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ORIGINAL ARTICLE

Closing the ring: historical biogeography of the salamander ring species *Ensatina eschscholtzii*

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ABSTRACT

Aim The salamander *Ensatina eschscholtzii* Gray is a classic example of a ring species, or a species that has expanded around a central barrier to form a secondary contact characterized by species-level divergence. In the original formulation of the ring species scenario, an explicit biogeographical model was proposed to account for the occurrence of intraspecific sympatry between two subspecies in southern California (the 'southern closure' model). Here we develop an alternative ring species model that is informed by the geomorphological development of the California Coast Ranges, and which situates the point of ring closure in the Monterey Bay region of central coastal California (the 'Monterey closure' model). Our study has two aims. The first is to use phylogenetic methods to evaluate the two competing biogeographical models. The second is to describe patterns of phylogeographical diversity throughout the range of the *Ensatina* complex, and to compare these patterns with previously published molecular systematic data.

Location Western North America, with a focus on the state of California, USA.

Methods We obtained mitochondrial DNA sequence data from 385 individuals from 224 populations. A phylogeny was inferred using Bayesian techniques, and the geographical distributions of haplotypes and clades were mapped. The two biogeographical ring species models were tested against our Bayesian topology, including the associated Bayesian 95% credible set of trees.

Results High levels of phylogeographical diversity were revealed, especially in central coastal and northern California. Our Bayesian topology contradicts the Monterey closure model; however, 0.08% of the trees in our Bayesian 95% credible set are consistent with this model. In contrast, the classic ring species biogeographical model (the southern closure model) is consistent with our Bayesian topology, as were 99.92% of the trees in our 95% credible set.

Main conclusions Our Bayesian phylogenetic analysis most strongly supports the classic ring species model, modified to accommodate an improved understanding of the complex geomorphological evolution of the California Coast Ranges. In addition, high levels of phylogeographical diversity in central and northern California were identified, which is consistent with the striking levels of allozymic differentiation reported previously from those regions.

Keywords

Bayesian analysis, biogeography, California, geomorphology, mitochondrial DNA, phylogeography, speciation, species concepts.

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INTRODUCTION

Ring species exhibit a circular arrangement of populations around a central barrier, with reproductively isolated parts overlapping at one point in the ring, yet with morphological and genetic intergradation elsewhere (Mayr, 1942, 1963). They arise when two or more lineages descend from a common ancestor and become reproductively isolated while maintaining their genetic connectivity through a chain of interbreeding populations. Mayr (1963, p. 507) stated that ring species are 'the perfect demonstration of speciation' because they illustrate how the microevolutionary processes generating intraspecific geographical variation can lead to species-level divergence. When geographical variation in characters has an adaptive basis, ring species present a natural example of how adaptive diversification interacts with geography to promote species formation (Mayr, 1942, 1963; Irwin *et al.*, 2001; Wake, 2006; Martens & Päckert, 2007). The intraspecific sympatry found in ring species also leads to serious taxonomic difficulties, drawing attention to the shortcomings of traditional Linnaean taxonomy and bringing the species problem into sharper focus (e.g. Highton, 1998; Wake & Schneider, 1998; Wake, 2006).

The salamander *Ensatina eschscholtzii* Gray, 1850 is a particularly influential example of a ring species (Ridley, 1996; Futuyma, 1998). Stebbins (1949) recognized seven subspecies in the complex, including four with a relatively uniform dorsal coloration (the 'unblotched' subspecies *picta*, *oregonensis*, *xanthoptica*, *eschscholtzii*) and three with bright dorsal patches of colour overlaid on a dark background (the 'blotched' subspecies *platensis*, *croceater*, *klauberi*) (for drawings and photographs see Stebbins, 2003; Wake, 2006). Together these subspecies are distributed in a ring around the Central Valley of California, which is hot and arid and currently presents an environment that is inhospitable to terrestrial salamanders (Fig. 1a). However, in the mountains of southern California, the unblotched subspecies *eschscholtzii* and the blotched subspecies *klauberi* are locally sympatric with either limited or no hybridization, indicating they have reached the species level of divergence (Fig. 1a; Stebbins, 1949, 1957; Brown, 1974; Wake *et al.*, 1986). Stebbins (1949) developed an explicit biogeographical model to account for this taxonomic oddity of sympatric subspecies. He postulated that the *Ensatina* complex originated in present-day northern California and southern Oregon, perhaps from a *picta*-like ancestor. This ancestral stock then expanded its distribution as two arms southward down the Coast Ranges (unblotched subspecies) and the inland ranges (blotched subspecies), the arms adapting and diverging as they spread, until they re-established contact in southern California as reproductively isolated entities (Fig. 1a). Broad zones of phenotypic intergradation between adjacent subspecies were interpreted as representative of ongoing genetic connectivity (Dobzhansky, 1958), and the two sympatric subspecies in southern California were thereby viewed as linked together by a continuous sequence of interbreeding populations, thus forming a ring species.

Much molecular systematic work has been done on the *Ensatina* complex since Stebbins (1949). The results are complex in detail, but support the major tenets of the ring species hypothesis in finding that secondary contacts between the coastal and inland arms are characterized by species-level divergence, while secondary contacts within the arms exhibit patterns of intergradation and genetic merger (Wake & Yanev, 1986; Wake *et al.*, 1986, 1989; Moritz *et al.*, 1992; Jackman & Wake, 1994; Wake, 1997; Alexandrino *et al.*, 2005). The only study to present a phylogenetic hypothesis for the *Ensatina* complex, however, was that of Moritz *et al.* (1992), which used 24 mitochondrial (mtDNA) cytochrome *b* sequences sampled throughout the range of the species. Their results supported the ring species scenario in that independent coastal (*xanthoptica*, *eschscholtzii*) and inland (southern *platensis*, *croceater*, *klauberi*) clades were identified. In their best-estimate phylogeny, these two clades were recovered as sister taxa, with northern lineages of *oregonensis* and *platensis* occupying basal positions (Fig. 1b).

