have also shown that the coastal arm of the *Ensatina* complex originated more than 2 mya (Parks, 2000; Kuchta

et al., in press). The geological formation of the Coast Ranges of California therefore presents a biogeographic obstacle because, as explained above, a continuous California Coast Range did not appear until 2–0.6 mya. A modification of the biogeography of the coastal arm of the *Ensatina* complex was thus developed to account for ring closure in southern California while accommodating the geomorphological evolution of the Coast Ranges (Wake, 1997). Under this scenario, the ancestor of the coastal clade colonized an island mass off the coast of central California (formerly a part of the Salinian terrain; Yanev, 1980; Hall, 2002) ca. 5 myr ago, and there the subspecies *xanthoptica* and *eschscholtzii* originated. This island was incorporated into the central Coast Range system by 2 myr (Yanev, 1980), and subsequently *xanthoptica* spread northward to form a secondary contact with *oregonensis*, and *eschscholtzii* expanded southward to the ultimate point of ring closure, the secondary contact with *klauberi* in southern California.

This biogeographical scenario was inspired in part by an allozyme survey of populations of *Ensatina* throughout central coastal California (Wake, 1997). The subspecies *oregonensis*, *xanthoptica*, and *eschscholtzii* are found in this region. Based on color patterns, Stebbins (1949) identified extremely broad zones of intergradation among the subspecies with *xanthoptica* intergrading with *oregonensis* northward into northern California, and intergrading with *eschscholtzii* southward into southern California. In contrast, Wake (1997), while acknowledging the descriptive accuracy of Stebbins' coloration analyses, found little evidence of broad-scale genetic intergradation among subspecies. Instead, contact zones were identified along the border of *xanthoptica* and the edge of the putative zones of intergradation between *oregonensis* and *eschscholtzii*. Sizeable levels of genetic divergence among populations were found (Nei's, 1978, genetic distances $\geqslant$0.4 in some comparisons). In several instances, however, genetic distances between subspecies were lowest close to contact zones, suggesting lineage merger or introgression. For example, Nei's D between *xanthoptica* and *eschscholtzii* drops from 0.32 to 0.15 as one narrows the geographic sampling gap between them (and a roughly 30 km gap remains unexplored for allozymes; Wake, 1997). High levels of genetic differentiation were documented within subspecies as well, and in some comparisons within subspecies divergence rivals between subspecies divergence. For instance, Nei's D within *xanthoptica* in the Santa Cruz mountains (southern San Francisco peninsula) ranges up to 0.31, while D between *xanthoptica* and *oregonensis* in these same mountains ranges from 0.16 to 0.32. Wake (1997) thus concluded that the separate subspecies in central coastal California are not independent evolutionary lineages, because while they represent genetic isolates evolved in allopatry (e.g., Baker and Bradley, 2006), they lack reproductive isolation and genetic independence at points of secondary contact.

In this paper we re-examine the phylogeography of *Ensatina* in central coastal California using mtDNA haplotypes. Our focus is on the documentation of population-level haplotype lineages and their historical and genetic interactions, and various population genetic and phylogenetic tests are employed to explore the histories of the recovered lineages. Population sampling in this study is relatively dense because mapping the complex distributions of haplotype lineages (many of which are phenotypically cryptic) was an objective of the study. Finally, we compare our results with allozyme patterns reported by Wake (1997).

2. Materials and methods

2.1. Population sampling

A fragment of the mitochondrial cytochrome *b* gene was sequenced for 309 individuals from 155 populations from the San Francisco Bay area (Fig. 2), including five sequences from an earlier study by Moritz et al. (1992). Populations are here defined as samples within one kilometer of each other that have mtDNA sequences belonging to the same haplotype lineage. Sampling was geared toward describing the geographic limits of haplotype lineages, with a particular focus on locating contact zones. Populations in this study are labeled from 32 to 182 to maintain consistency with Kuchta et al. (in press), and DNA laboratory methods are provided in Kuchta et al. (in press). Detailed locality information is reported in Appendix A of the online Supplementary materials.

2.2. Phylogenetic analysis

For the phylogenetic analysis, the data set was divided into 1st, 2nd, and 3rd codon positions, and the best-fitting model of nucleotide substitution for each partition was selected using the Akaike Information Criterion (AIC) as implemented in MrModelTest (v.1.1b) (Nylander, 2004). The models chosen were HKY + Γ, HKY + I + Γ, and GTR + Γ for the1st, 2nd, and 3rd codon positions, respectively. Bayesian phylogenetic analyses were performed using MrBayes v.3.04b (Huelsenbeck and Ronquist, 2001), including flat Dirichlet prior distributions for substitution rates and base frequencies, and default flat prior distributions for all other parameters. The tree was rooted with 12 outgroup sequences, selected based on their inferred relationships to *Ensatina* (Mueller et al., 2004; Chippindale et al., 2004; Vieites et al., 2007). See Kuchta et al. (in press) for further methodological details.

We explicitly distinguish between lineages and clades in this paper. Lineages designate groups of populations characterized by a single unbranched pattern of ancestry and descent. They are here diagnosed as mtDNA haplotype clades within which there is only weak divergence among haplotypes. Clades form a nested hierarchy composed of two or more haplotype lineages. The correspondence among haplotype lineages and patterns of population structure as measured by allozymes is considered in the discussion.

2.3. Phylogeographic diversity

2.3.1. Population structure: indices of genetic diversity and demographic analyses

Genetic diversity indices were used to compare patterns of genetic differentiation among haplotype lineages. Computed diversity indices include haplotype diversity (h), sequence diversity (κ), and nucleotide diversity (π) (Nei, 1987). An analysis of molecular variation (AMOVA; Excoffier et al., 1992) was used to summarize patterns of genetic differentiation within and among clades, lineages, and populations (e.g., Fontanella et al., 2008). Arlequin v.3.11 (Excoffier et al., 2005) was used for all of these calculations.

Histograms of the number of pairwise mutational differences among haplotypes (i.e. mismatch distributions) were used to infer changes in population demography. Assuming an infinite-sites model, recent demographic expansion will generate a mismatch distribution that is unimodal and resembles a Poisson distribution (Slatkin and Hudson, 1991). Conversely, with demographic stability mismatch distributions become multimodal or "ragged" (Rogers and Harpending, 1992). We generated mismatch distributions for all the recovered haplotype lineages and compared these distributions to the expected distribution under a step-wise expansion model (Schneider and Excoffier, 1999). Mismatch analyses were performed with Arlequin 3.11 (Excoffier et al., 2005), and significance was assessed via parametric bootstrapping of the dataset (1000 replicates). Mismatch figures were created using DNAsp 4.50.1 (Rozas et al., 2003).