A detailed follow-up study to Moritz *et al.*'s (1992) is needed because the substantial phylogeographical structure within the *Ensatina* complex remains poorly demarcated, and because allozyme studies have uncovered a multifaceted biogeographical history, the hierarchical organization of which remains unclear (Wake & Yanev, 1986; Jackman & Wake, 1994; Wake, 1997). In addition, following the publication of Moritz *et al.* (1992), Parks (2000) estimated a Miocene origin for the *Ensatina* complex. The geomorphology of western North America differed dramatically in the Miocene from that of today (Yanev, 1980; Hall, 2002), and a full understanding of the ring species biogeography for *Ensatina* must incorporate knowledge of the geomorphological evolution of the region (e.g. Wake, 1997).

Here we present a new phylogeny for the *Ensatina* complex generated using mitochondrial DNA sequences. The current study expands on that of Moritz *et al.* (1992) by including 385 sequences from 224 populations. One aim of the study is to introduce a novel biogeographical model based strictly on the geomorphological evolution of the California Coast Ranges, developed as an alternative to the classic ring species model of Stebbins (1949). Our second aim is to provide a description of patterns of phylogenetic and phylogeographical diversity, including mapping the distribution of mtDNA haplotype lineages in California. These results are used to distinguish between the two competing biogeographical models.

The biogeography of ring closure

The Coast Ranges of California are composed of northern and southern elements that have only recently been made continuous (Fig. 2). Orogeny of the northern Coast Ranges was the result of uplift caused by interactions between the Pacific and North American plates (Atwater, 1970). In contrast, assorted elements of the central and southern Coast Ranges were initially part of a land mass known as the Salinian terrain. Thirty million years ago, during Oligocene times, the Salinian terrain was located off the coast of present-day southern

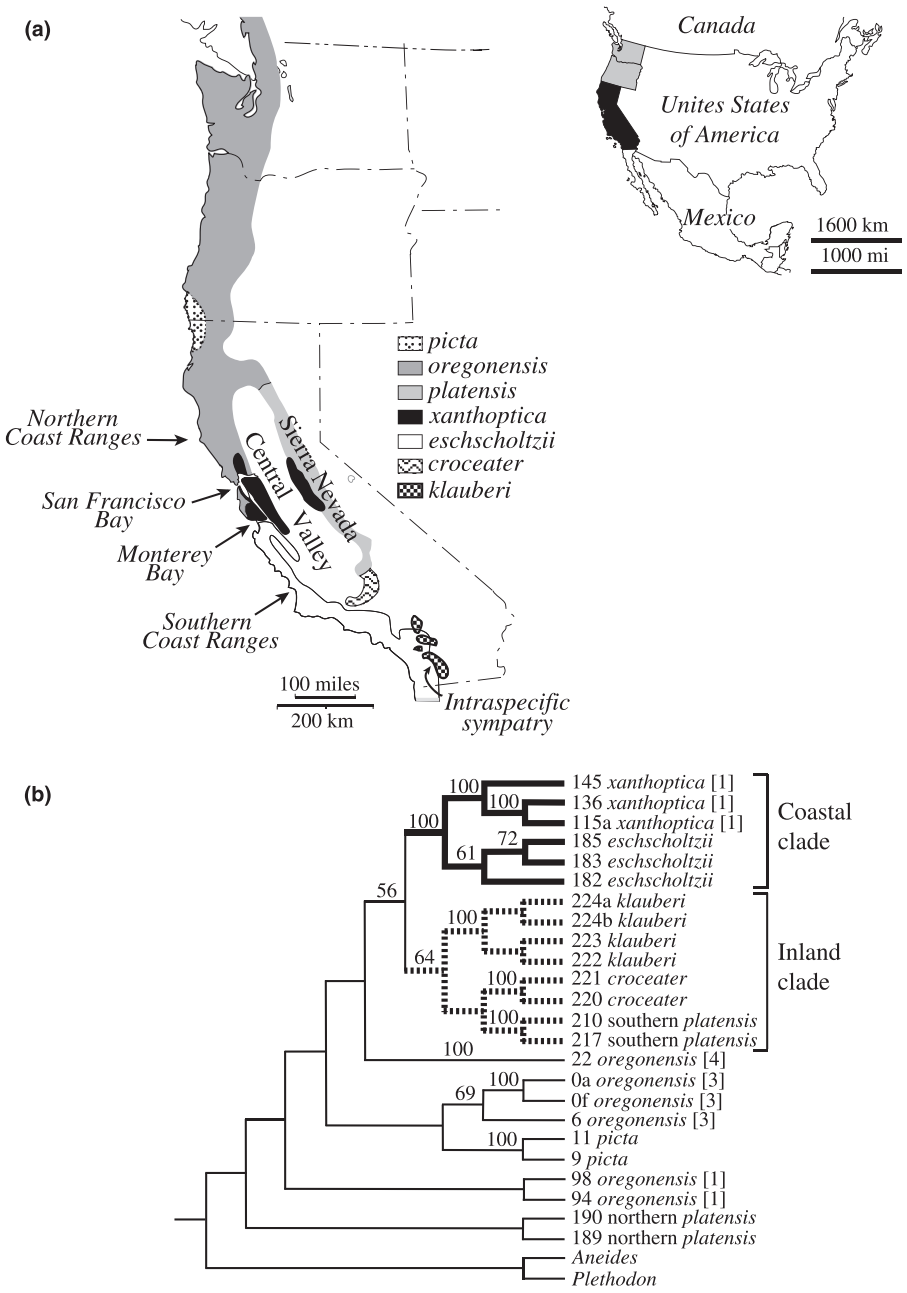

Figure 1 (a) Map showing the distribution of subspecies of *Ensatina eschscholtzii* in western North America. In the USA, the state of California is highlighted in black; Washington and Oregon are shown in grey. Subspecies, which circumscribe patterns of phenotypic variation (Stebbins, 1949), are differentially shaded. In southern California, the subspecies *eschscholtzii* and *klauberi* are locally sympatric in places with limited or no interbreeding. Note the locations of San Francisco Bay and Monterey Bay. (b) Best-estimate phylogeny from Moritz *et al.* (1992), showing the recovered relationships among 24 mtDNA cytochrome *b* sequences, plus two outgroup taxa. Individual samples are relabelled to correspond with the names and population numbering scheme used in this paper. This tree was constructed using parsimony analysis (see Moritz *et al.*, 1992, for details); numbers above branches indicate bootstrap support (100 replicates) for values > 50%.

California (Hall, 2002). Thereafter, from the mid-Miocene (*c.* 18 Ma) onward, fragments of this land mass formed islands that slid northward and were incorporated into the Coast Ranges of central coastal California (Hall, 2002; Wake, 2006). Nonetheless, as recently as 2 Ma the Coast Ranges remained divided by the outlet of a large marine embayment that extended into the Central Valley of California through the present-day Monterey Bay region (Yanev, 1980; Hall, 2002) (Fig. 2b). This barrier was altered when continuing uplift closed off the marine embayment, transforming the southern

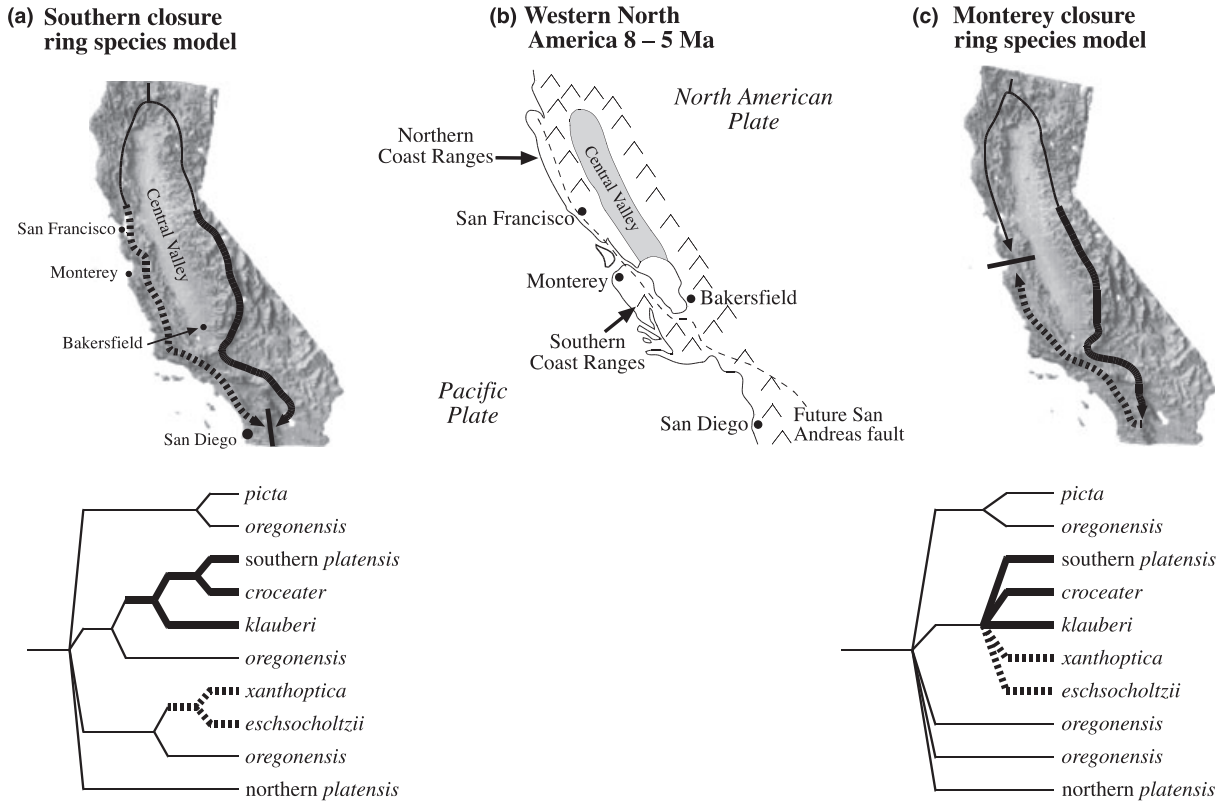

Figure 2 Biogeographical models and corresponding phylogenetic hypotheses. (a) Stebbins's (1949) southern closure ring species hypothesis. This model is supported if the recovered phylogeny identifies independent coastal (*xanthoptica*, *eschscholtzii*) and inland (southern *platensis*, *croceater*, *klauberi*) clades, with northern lineages (*oregonensis* and *picta*) basal. (b) The coast of California 8–5 Ma, when the present day Coast Ranges were divided into northern and southern halves by a marine embayment (Hall, 2002). (c) The Monterey closure ring species hypothesis. This model is supported if the coastal and inland clades are most closely related, with northern lineages basal. In both (a) and (c), the phylogenetic models account for what is already understood about the phylogeny of the *Ensatina* complex: that *oregonensis* is a deeply diverged, paraphyletic assemblage of lineages, that *picta* is nested within *oregonensis*, and that *platensis* is composed of two unrelated mtDNA clades (Moritz *et al.*, 1992).