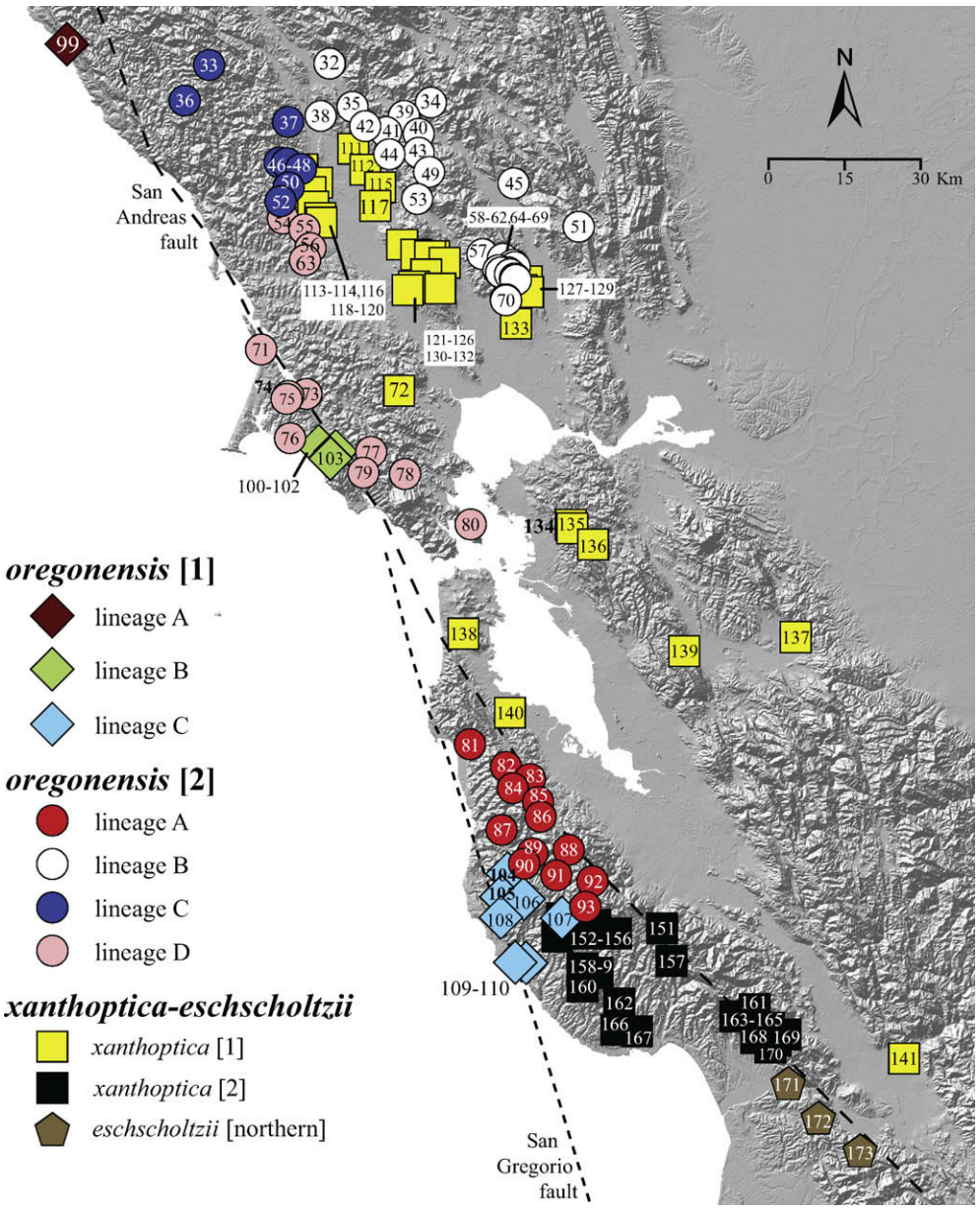

Fig. 2. Map showing the distribution of samples of *Ensatina eschscholtzii* in central coastal California. Samples assigned to the same clade are given a common symbol, and colors are used to identify lineages within clades. Population numbers correspond to locality information provided in Appendix A, and are used throughout the text. The dotted lines trace the San Andreas and San Gregorio faults.

The R_2 raggedness index (Ramos-Onsins and Rozas, 2002) and Fu's F_s (Fu, 1997) were used to test for demographic expansion against a null model of demographic stability, assuming selective neutrality of the mtDNA haplotypes. Ramos-Onsins and Rozas (2002) have shown that R_2 has higher statistical power than F_s with small samples, while F_s is superior for large samples. R_2 and F_s were calculated using DNAsp 4.1.7 (Rozas et al., 2003), and significance was assessed via parametric bootstrapping of the dataset (50,000 replicates). Note that the null model of R_2 and F_s is demographic stability, whereas the null model of the mismatch distributions is demographic expansion.

2.3.2. Haplotype networks

Haplotype networks were constructed to evaluate relationships among haplotypes within lineages and clades. Prior to analysis, sequences were truncated to be of equal length, and some particularly short sequences were eliminated from the analysis (Appendix A). Haplotype networks were calculated using the statistical parsimony procedure (Templeton et al., 1992) as implemented in TCS 1.13 (Clement et al., 2000).

2.3.3. Isolation by distance

Isolation by distance (IBD) was examined by plotting maximum likelihood estimates of genetic distance against geographic distance for all pairwise comparisons. The model of nucleotide substitution was selected using the Akaike Information Criterion (AIC) as implemented in ModelTest v.3.7 (Posada and Crandall, 1998). Mantel tests (100,000 randomizations), which correct for the non-independence among pairwise comparisons, were used to test for a significant correlation between geographic and genetic distance. Reduced Major Axis (RMA) regression was used to calculate the slope, y-intercept, and coefficient of determination (r^2) of the IBD plots. Based on simulation data, Hellberg (1994) has shown that RMA regression is superior to ordinary least squares regression

for the purpose of documenting IBD. Nonetheless, because of the non-independence of data points, the regression statistics presented here should be viewed as heuristic in nature. The web-based computer program IBDWS v.3.14 (Jensen et al., 2005) was used to calculate Mantel tests and RMA regression statistics.

3. Results

3.1. Phylogenetic relationships

Three basal clades were recovered in the phylogenetic analysis, each of which contains multiple haplotype lineages in central coastal California (Fig. 1B; Appendix A). The first clade includes the subspecies *xanthoptica* and *eschscholtzii*, and we call this the 'coastal clade' (Fig. 1B); the second clade is a lineage currently assigned to the subspecies *oregonensis*, and we call it '*oregonensis* [1]' (Fig. 1B); and the third clade is a clade we call 'Clade A.' This last clade includes a clade we call '*oregonensis* [2]', and although a member of the subspecies *oregonensis* (a phenotypic designation), *oregonensis* [2] is distantly related to *oregonensis* [1] (Fig. 1B; Appendix A). The coastal clade, *oregonensis* [1], and *oregonensis* [2] are all found in central coastal California. Maximum likelihood estimates of sequence divergence between these clades ranges from 5.9% (*xanthoptica* [1] vs. *xanthoptica* [2]) to 16.1% (*oregonensis* [2] vs. *xanthoptica* [1]) (Appendix A).

3.2. Molecular analysis of variance

An AMOVA was used to describe the variance structure among mtDNA sequences at three hierarchical levels: among the three clades of central coastal California *Ensatina* identified in the phylogenetic analysis, among the haplotype lineages within these clades, and among populations within lineages. The clades in the analysis were as follows (lineages in parentheses; Fig. 2): coastal clade (*xanthoptica* [1], *xanthoptica* [2], *eschscholtzii* [northern lineage]), *oregonensis* [1] (A–C), and *oregonensis* [2] (A–D). Haplotype lineage diversity within clades is discussed in detail below. The largest fraction of the variance, 66%, was attributed to variation among clades, while 31% of the variance was explained by variation among lineages within clades. Only 3% of the variance was due to differences among populations within haplotype lineages (Table 1).

3.3. Patterns of differentiation by clade

3.3.1. Divergence within oregonensis [1]

The *oregonensis* [1] clade is distributed north and south of San Francisco Bay, with three strongly supported, allopatric haplotype lineages. From north to south, the three lineages are located (1) in north coastal California, from northern Sonoma County northward to central Mendocino County (lineage A); (2) at the southern end of Point Reyes peninsula, north of San Francisco Bay (lineage B); and (3) along the coast of the San Francisco Peninsula (lineage C) (Figs. 2 and 3A). Maximum likelihood estimates of sequence divergence among lineages within *oregonensis* [1] range from 3.2% (lineage A vs. B) to 8.1% (lineage A vs. C) (Appendix A). Using statistical parsimony to construct a haplotype network, lineage A does not connect to the other lineages of *oregonensis* [1] at the 95% confidence level; lineages B and C do connect, but are separated by eight mutations (Fig. 3B). Different haplotypes are found within every population of lineage A, which has higher indices of genetic diversity than the other lineages (Table 2), while levels of intrapopulational variation are higher within lineage B (Fig. 3B). Nonetheless, neither F_s, R_2, nor the mismatch distribution is significant within lineages A or B. (Table 2). The range of lineage B is currently highly restricted, however, with only 3.5 km separating the most widespread samples (Fig. 2). Based on these distributional data alone, it seems likely that lineage B has experienced range contraction. Sequences from lineage C show weak geographic structuring, yet high intrapopulational variation (Fig. 3B). Fu's (1997) F_s is significantly negative, suggesting recent demographic expansion (Table 2), and a mismatch distribution is visually similar to the expectations under an expansion model (Fig. 4A).

Isolation by distance (IBD) was not significant within either lineage B or C, both of which have small geographic ranges. In contrast, IBD was significant in lineage A (Mantel test, $P = 0.03$). In this case, the larger geographic range of lineage A may have improved the statistical power of the test. When the entire *oregonensis* [1] clade is evaluated, IBD within and among lineages is strong ($r^2 = 0.91$; $P < 0.0001$), but flattens out at high geographic distances, indicating a departure from IBD expectations (Fig. 3C). This is because the level of divergence between lineages A and B is similar to the level of divergence between lineages A and C.

3.3.2. Divergence within oregonensis [2]

The other clade of oregonensis in central coastal California is *oregonensis* [2] (Fig. 2). Five geographically bounded haplotype lineages are found within *oregonensis* [2] (Fig. 5A). Four of these are located in central coastal California: on the San Francisco peninsula (lineage A), in the region directly north of San Francisco Bay (lineage B), on the west side of the Cotati Valley in Sonoma County (lineage C), and on the Point Reyes Peninsula (lineage D) (Fig. 2). All of these lineages are strongly supported ($pp \geqslant 95\%$), although relationships among them are not resolved (Fig. 5A). The fifth lineage, lineage E, is located in northern coastal California, outside the range of the current study (see Kuchta et al., in press). Among central coastal lineages, maximum likelihood estimates of sequence divergence range from 2.8% (lineage A vs. B) to 3.7% (lineage C vs. D) (Appendix A).