Central Valley into an enormous freshwater lake (Dupré, 1990; Sims, 1993). Like the marine embayment, this lake drained into Monterey Bay via the wide Salinas and Pajaro River valleys (Sarna-Wojcicki *et al.*, 1985), and the Monterey Bay region probably remained a dispersal barrier for many terrestrial organisms. Finally, 600,000 years ago the drainage of the Central Valley shifted northward to exit just north of present-day San Francisco (Fig. 2a), eliminating the geographical barrier at Monterey Bay (Sarna-Wojcicki *et al.*, 1985).

The Monterey Bay region thus constitutes a historical barrier, and today many taxa show concordant phylogeographical breaks there (Calsbeek *et al.*, 2003; Lapointe & Rissler, 2005; Feldman & Spicer, 2006; Kuchta & Tan, 2006; Rissler *et al.*, 2006). Because the origins of the coastal and inland arms of the *Ensatina* complex are thought to pre-date the formation of a continuous California Coast Range system (Wake, 1997), the Monterey Bay region should represent a fundamental biogeographical barrier for *Ensatina* as well. Recognizing this, Wake (1997) proposed that the ancestors of the coastal clade (*xanthoptica*, *eschscholtzii*) dispersed out to a piece of the Salinian terrain prior to its merger with the North American plate. Later, after the Coast Ranges became contiguous, *eschscholtzii* would have expanded southward to form a secondary contact with *klauberi* in present-day southern California. This scenario is consistent with the geomorphological evolution of the California Coast Ranges, and is also consistent with the classic ring species interpretation of the *Ensatina* complex because the coastal and inland arms evolved in the north and dispersed southward to form a secondary contact in southern California. From a phylogenetic perspective, the ring species hypothesis of Stebbins (1949) predicts that the coastal clade (*xanthoptica*, *eschscholtzii*) and the inland clade (southern *platensis*, *croceater*, *klauberi*) are each derived independently from a northern ancestor (Fig. 2a). We call this scenario the southern closure model because it situates the point of ring closure in southern California.

An alternative biogeographical hypothesis, based on the geological formation of the California Coast Ranges, is that the Monterey Bay region, rather than the mountains of southern California, is the ultimate point of ring closure in the *Ensatina* complex. Under this scenario, *Ensatina* originated in northern California and expanded southward, yet was prevented from

dispersing the length of the California coastline by the Monterey embayment (Fig. 2c). The inland clade (the ancestors of southern *platensis*, *croceater* and *klauberi*), on the other hand, was free to expand into southern California, where it gave rise to the coastal clade (*xanthoptica*, *eschscholtzii*). Ancestors of the coastal clade (which either lost their dorsal blotching or pre-dated the origin of the blotched phenotype) then expanded northward to the southern limit of the Monterey embayment. When the drainage of the Central Valley shifted to the Golden Gate north of San Francisco, the ring of populations closed. We call this new ring species scenario the Monterey closure model. The key phylogenetic prediction of the Monterey closure model is that the coastal and inland clades are closely related, with lineages in northern California ancestral to them (Fig. 2c). Indeed, this is exactly the pattern of relationships recovered by the best-estimate phylogeny of Moritz *et al.* (1992), although it was weakly supported (bootstrap = 56%; Fig. 1b).

MATERIALS AND METHODS

Population sampling and laboratory techniques

Populations are defined here as samples within 1 km of each other that belong to the same mtDNA haplotype lineage. For this study, a fragment of the cytochrome *b* (cyt *b*) gene was obtained from 224 populations (385 individuals) throughout the range of *E. eschscholtzii*, including 23 populations (24 individuals) sampled by Moritz *et al.* (1992) (see Appendix S1 in Supporting Information; Fig. 1b). The complete data set includes overlapping mtDNA haplotypes collected using two different sequencing technologies. For 39 haplotypes, the primers MVZ15 and Cytb2 were used to amplify the region between nucleotide positions 19–405 of the mtDNA cyt *b* locus (Moritz *et al.*, 1992). Sequences were obtained by running labelled single-strand polymerase chain reaction (PCR) products on acrylamide gels (manual sequencing; see Moritz *et al.*, 1992 for laboratory details). The average length of these sequences was 439 bp (range 242–625).

For the remaining 322 haplotypes, whole genomic DNA was extracted from ethanol-preserved or frozen tissues (tail tips, liver, heart) using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). The primers MVZ15 and MVZ16 (Moritz *et al.*, 1992) were used to amplify the region of the mtDNA *cyt b* gene between nucleotide positions 19 and 804. Amplifications were carried out in a PTC-100 Thermal Cycler (M.J. Research, Waltham, MA, USA) as follows: 94°C for 1.5 min (initial denaturation); 35 cycles at 94°C for 1 min, 49°C for 1 min, and 72°C for 1 min.

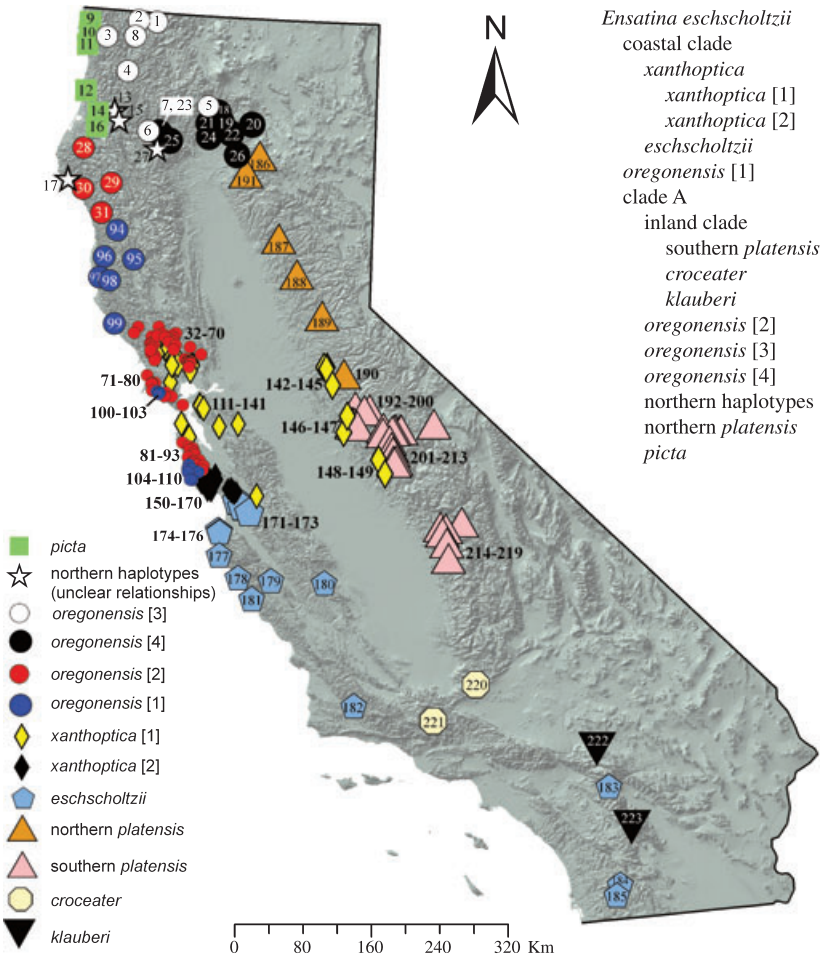

Figure 3 Map showing the distribution of samples of *Ensatina eschscholtzii* in California. Samples assigned to the same subspecies are given the same symbol, and colours are used to separate clades within subspecies. Nine populations north of California are not shown. Population numbers correspond to those in Appendix S1, and are used throughout the manuscript. The table (upper right) provides a reference for the names used here, with the pattern of indentation corresponding to nested clades (Fig. 4a).

Amplification reaction mixtures consisted of 1× PCR buffer with 1.5 mM $MgCl_2$, 40 mM of each dNTP, 10 μM of each primer, and 0.75 U *Taq* DNA polymerase in a total volume of 25 μL. PCR experiments included non-template controls to monitor contamination. Double-stranded PCR products were purified using the QIAquick PCR Purification kit (Qiagen). All samples were sequenced in both directions in a 10-μL reaction mixture using dRhodamine and a 377 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were visually aligned in SEQUENCHER (Gene Codes, Ann Arbor, MI, USA). The average sequence length was 664 bp (range: 331–784). All GenBank accession numbers are listed in Appendix S1.

Phylogenetic analysis

Partitioned Bayesian phylogenetic analyses were carried out using MRBAYES ver. 3.04b (Huelsenbeck & Ronquist, 2001). The data set was divided into three partitions: 1st, 2nd and 3rd codon positions. For each partition the best-fitting model of nucleotide substitution was selected using the Akaike information criterion as implemented in MRMODELTEST ver. 1.1b (Nylander, 2004). The models selected were: HKY + Γ, HKY + I + Γ, and GTR + Γ for the 1st, 2nd and 3rd codon positions, respectively. Flat Dirichlet prior distributions were used for substitution rates and base frequencies, and default flat prior distributions were used for all other parameters. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold and three heated chains (temperature set to the default value of 0.2) for 20 million generations and sampled every 1000 generations. Stationarity was confirmed by examining plots of $-\ln L$ scores and parameter values. The phylogeny was rooted with 10 outgroups from Mueller *et al.* (2004): *Plethodon cinereus*, *P. petraeus*, *P. elongatus*, *Desmognathus wrighti*, *D. fuscus*, *Phaeognathus hubrichti*, *Hydromantes brunus*, *Speleomantes italicus*, *Aneides hardii* and *A. flavipunctatus* (GenBank accession numbers NC006343–NC006345, NC006334, NC006335, NC006337–NC006339, AY728215, NC006327). Two additional outgroup sequences from Moritz *et al.* (1992) were also included: *Aneides lugubris* (L75820) and *Plethodon elongatus* (L75821). The sister taxon of *Ensatina* is currently unclear, but these outgroups include representatives of all the genera that have been inferred to be most closely related to *Ensatina* (Chippindale *et al.*, 2004; Mueller *et al.*, 2004; Vieites *et al.*, 2007). In addition, the inclusion of multiple outgroups was necessary for calibrating our divergence time estimates (described below).

Twelve haplotypes in our complete data set were excluded from the Bayesian phylogenetic analysis because they were very short, or were of low quality (Appendix S1). These were later assigned to clades using a neighbour-joining analysis with maximum likelihood distances, and in all cases the haplotypes were assigned to a geographically logical clade with high confidence (> 95% bootstrap support; data not shown). Individuals from these localities are plotted in Fig. 3 because they help to identify the geographical bounds of haplotype lineages.