A haplotype network of *oregonensis* [2] failed to incorporate lineage E at the 95% confidence level. The other lineages form a network with 9–17 mutations separating them, with lineage D connecting to the other lineages at two points (Fig. 5C). A mismatch distribution of the *oregonensis* [2] clade deviates from a Poisson distribution (Fig. 4; $P = 0.01$), which is consistent with the high level of structure observed in the phylogenetic analysis and haplotype network. Demographic statistics within lineages are heterogeneous. Lineages A and B form star phylogenies, with one common haplotype present in multiple populations. In lineage B, F_s and R_2 are both significant, suggesting recent expansion (Table 2). Lineage C, in contrast, is dominated by a single haplotype, with one other haplotype recovered that is three mutational steps divergent; the mismatch distribution differs significantly from a Poison distribution, which is inconsistent with an expansion model for the populations within this lineage (Table 2). Lineage D has substantially higher genetic diversity indices than the other lineages, with a number of highly divergent haplotypes present in the network (Table 2). F_s and R_2 are both significant (Table 2). Individually, none of lineages A–D exhibit significant IBD (data not shown). In contrast, significant IBD is observed when all of the lineages of *oregonensis* [2] are considered together ($P < 0.0001$; $r^2 = 0.32$; Fig. 5C), although at distances over ~100 km the plot of genetic distance on geographic distance levels off.

Table 1
Results of the hierarchical analysis of molecular variance (AMOVA) among clades, lineages, and populations in central coastal California.

Source of variation	df	SS	Percentage of variation
Among clades	2	4146.7	66.0%
Among lineages within clades	9	1890.3	31.0%
Within lineages	234	262.1	3.0%

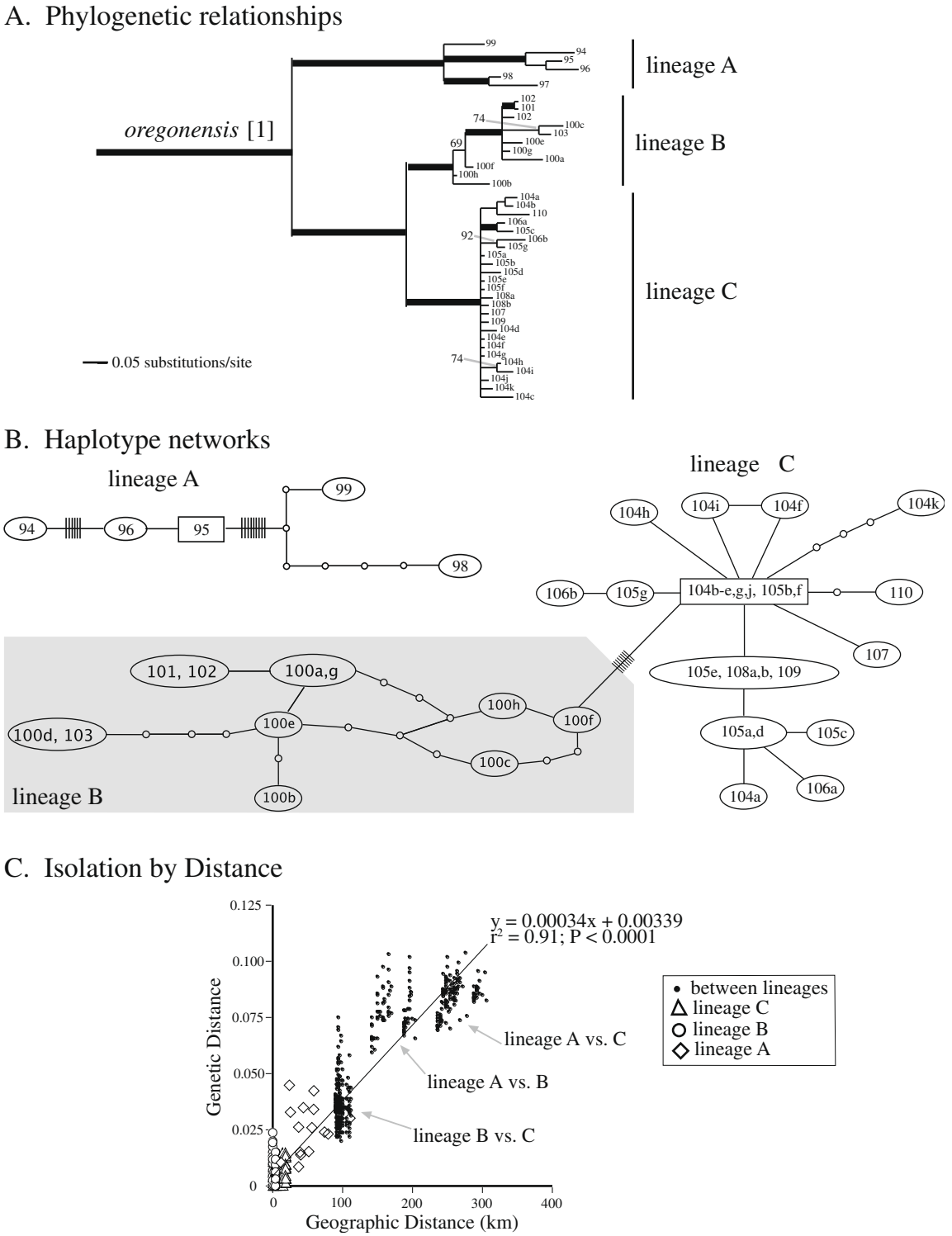

Fig. 3. Diversification within the *oregonensis* [1] clade. Population numbers refer to Fig. 2, Appendix A. (A) Phylogenetic relationships inferred using a Bayesian analysis. Thick lines designate clades with posterior probabilities ⩾95%. (B) Haplotype networks. The two separate networks do not connect at the 95% confidence level. (C) Isolation by distance plot of all the populations within *oregonensis* [1].

3.3.3. Divergence within the coastal clade

The subspecies *xanthoptica* is comprised of two lineages (Fig. 6A). One of these, which we call *xanthoptica* [1], is found east and north of San Francisco Bay, as well as in the foothills of the Sierra Nevada (Fig. 2). In our Bayesian phylogeny, the San Francisco Bay populations of *xanthoptica* [1] form a monophyletic group, while Sierra Nevada populations are reconstructed as three separate basal lineages (Fig. 6A); statistical support for these relationships is weak, however. A haplotype network gives similar results, with populations north and east of San Francisco Bay localized to different regions of the network, and coastal populations recovered as distinct from populations in the Sierra Nevada

Table 2
Diversity indices and population expansion statistics.

Clades (haplotype lineages)	Number pops	Number individuals	S[a]	h[b]	κ[c]	π[d]	R_2[e]	F_s[f]	Mismatch SSD[g]
oregonensis [1]	15	35	64	0.94	13.51	0.025	0.102	−2.479	0.027
Lineage A	5	5	26	1.00	13.00	0.020	0.161	<0.001	0.045
Lineage B	3	5	8	0.90	4.40	0.008	0.233	0.286*	0.116
Lineage C	7	25	14	0.88	2.01	0.003	0.087	**−7.939***	0.002
Lineages B + C	10	30	26	0.91	5.38	0.010	0.010	−3.891	0.023
oregonensis [2]	55	92	70	0.91	11.78	0.020	0.097	−3.457	**0.023***
Lineage A	8	21	9	0.27	1.03	0.002	0.089**	0.109**	0.049
Lineage B	27	40	21	0.77	1.92	0.003	**0.047****	**−10.187****	0.0017*
Lineage C	8	9	7	0.22	1.56	0.002	0.271*	3.095*	**0.071**
Lineage D	12	22	23	0.97	3.23	0.006	**0.076***	**−5.547***	0.0047
xanthoptica	50	106	84	0.89	18.32	0.029	0.118**	−0.379**	**0.052****
San Francisco Bay pops of *xanthoptica* [1]	30	47	25	0.69	1.98	0.003	**0.046**	**−8.886**	0.245**
Sierra Nevada pops of *xanthoptica* [1]	8	13	27	0.82	8.08	0.012	0.133*	2.806*	**0.110**
All of *xanthoptica* [1]	38	60	52	0.80	4.69	0.007	**0.051***	**−6.321*****	0.020
xanthoptica [2]	12	46	18	0.73	1.37	0.002	**0.051**	**−10.981**	0.005
eschscholtzii	15	16	21	0.98	6.50	0.021	0.144	−3.042*	0.015
eschscholtzii [northern]	12	13	12	0.97	4.03	0.013	0.151	**−3.844***	0.004

Results with P-values <0.05 are indicated in bold. Symbols: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

[a] S = number of segregating sites in a sample.

[b] h = haplotype diversity (the probability that two randomly sampled sequences are different).

[c] κ = sequence diversity (the average number of nucleotide differences between pairs of sequences).

[d] π = nucleotide diversity (the probability that two randomly sampled homologous nucleotides are different).

[e] Ramos-Onsins and Rozas (2002) R_2. The null hypothesis is population stability; significant results are indicative of demographic expansion.

[f] Fu's F_s (Fu, 1997). The null hypothesis is population stability; significant results are indicative of demographic expansion.

[g] Sum of squared deviations between the observed mismatch distribution and the mismatch distribution expected under a sudden demographic expansion model. Significant values are consistent with population stability (in contrast with F_s and R_2).