Divergence time estimates

Using a simple molecular clock, Parks (2000) estimated that the coastal clade (*xanthoptica*, *eschscholtzii*) of the *Ensatina* complex originated at least 10 Ma. If this is correct, the coastal clade pre-dates the development of a continuous Coast Range system in central coastal California (which formed 2–0.6 Ma; see Introduction), and thus could not have evolved *in situ* as postulated by Stebbins (1949). We estimated the age of the coastal clade using a Bayesian approach that does not assume constant evolutionary rates, as implemented in the software package 'multidistribute' (Thorne *et al.*, 1998; Thorne & Kishino, 2002). The fossil record for plethodontid salamanders is meagre, and no *Ensatina* fossils have been found (Holman, 2006). However, two fossils were useful for constraining divergence dates among outgroup taxa: the common ancestor of *H. brunus* and *S. italicus* was constrained to be at least 13.75 Myr old (Venczel & Sanchíz, 2005), and the common ancestor of *A. hardii* and *A. flavipunctatus* was constrained to be at least 23 Myr old (Tihen & Wake, 1981).

Comparing biogeographical models

Our two biogeographical models are the southern closure model (Stebbins, 1949; Fig. 2a) and the Monterey closure model (Fig. 2c). In evaluating these hypotheses, we take into account the composite nature of the subspecies *platensis*, and the fact that *picta* was postulated previously to be nested within a deeply diverged, multiply paraphyletic *oregonensis* (Moritz *et al.*, 1992; Jackman & Wake, 1994). The two competing biogeographical models (Fig. 2a,c) were compared with our Bayesian topology and with the topologies present in the Bayesian 95% credible set using MESQUITE ver. 1.1 (Maddison & Maddison, 2006). The 95% credible set of trees includes all the topologies that are statistically indistinguishable from the recovered Bayesian topology. Consequently, topologies not present in the 95% credible set are statistically rejected, while topologies within the credible set are not rejected (Huelsenbeck & Rannala, 2004).

RESULTS

Phylogenetic relationships

Plots of $-\ln L$ scores and other parameter values suggested that stationarity was achieved in the Bayesian phylogenetic analysis. To be conservative, the first five million generations were discarded as burn-in, leaving 15 million generations and 15,000 topologies in the data set. Branch lengths for a consensus phylogram were calculated from the means of the posterior probabilities, and the posterior probabilities of clades were calculated as the fraction of instances that each clade was recovered. Three major, basal clades are recovered in the

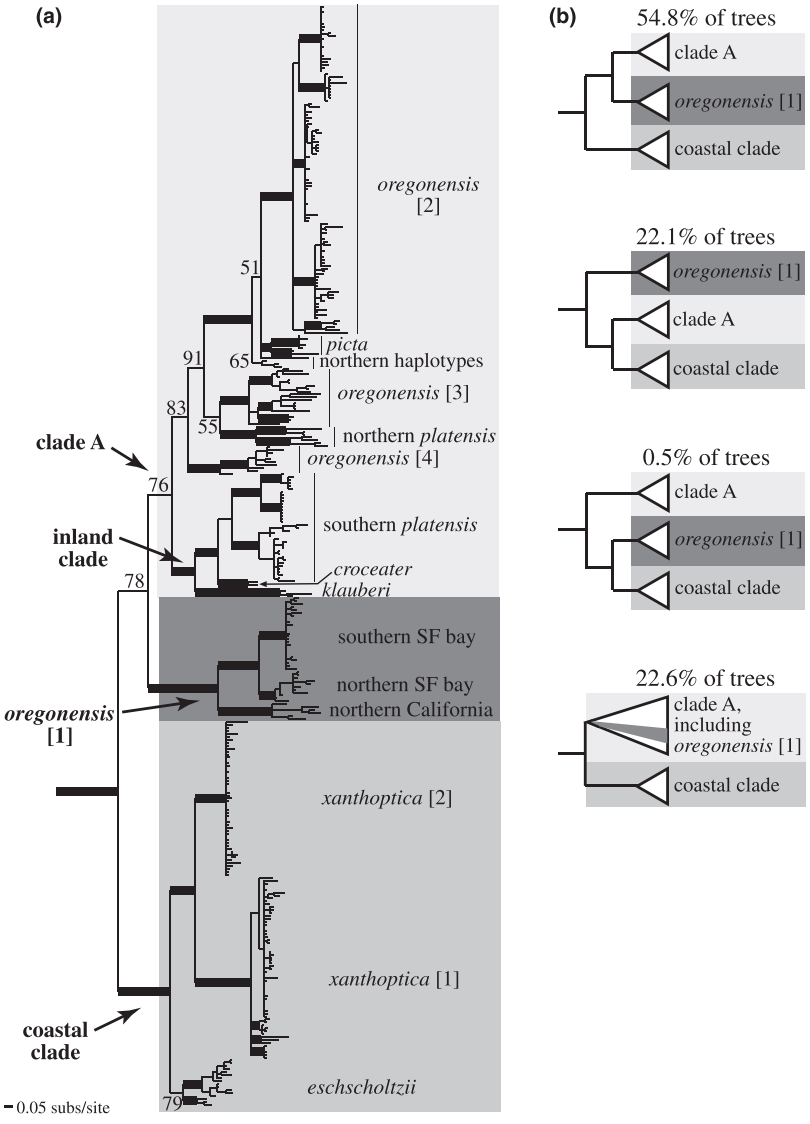

Figure 4 (a) Bayesian phylogenetic hypothesis for *Ensatina eschscholtzii*. Subspecies and the names of clades used in this paper are labelled to the right. Thick branches have posterior probabilities ≥ 95%. Branches of interest with posterior probabilities < 95% are labelled above the branch. Note that branches labelled 'northern haplotypes' have poor phylogenetic support and are not assigned to any particular haplotype lineage. (b) Basal branching patterns recovered in the Bayesian 95% credible set, illustrating the four possible phylogenetic placements of *oregonensis* [1]. The shading scheme matches (a). In the last reconstruction, which shows a lack of resolution at the base of the tree, eight trees (0.08% of the total) recover the coastal clade and the inland clade as sister taxa. The remaining 99.92% of the trees in the 95% credible set do not recover them as sister taxa.

Bayesian phylogeny, each of which contains substantial substructure (Fig. 4a). We refer to the clade including the subspecies *xanthoptica* and *eschscholtzii* as the coastal clade; a second clade, currently assigned to the subspecies *oregonensis*, is referred to as *oregonensis* [1]; and the third clade, including the rest of the *Ensatina* complex, we call clade A.

Coastal clade

The coastal clade includes the subspecies *xanthoptica* and *eschscholtzii*, and is strongly supported with a posterior probability (hereafter, PP) ≥ 95% (Fig. 4a). The subspecies *xanthoptica* (PP ≥ 95%) includes two haplotype lineages (both PP ≥ 95%), one of which is limited to the southern San Francisco peninsula (*xanthoptica* [1]; Figs 3 & 4a), and the other of which is found east and north of the San Francisco Bay region, as well as in the foothills of the Sierra Nevada (*xanthoptica* [2]; Figs 3 & 4a). The subspecies *eschscholtzii* also forms a monophyletic group, although, surprisingly, statistical support is weak (PP = 79%; Fig. 4a). Two well supported lineages (PP ≥ 95%) are recovered within *eschscholtzii,* however. One is found in southern California, and the other is located in central coastal California as far northward as the Pajaro river in the Monterey Bay area (Fig. 3).

oregonensis [1]

This clade is distributed along the coast of the northern half of California (Fig. 3). Within *oregonensis* [1] we recover three strongly supported lineages (Fig. 4a; PP ≥ 95%) with allopatric distributions: (1) along the coast of the southern San Francisco Peninsula; (2) a small patch of populations restricted to the Point Reyes peninsula, north west of San Francisco Bay; and (3) along the coast from northern Sonoma County northward to central Mendocino County in northern California (Fig. 3).

Clade A: northern lineages belonging to the subspecies oregonensis *and* picta

The third major clade recovered in the Bayesian analysis is clade A. This clade is not strongly supported (PP = 76%), but we recognize it here for communication purposes (see Comparing Biogeographical Models below). Multiple lineages possessing the unblotched *oregonensis* phenotype are members of clade A. One of these we call *oregonensis* [2] (Fig. 4a). Five geographically demarcated haplotype lineages are found within *oregonensis* [2], four of which are restricted to the San Francisco Bay region (Fig. 4a). A second clade, *oregonensis* [3] (Figs 3 & 4a), is distributed from northern California northward to central Washington State. It is most likely that this clade extends to the northern limit of the distribution of *Ensatina* in central coastal British Columbia, Canada. The final clade is *oregonensis* [4] (Fig. 4a), and it occupies a central position in the ring at the northern end of the Central Valley, where it forms a secondary contact with the northern clade of *platensis* (Fig. 3; Jackman & Wake, 1994; Wake & Schneider, 1998).

The northwestern-most clade in California, representing *picta*, is complex (Figs 3 & 4). Populations 9–12 form a lineage (PP ≥ 95%) within the traditional range of *picta*. Populations 14 and 16 to the south are sister to populations 9–12 (PP ≥ 95%), and are located within the range of Stebbins's (1949) *picta*/*oregonensis* intergrade zone. In contrast, population 3, located 11 km south east of *picta* population 10, is positioned within the eastern range limit of *picta* (Stebbins, 1949) yet belongs to the *oregonensis* [3] clade (Fig. 3).

Finally, not all the sequences from northern California are of clear phylogenetic affinity. We label these the 'northern haplotypes' (Fig. 4). One haplotype from Trinity County (population 27) is recovered as closely related to *picta* and *oregonensis* [2] (Fig. 4a). Three other haplotypes in north coastal California (populations 13, 15, 17) form a weakly supported clade (PP = 65%; Fig. 4a). These three populations are geographically close to population 27, *oregonensis* [2], *oregonensis* [3] and *picta* (Fig. 3).

Clade A: clades within the inland arm of the Ensatina *complex*

The inland clades (including northern *platensis*, as well as southern *platensis* + *croceater* + *klauberi*) possess blotched phenotypes and are distributed from the northern Sierra Nevada mountains southward to southern California (Fig. 1a). Haplotypes from the subspecies *platensis* form two unrelated clades corresponding to the northern and southern portions of the distribution of the subspecies; we call these two clades northern *platensis* and southern *platensis* (Figs 3 & 4a). In our analysis, northern *platensis* is strongly supported (PP ≥ 95%). Southern *platensis* is weakly supported (PP = 68%), but it is composed of two strongly supported subclades (PP ≥ 95%; Fig. 4a). Northern and southern *platensis* meet between populations 190 and 192 in the central Sierra Nevada (Fig. 3), *c.* 75 km north of a transition zone in allozymes (Jackman & Wake, 1994; Wake & Schneider, 1998).