(Fig. 6B). Maximum likelihood estimates of sequence divergence between coastal and Sierra Nevada populations average 1.6% (Appendix A).

Among north bay populations, a single haplotype is widely distributed, while all other haplotypes occur at low frequency and are, at most, two base pairs different from the most common haplotype (Fig. 6B). East bay populations are more diverse, with several east bay populations possessing unique haplotypes and no single haplotype distributed throughout the region. In addition, two populations sampled from the eastern edge of the San Francisco peninsula (populations 138 and 140; Fig. 2) are shown to be independently derived from east bay populations. The relationship of north bay haplotypes to east bay haplotypes is ambiguous, with two different possible links represented (Fig. 6B). Maximum likelihood estimates of sequence divergence between east bay and north bay populations are low, averaging 0.6% (Appendix A). Within all San Francisco Bay area populations of *xanthoptica* [1], F_s and R_2 are significant (Table 2), suggesting recent expansion throughout the region. This is also true if populations north and east of San Francisco Bay are treated separately (data not shown).

Haplotypes from Sierra Nevada populations are more deeply differentiated than San Francisco Bay populations, with higher indices of genetic diversity (Table 2). A mismatch distribution differs significantly from that expected under demographic expansion (Fig. 7A). The three clades of Sierra Nevada populations recovered in the Bayesian analysis are also visible in the haplotype network, and are most closely related to each other assuming the network roots outside the Sierra Nevada haplotypes (Fig. 6B). Sierra Nevada haplotypes connect to east bay populations of *xanthoptica*, not north bay populations, consistent with east bay populations as the source for colonization of the Sierra Nevada (Wake and Yanev, 1986; Wake et al., 1989).

Both San Francisco Bay area populations and Sierra Nevada populations of *xanthoptica* [1] individually show significant IBD ($P \leqslant 0.0006$; Fig. 6E). When these two regions are combined into a single analysis, IBD is also strongly supported (Fig. 6E; $P < 0.0001$), with more of the variation explained than when either region is analyzed separately ($r^2 = 0.59$ vs. $r^2 = 0.29$ and 0.15, respectively).

The other clade of *xanthoptica*, which we call *xanthoptica* [2], is endemic to the San Francisco peninsula (Fig. 2). This clade constitutes a polytomy with no meaningful phylogenetic structure (Fig. 6A), and has lower indices of genetic diversity than *xanthoptica* [2] (Table 2). A haplotype network is star shaped, with most populations containing one of two haplotypes that span the geographical distribution of the clade (Fig. 6D). Sequence divergence among haplotypes within this lineage averages 0.4%. Given the lack of structure, it is somewhat surprising that IBD is significant ($P < 0.001$). However, geographic distance is a poor predictor of genetic distance ($r^2 = 0.04$), and IBD is visually absent in a plot of the data (Fig. 6E). In this case, significance is attained because of dense sampling (59 haplotypes from 21 populations) rather than a large IBD effect. Consistent with this interpretation, a mismatch distribution of the haplotypes closely matches a Poisson distribution (Fig. 7A), which, assuming neutrality, is the expected result under a model of range expansion. F_s and R_2 are both significant (Table 2).

Two lineages are recovered within *eschscholtzii* (Fig. 6A). One is restricted to southern California, and the other is located in central coastal California as far northward as the Pajaro River in the Monterey Bay area (Fig. 2). We call this latter lineage *eschscholtzii* [northern]. Haplotype networks and mismatch distributions were not calculated for the southern lineage of *eschscholtzii* because the sample size is small ($n = 3$ sequences), and because this lineage is located outside the central coastal California region. Within the northern lineage, most populations possess unique haplotypes, but those haplotypes differ from one another by only one or a few base pairs (Fig. 6C). Sequence divergence among haplotypes averages 1.3% (Appendix A). IBD was not significant, but F_s is significant (Table 2), suggesting recent demographic expansion.

4. Discussion

The salamander *E. eschscholtzii* is a noted example of a ring species, yet has a more intricate biogeographic history than an idealized ring species model (e.g., Irwin et al., 2001). Previous

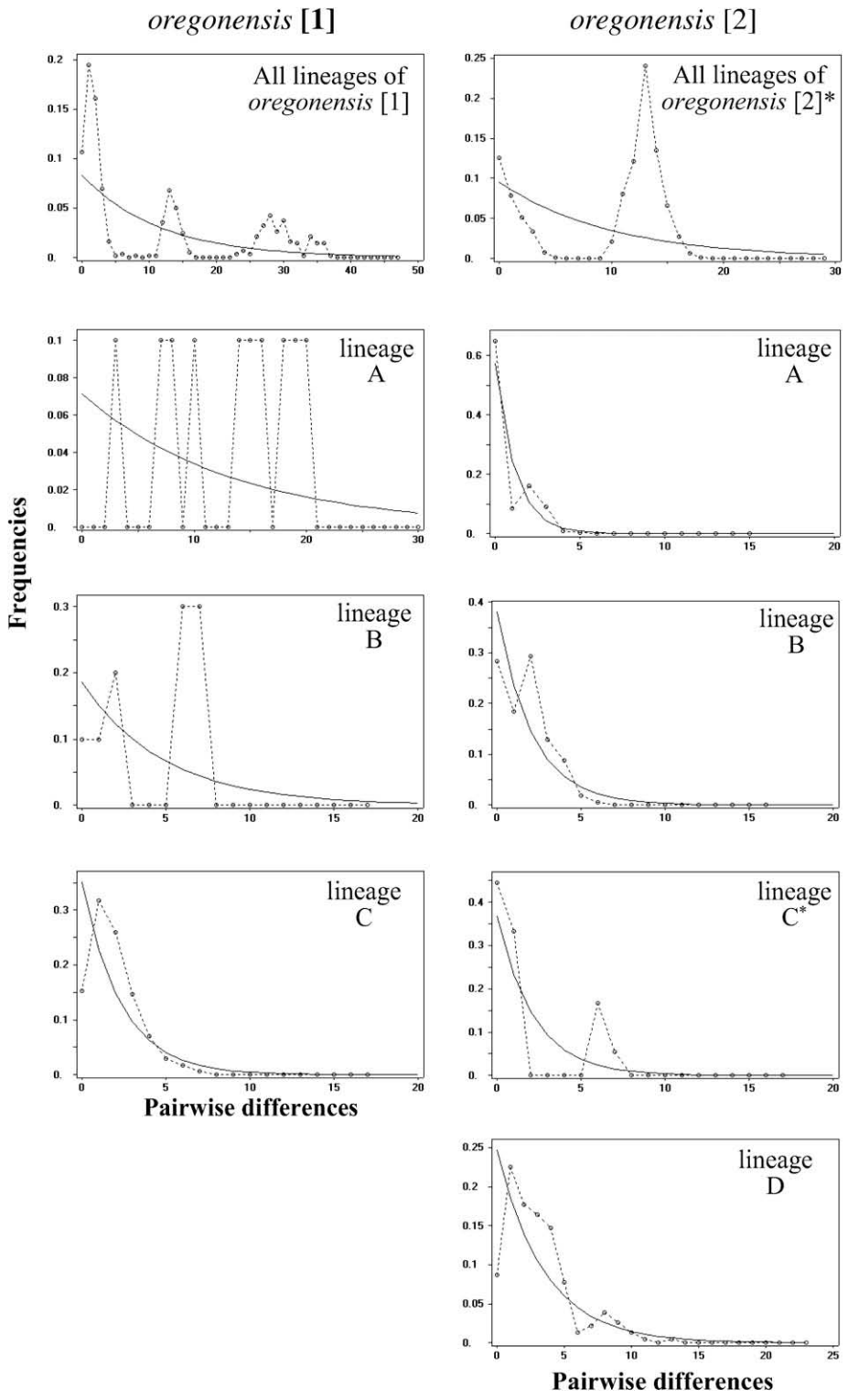

Fig. 4. Mismatch distributions within (A) *oregonensis* [1] and (B) *oregonensis* [2]. Dotted lines show the observed distribution of mismatches, and solid lines show the expected distribution under an expansion model. Asterisks (∗) designate distributions that deviate significantly from a model of demographic stability (Table 2).

molecular systematic studies have revealed that the complex is composed of several genetic lineages that have undergone multiple episodes of isolation, differentiation, range expansion, and secondary contact (reviewed in Wake, 2006). Consequently, the current ring-like distribution stems from the reassembly and merger of formerly independent elements, with the exception of secondary contacts between the coastal and inland arms of the ring, which exhibit species-level divergence as predicted by the ring species hypothesis (Wake et al., 1989; Alexandrino et al., 2005). Midway down the coastal arm of the ring, in central coastal California, the subspecies *oregonensis, xanthoptica,* and *eschscholtzii* meet. Wake (1997) reported on patterns of allozyme diversity and contact zone interactions in this region, and found notable levels of genetic differentiation (Nei's $D > 0.4$ in some comparisons). Using mtDNA haplotypes, our study has also uncovered striking levels of phylogeographic structure in the region, including two unre-

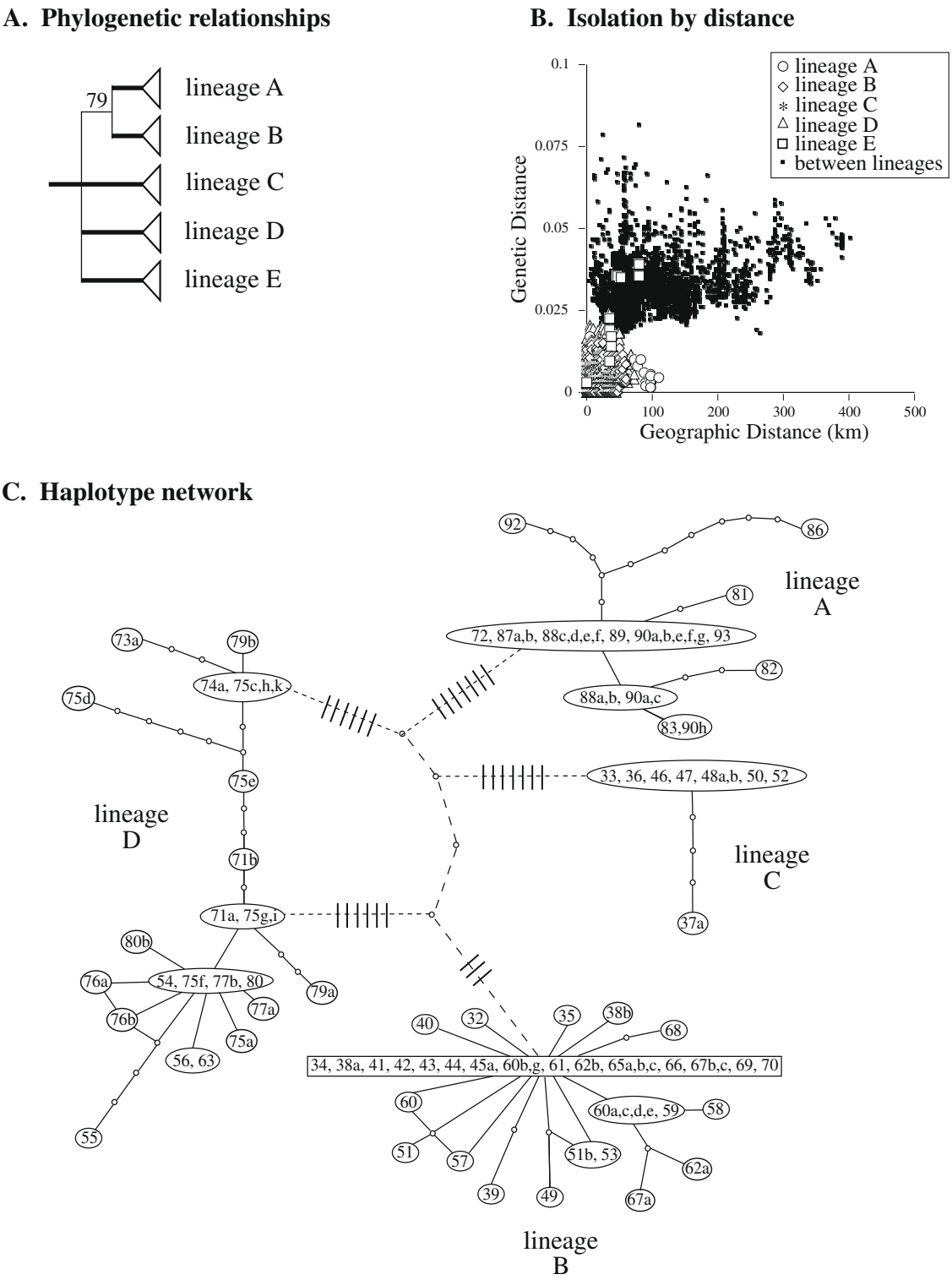

Fig. 5. Diversification within the *oregonensis* [2] clade. Population numbers refer to Fig. 2, Appendix A. (A) Phylogenetic relationships inferred using a Bayesian analysis. Thick lines designate clades with posterior probabilities ⩾95%. (B) Isolation by distance plot of all the populations within *oregonensis* [2]. RMA regression statistics are not shown due to the strong departure from a linear relationship. (C) Haplotype network. Lineage E (not shown) does not connect in at the 95% confidence level (see text).

lated clades of *oregonensis* (which together contain a total of seven lineages), and two lineages of *xanthoptica* (Figs. 3, 5 and 6). Two lineages of *eschscholtzii* were found as well, one of which is present in the Monterey Bay area. Maximum likelihood estimates of average percent sequence divergence between subspecies range from 5.9% to 16.1% (Appendix A).

This is a tremendous amount of genetic structure for a region this size (ca. 250 × 75 km; Fig. 2), even for a relatively dispersal-limited amphibian (Avise, 2000; Vences and Wake, 2007), and highlights the contribution intraspecific variation can make to regional biodiversity (Rissler et al., 2006; Avise and Hamrick, 1996). Of the ten haplotype lineages documented in the current

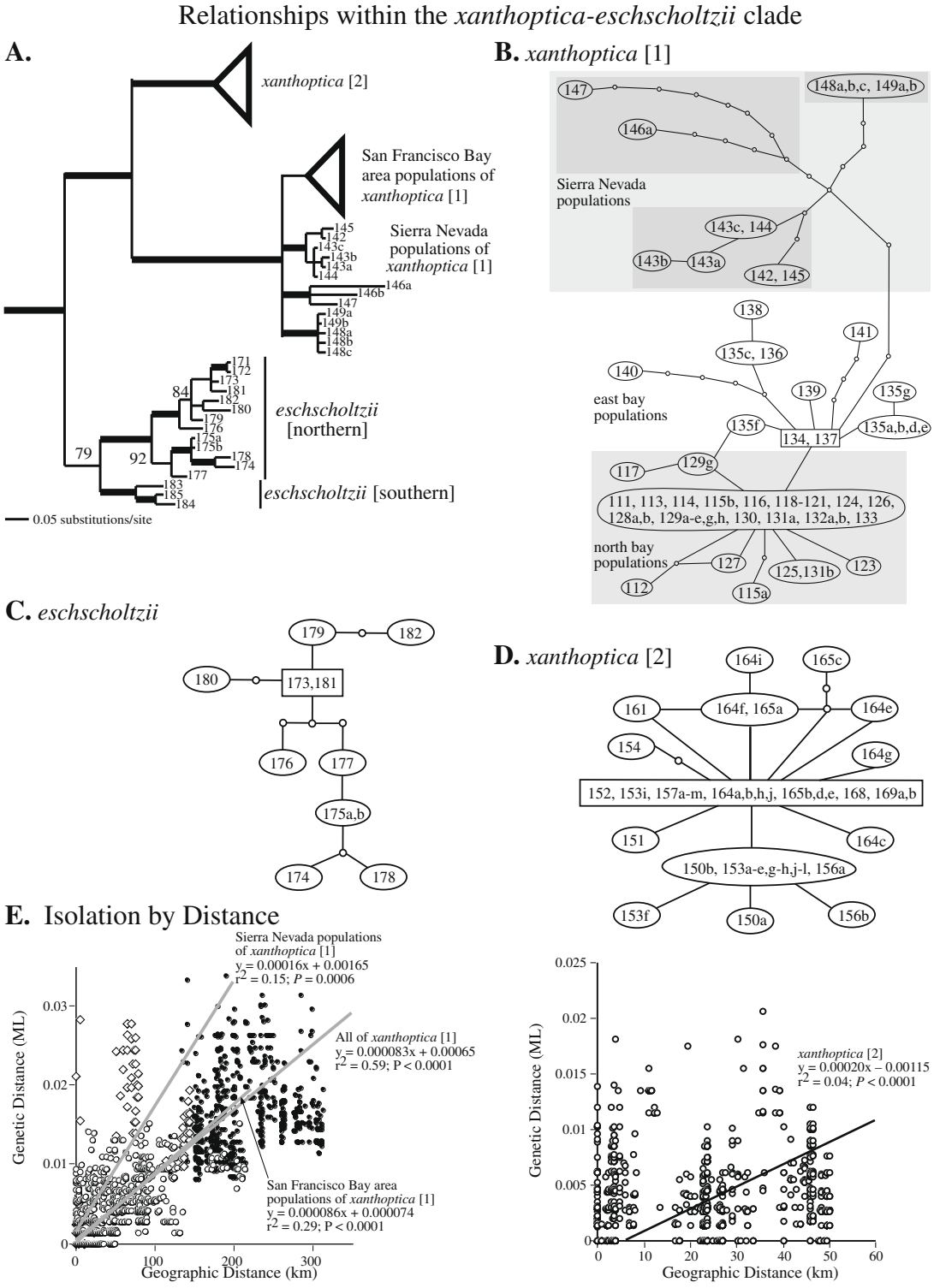

Fig. 6. Diversification within the *xanthoptica-eschscholtzii* clade. Population numbers refer to Fig. 2, Appendix A. (A) Phylogenetic relationships inferred using a Bayesian analysis. Thick lines designate clades with posterior probabilities ⩾95%. Relationships within *xanthoptica* [2] and the San Francisco Bay area haplotypes of *xanthoptica* [1] are not shown because they form polytomies. (B–D) Haplotype networks of *xanthoptica* [1], *eschscholtzii*, and *xanthoptica* [2]. (E) Isolation by distance plot of all the populations within *xanthoptica* [1] (left) and *xanthoptica* [2] (right).

study, six show evidence of recent demographic expansion, whereas one shows evidence of demographic stability (Table 2). This suggests that the distributions of haplotype lineages in central coastal California are dynamic, and that many secondary contacts between haplotype lineages (see Section 4.4) may not be old. Rapidly shifting range limits may seem counterintuitive given the extremely limited dispersal abilities of *Ensatina* (Staub et al., 1995). However, measures of dispersal potential in the field and the evolutionary ecology of dispersal dynamics at the limits of a species' range may differ strongly (Simmons and Thomas, 2004; Cabe et al., 2007). For instance, the Eastern red-backed salamander, *Plethodon cinereus,* has small home range sizes (5–25 m^2, depending on the study; Cabe et al., 2007) and limited dispersal (Mathis, 1991), yet has accomplished a dramatic post-Pleistocene range

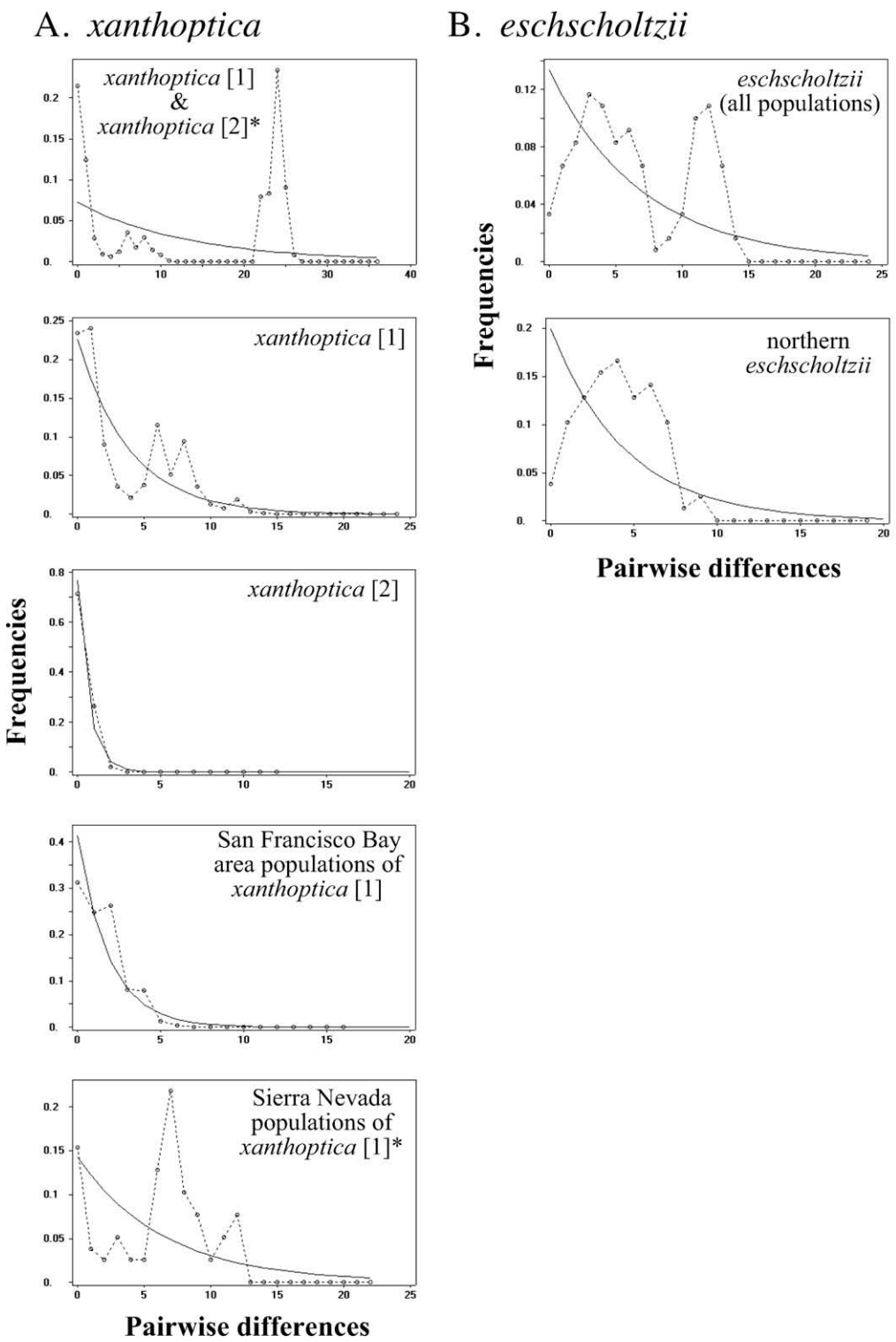

Fig. 7. Mismatch distributions within (A) *xanthoptica* and (B) *eschscholtzii*. Dotted lines show the observed distribution of mismatches, and solid lines show the expected distribution under and expansion model. Asterisks (∗) designate distributions that deviate significantly from a model of demographic stability (Table 2).

expansion—roughly 75% of the range of *P. cinereus* was under ice at the height of the last glacial maximum (Highton, 1995).

4.1. The subspecies oregonensis: diversity and distribution

4.1.1. Phylogeographic divergence

In the central coastal California, the subspecies *oregonensis* is a paraphyletic taxon represented by two distantly related clades that we have labeled *oregonensis* [1] and *oregonensis* [2] (Fig. 1B, 2). The average percent divergence between these two clades was 13.8%. Both lineages possess a generalized camouflaged color pattern with a light brown back and a pale belly, and are very similar in appearance. In general, *oregonensis* [1] is particularly drab in color, while the limbs, tail, and torso of *oregonensis* [2] possess subdued yellow and orange elements, which is why Stebbins (1949) interpreted them as intergrades between *oregonensis* and *xanthoptica*

(one lineage of *xanthoptica* possesses a vibrant orange ventral coloration; see Section 4.2.1 below).

The *oregonensis* [1] clade includes three allopatric lineages (A–C; Fig. 2 and 3), all of which occupy restricted distributions along the central California coast. An earlier phylogeographic study that examined mtDNA variation throughout the *Ensatina* complex sampled two haplotypes from *oregonensis* [1] (Moritz et al., 1992), both of which were members of lineage C to the north (Fig. 2). Our study has revealed two additional lineages, one occupying a very restricted range north of the Golden Gate on the Point Reyes peninsula (lineage B), and one on the southwestern edge of the San Francisco peninsula (lineage C) (Fig. 2).

The second clade possessing the *oregonensis* phenotype, *oregonensis* [2], is found inland of *oregonensis* [1], and includes five geographically separate lineages, four of which are located in the San Francisco Bay area (Figs. 2 and 5). Lineage A is endemic to the San Francisco peninsula, while lineages B–D form a distributional patchwork north of San Francisco Bay. In most instances, the different lineages of *oregonensis* [2] are separated by inappropriate habitat, such as arid valleys, while within lineages the habitat is largely continuous (excluding recent human development; see Section 4.4 for a potential exception involving lineages C and D).

4.1.2. Biogeography of oregonensis

The subspecies *oregonensis* contains two separate clades, one of which includes three allopatric coastal lineages (*oregonensis* [1]), while the other possesses a more continuous distribution (*oregonensis* [2]; Fig. 2). The odd distribution of *oregonensis* [1] may be a consequence of Pleistocene sea level fluctuations. During the height of the last glacial maximum the sea level along coastal California was up to 120 m lower than it is today, which shifted the California coastline several km westward (Sloan, 2006). The three lineages of *oregonensis* [1] may have formed a continuous coastal distribution at that time, or during previous Pleistocene glacial maxima. Indeed, there is significant isolation by distance within *oregonensis* [1] (Fig. 3C), suggesting that the current distribution represents relict fragments of a formerly continuous distribution.

There is a notable association between geology and distribution in the subspecies *oregonensis*. In central California, geological substrates are distinct east and west of the San Andreas and San Gregorio faults. Pacific plate exposures include the western San Francisco peninsula, the Pt. Reyes peninsula, and a sliver of land in Mendocino County extending from Fort Ross in the south to Point Arena in the north. The three parts of the distribution of *oregonensis* [1] correspond with these three fragments of Pacific Plate (Fig. 2). The northwest movement of the Pacific Plate relative to the North American plate contrasts with the northern origin of *oregonensis* (Moritz et al., 1992; Kuchta et al., in press), and it is therefore unlikely that *oregonensis* [1] originated on Pacific Plate exposures. Instead, the current distribution of *oregonensis* [1] is most likely a byproduct of colonization of the habitats found on these exposures, which are low elevation terraces with a relatively cool, moist climate.

The parallel coastal-inland distributions of *oregonensis* [1] and *oregonensis* [2] (Fig. 2) were unanticipated because most taxa that exhibit phylogeographic disjunctions in the Coast Ranges are split into northern and southern components (e.g., Calsbeek et al., 2003; Feldman and Spicer, 2006; Rissler et al., 2006). Nonetheless, some taxa share distributional similarities with *oregonensis* [1] and *oregonensis* [2]. The California slender salamander (*Batrachoseps attenuatus*) possesses phylogenetically distinct coastal and inland lineages (Martínez-Solano et al., 2007), for example, as does a complex of Trap-door spiders (*Promyrmekiaphila*) (Stockman and Bond, 2007). Sharp-tailed snakes, *Contia tenuis*, have a phylogeographic pattern that is perhaps the closest match to *oregonensis* (Feldman and Spicer, 2002, 2006). Like *oregonensis*, *C. tenuis* possesses separate coastal and inland clades, with the coastal clade including allopatric populations found on the San Francisco peninsula as well as north of San Francisco Bay. No samples of *C. tenuis* from the Pt. Reyes peninsula have been collected, but it would be of interest to conduct a systematic search to determine if the coastal clade is present, as with *oregonensis* [1].

4.2. The coastal clade: diversity and distribution in xanthoptica and eschscholtzii

4.2.1. Phylogeographic divergence

Like *oregonensis*, the subspecies *xanthoptica* was found to be composed of geographically distinct lineages. One of these, which we call *xanthoptica* [1], is found north and east of San Francisco Bay. Members of this lineage differ from *oregonensis* by possessing a dark brown back, a vibrant orange belly, orange proximal limb segments, and a striking yellow eye patch. This lineage is thought to be Batesian mimetic of Pacific newts, genus *Taricha* (Kuchta, 2005; Kuchta et al., 2008), which are highly toxic and possess a similar aposematic coloration (e.g., Brodie et al., 2005; Hanifin et al., 2008). The second lineage of *xanthoptica* is endemic to the San Francisco peninsula (Fig. 2). This lineage, which we call *xanthoptica* [2], has a less conspicuous coloration than *xanthoptica* [1], and was considered by Stebbins (1949) to be *xanthoptica* × *oregonensis* intergrades.

The levels of mtDNA differentiation within *xanthoptica* [1] in the San Francisco Bay area are relatively low (average mtDNA haplotype divergence <1%), and haplotypes north and east of San Francisco Bay, even though separated by a marine barrier, differ by as little as a single base pair (Fig. 5B). Haplotypes from eastern San Francisco Bay and the Sierra Nevada are more divergent, with at least five base pair differences separating them (Fig. 6B).

The subspecies *eschscholtzii* and *xanthoptica* form sister taxa in our phylogenetic analysis (Fig. 6A). Within *eschscholtzii*, northern and southern lineages were recovered, with the northern lineage distributed northward into the northern Monterey Bay region (Fig. 2). Unlike *oregonensis* and *xanthoptica*, *eschscholtzii* was not found to possess high levels of phylogeographic structure, although there is variation among populations (Table 2). A test of demographic expansion in northern *eschscholtzii* is significant (Table 2). This result corresponds with the predictions of Stebbins (1949) and Wake (1997), both of whom envision *eschscholtzii* dispersing from north to south to form a secondary contact with *klauberi*. More sampling in the southern portion of the range of *eschscholtzii*, as well as across the range of *klauberi*, is needed to determine if the secondary contacts between these two lineages are the consequence of relatively recent range expansions.

The range of *eschscholtzii* approaches *xanthoptica* [2] at the northern end of Monterey Bay. An active secondary contact may have existed in the recent past, but large-scale agricultural development has severely disrupted the habitat in this area (Wake, 1997) and the contact zone between *xanthoptica* and *eschscholtzii* is no longer amenable to detailed investigation. We found sequences from *eschscholtzii* and *xanthoptica* [2] within 7.8 km of each other at the northern end of Monterey Bay (populations 170 and 171); *eschscholtzii* was also sampled within 23.6 km of *xanthoptica* [1] (populations 170 and 141) (Fig. 2). In neither case was their evidence of introgression; however, sample sizes are low, and mtDNA is an unreliable marker for analyses of contact zone interactions because it is haploid and only maternally inherited, and because it is but a single marker (Ballard and Whitlock, 2004). Hybrid zones are best characterized with the use of multiple nuclear markers (e.g., Harrison, 1993). Indeed, mtDNA is commonly discordant with nuclear markers (e.g., Ruedi et al., 1997; Mead et al., 2001; Jockusch and Wake, 2002; Funk and Omland,

2003; García-París et al., 2003; Kuchta and Tan, 2005, 2006), including within the *Ensatina* complex itself (Wake and Schneider, 1998).

4.2.2. Biogeography of the subspecies xanthoptica

In an earlier allozyme study, Wake and Yanev (1986) estimated that the Nei's genetic distance (Nei, 1978) between east bay and Sierra Nevada populations was 0.02, far below the level of differentiation measured in most other nearest-neighbor comparisons within *Ensatina*. Accordingly, they inferred that the Sierra Nevada was colonized during the Pleistocene epoch by dispersal across the alluvial plains of the Central Valley (see also Stebbins, 1949; Wake, 1997). The overall levels of divergence found in the current study are low, as would be expected with a Pleistocene dispersal event. Strong evidence for the recent colonization of the Sierra Nevada by San Francisco Bay area populations would be provided if haplotypes from the Sierra Nevada were phylogenetically nested within a clade of San Francisco Bay area populations. Our phylogenetic analysis, however, recovered central coastal California populations as monophyletic, with three lineages of Sierra Nevada haplotypes at the base. Indeed, this pattern of branching is consistent with a colonization of the central Coast Ranges by a Sierran ancestor. Nonetheless, these results fail to refute the earlier hypothesis of a central coastal origin for *xanthoptica* [1] because the relationships are not statistically supported ($pp < 0.95$ for the central coastal California clade of *xanthoptica* [1]; Fig. 6A).

The "transvalley leak" of *xanthoptica* [1] between the east San Francisco Bay region and the foothills of the Sierra Nevada occurred across intervening habitat in the Central Valley that is currently arid and inhospitable to terrestrial salamanders. Comparative studies have shown that the Central Valley is a general biogeographic boundary within California (Calsbeek et al., 2003; Rissler et al., 2006). On the other hand, phylogeographic studies have shown that the California slender salamander (*B. attenuatus*; Martínez-Solano et al., 2007), the Arboreal salamander (*Aneides lugubris*; Lapointe and Rissler, 2005), and the California mountain kingsnake (*Lampropeltis zonata*; Rodríguez-Robles et al., 1999) have, like *xanthoptica* [1], accomplished similar dispersal events across the Central Valley. In all of these taxa, San Francisco Bay and Sierra Nevada populations are weakly diverged, strengthening the case for a Pleistocene dispersal corridor across the Central Valley.

4.3. Concordance between mtDNA and allozymes

In Fig. 8 we present a summary of some of the patterns of allozyme variation reported by Wake (1997) in combination with the results of our mtDNA study. In most instances, mtDNA lineages are narrowly parapatric without sympatry (see Section 4.4 for an exception), and we observe broad agreement between our mtDNA studies and the allozyme data of Wake (1997). Most of the large genetic distances reported by Wake (1997) are shown to involve comparisons between populations represented by divergent mtDNA clades. For example, on the San Francisco peninsula, Nei's (1978) genetic distances between a population of *oregonensis* [1] and nearby populations of *oregonensis* [2] and *xanthoptica* [2] range from 0.17 to 0.23. In contrast, on the east side of the San Francisco peninsula, $D = 0.02$ between two populations of *oregonensis* [2]. Similarly, north of San Francisco Bay, Nei's D between *xanthoptica* [1] and *oregonensis* [2] ranges from 0.37 to 0.40, but D within *oregonensis* [2] is 0.04–0.05. We caution, however, that the broad-scale correspondence between allozymic and mtDNA variation reported here is a poor measure of genetic interactions where lineages meet. Finer-scale analyses are required. For example, using allozymes, Wake (1997) found the central coastal California lineages of *Ensatina* were not maintaining their genetic independence upon secondary contact, but instead showed evidence of introgression and genetic merger.

The evolutionary dynamics of secondary contacts within the *Ensatina* complex are of fundamental importance to the ring species interpretation. Secondary contacts between lineages derived from the coastal and inland axes form either narrow hybrid zones (Wake et al., 1989; Alexandrino et al., 2005), or to exhibit sympatry with limited to no hybridization (Wake et al., 1986). In either scenario, the lineages constitute genetically and reproductively isolated species (Wake, 2006). On the other hand, preliminary studies of contact zone interactions within the coastal and inland axes suggest that the interacting lineages merge upon recontact (Wake, 1997, 2006; Wake and Schneider, 1998). Thus, while the ring distribution of *Ensatina* has been assembled following multiple periods of isolation and differentiation, the evolutionary dynamics at zones of secondary contact (genetic merger vs. reproductive isolation) are consistent with the ring species interpretation. Examples of ring species are rare in nature (Irwin and Irwin, 2002; Martens and Päckert, 2007), and recent studies of other potential ring species have concluded that they fail to fit stringent ring species criteria (Liebers et al., 2004; Päckert et al., 2005; Joseph et al., 2008). Within the *Ensatina* complex, more detailed explorations of patterns of introgression within the coastal and inland arms are underway (Pereira and Wake, submitted for publication). In general, the process of lineage merger following periods of isolation and differentiation is an underexplored topic in evolutionary biology (e.g., Jockusch and Wake, 2002).

4.4. Secondary contacts

A key prediction of the ring species scenario is that secondary contacts within the ring (as opposed to contacts between the coastal and inland arms of the ring) are characterized by interbreeding and a lack of genetic independence. The work of Wake (1997) disclosed many secondary contacts within central coastal California, and our studies of mtDNA sequence variation have identified additional contact zones. These provide possibilities for the analysis of contact zone dynamics within the coastal arm of the complex. One new contact zone between haplotype lineages within a clade was identified. This is within *oregonensis* [2], where lineages C and D north of San Francisco Bay were sampled within 3.5 km of each other (Fig. 2; Appendix A). They are joined by continuous habitat, and should contact one another. The average mtDNA sequence divergence between these two clades is 3.7%.

Several new potential contacts between clades were also located, including three new contacts between *oregonensis* [1] and *oregonensis* [2] north and south of San Francisco Bay. To the north, on the Pt. Reyes peninsula, lineage B of *oregonensis* [1] retains a relict distribution, with only 3.5 km separating our most widely spaced samples; populations of *oregonensis* [2] were sampled within 6–7 km to the north and east (Fig. 2). The habitat in this area is continuous, and secondary contacts are expected. On the San Francisco peninsula, lineage C of *oregonensis* [1] and lineage A of *oregonensis* [2] were sampled within 3.3 km of each other along Pescadero Road in San Mateo County (populations 90 and 104, respectively; Fig. 2). These same two clades were also found within 4.2 km of each other near Waterman's Gap in northern Santa Cruz County (populations 93 and 107, respectively, Fig. 2). This latter contact zone is particularly interesting because a haplotype belonging to *xanthoptica* [2] (population 150) was found in sympatry with population 107 of *oregonensis* [2], making Waterman's Gap a three-way contact between lineages of *Ensatina*, two of which are genetically distinct, but phenotypically cryptic (*oregonensis* [1] and [2]), and one of which is genetically and phenotypically distinct from the other two (*xanthoptica* [2]). Finally, lineages of *xanthoptica* [1] and *oregonensis* [2] meet in a series of

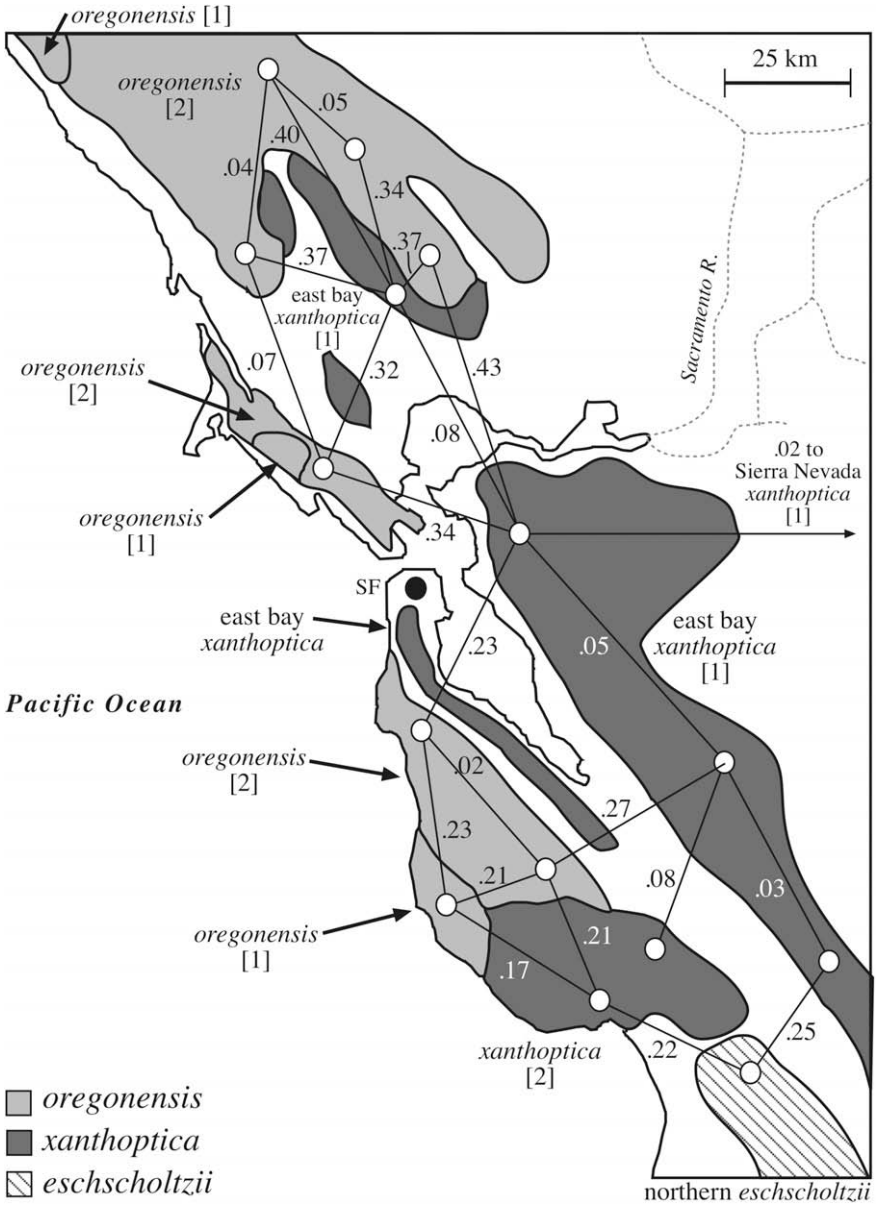

Fig. 8. Map showing the relationship between the major mtDNA clades identified in this study and a sample of Nei's genetic distances reported in the allozyme study of Wake (1997). Subspecies share a common pattern, and the mtDNA lineages within subspecies are demarcated with lines. The black dot at the tip of the San Francisco peninsula denotes the city of San Francisco.

secondary contacts north of San Francisco Bay, where an extension of *xanthoptica* [1] projects into the range of *oregonensis* [1]. Several of these contacts were analyzed by Wake (1997). This study has located yet another secondary contact east of the town of Napa, between population 129 of *xanthoptica* [1] and population 68 of lineage B of *oregonensis* [2], where two mtDNA lineages were found 1 km apart and are connected by continuous habitat (Fig. 2).

5. Summary

Ensatina eschscholtzii is a classic, much studied example of a ring species. In an ideal ring species, a string of intergrading populations come together to form a secondary contact. At this point of secondary contact, the terminal differentiates interact as distinct species, whereas populations within the ring show free interbreeding. In *Ensatina,* earlier studies indicate that the "ring" did not evolve *in situ*, as postulated by Stebbins (1949), but rather has been assembled following periods of range fragmentation. Nonetheless, it appears that secondary contacts within the coastal and inland arms of the ring, which display lower levels of divergence than contacts between the distributional arms, are largely characterized by introgression and genetic merger (Wake and Schneider, 1998). The Coast Ranges of central coastal California are of particular interest for the ring species scenario because they were the final element to form in the evolution of continuous Coast Range system (Kuchta et al., in press). Three subspecies are found in this area: *oregonensis*, *xanthoptica*, and *eschscholtzii*. Wake (1997) studied patterns of allozymic differentiation and uncovered substantial levels of divergence, both within and between these subspecies. He also presented preliminary evidence of genetic merger at points of secondary contact between subspecies. Nonetheless, the complex patterns of differentiation revealed by the allozyme studies suggested a more detailed regional history than previously imagined, and the hierarchical organization of the genetic variation was unclear. Our mtDNA phylogeography study found that the central coastal region consists of three subspecies, three clades

(which do not correspond with the three recognized subspecies), and 10 haplotype lineages. Two highly divergent, non-sister clades of *oregonensis* were found, and divergence between these clades approaches the levels recorded between *oregonensis* and the coastal clade (*xanthoptica/eschscholtzii*). At a finer scale, mtDNA haplotype lineages were found to form a patchwork of largely non-overlapping distributions, and several secondary contacts were identified. Our results are consistent with what is known of the geomorphological development of the California Coast Ranges, and verify that the evolutionary dynamics among central Coast Range populations are of fundamental importance for the ring species interpretation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.10.019.

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