The final two lineages in clade A represent the subspecies *croceater* and *klauberi*. The *croceater* lineage (PP ≥ 95%) is recovered as sister to southern *platensis* (PP ≥ 95%), with *klauberi* (PP ≥ 95%) sister to this clade (PP ≥ 95%; Fig. 4a). Together, these three lineages form a strongly supported inland clade (Fig. 4a). The relationship of this clade to the coastal clade (*xanthoptica*, *eschscholtzii*) is key to distinguishing between our two competing biogeographical models (Fig. 2a,c; see below).

Divergence time estimate

We estimated the age of the split between the coastal clade and clade A + *oregonensis* [1] at 21.5 Ma (95% CI = 8.9–51.1 Ma). A wide confidence interval was obtained because we were unable to constrain any of the nodes within the *Ensatina* complex with fossil calibrations, limiting our fossil dates to outgroup taxa. In addition, we were unable to put an upper bound on any node in the phylogeny, which is responsible for the large upper bound on the CI. Consequently, our estimate of the age of the coastal clade should be viewed with a high level of caution. Nonetheless, the lower estimate of 8.9 Myr for the origin of the coastal clade is in accordance with the simple molecular clock estimates of Parks (2000), and greatly precedes the formation of a continuous Coast Range system in central California, which formed no sooner than 2 Ma (Fig. 2b).

Comparing biogeographical models

We recovered separate, strongly supported coastal (*xanthoptica*, *eschscholtzii*) and inland (southern *platensis*, *croceater*, *klauberi*) clades in our phylogeny. The basal pattern of branching in our phylogeny has low statistical support (PP < 95%), however, which complicates the comparison of biogeographical models. Reference to the 95% credible set of trees provides insight into the statistically equivalent set of topologies; any topology present in this credibility set cannot be statistically rejected (Huelsenbeck & Rannala, 2004). There were 9500 trees in the Bayesian 95% credible set, and all identify separate coastal and inland clades (Fig. 4b). In 99.92% of these trees, the coastal and inland clades are not sister taxa, and thus they support the southern closure model (Fig. 2c). In eight of the topologies (0.08%), however, the inland and coastal clades are sister taxa, which is consistent with the southern closure model. Thus, while the Monterey closure model does not receive strong support, we are unable to reject it with statistical confidence.

The southern closure model (Stebbins, 1949) is more complex. Our Bayesian topology recovers the coastal clade (*xanthoptica*, *eschscholtzii*) as sister to the remainder of the *Ensatina* complex. This is consistent with the southern closure model (Stebbins, 1949) because it allows for the independent evolution of separate coastal and inland clades from a northern ancestor (Fig. 2a). Inspection of the Bayesian 95% credible set

of trees revealed four sets of statistically indistinguishable topologies that differed in their branching patterns at the base of the tree, all four the result of an unstable placement of the *oregonensis* [1] clade (Fig. 4b). This clade is recovered as: (1) sister to clade A, as in our majority rule Bayesian topology (Fig. 4a); (2) sister to the coastal clade (*xanthoptica*, *eschscholtzii*); (3) sister to the rest of the *Ensatina* complex; or (4) nested within clade A (Fig. 4b). This latter reconstruction explains why clade A has low statistical support, despite the fact that no members of clade A are ever recovered outside that clade in the 95% credible set of trees. The strongest support for the southern closure hypothesis is provided by those topologies in which lineages of *oregonensis* are recovered as basal to both the coastal clade and the inland clade (sets 2 and 3 above; Fig. 4b).

DISCUSSION

Assembling a ring species

The phylogeographical complexity within *Ensatina* revealed by our study is likely to be a consequence of the old age of the complex (Maxson *et al.*, 1979; Larson *et al.*, 1981; Parks, 2000), combined with a vast geographical range relative to dispersal ability (Staub *et al.*, 1995) and the geomorphological complexities of the tectonically active California landscape (Yanev, 1980; Hall, 2002; Burnham, 2005). This set of circumstances has influenced patterns of diversification in diverse taxa (Kuchta & Tan, 2005; Feldman & Spicer, 2006; Chatzimanolis & Caterino, 2007; Rich *et al.*, 2008), including adaptive differentiation accompanying lineage divergence within the *Ensatina* complex itself (Kuchta, 2005; Wake, 2006; Kuchta *et al.*, 2008). Using a Bayesian approach, we estimated that the coastal clade (*xanthoptica*, *eschscholtzii*) originated prior to the formation of a continuous California Coast Range system, which closed between 2 Ma and 600,000 yr ago. Consequently, a strict interpretation of the southern closure ring species model (Fig. 2a) is problematic, because Stebbins (1949) explicitly predicted that the coastal arm of the *Ensatina* complex evolved within the present-day Coast Ranges. One solution that is consistent with the southern closure model was presented by Wake (1997), who postulated that the ancestor of the coastal clade colonized an island mass that was a geological precursor to part of the central Coast Ranges of California. An alternative solution, developed in this paper (Fig. 2c), postulates that ring closure in the *Ensatina* complex is located in the Monterey Bay region rather than southern California, and formed after uplift of the Coast Ranges created a continuous Coast Range system 2–0.6 Ma.

The southern closure and Monterey closure biogeographical models were evaluated by comparing their predicted phylogenetic topologies with our Bayesian topology and the associated Bayesian 95% credible set of trees. Our Bayesian topology is not consistent with the Monterey closure model because the coastal clade (*xanthoptica*, *eschscholtzii*) is not recovered as closely related to the inland clade (southern *platensis, croceater*, *klauberi*; cf. Figs 2c & 4a). In the 95% credible set of trees, however, a small number of the topologies (0.08%) recover the coastal and inland clades as sister taxa. This result precludes rigorous rejection of the Monterey closure model. The southern closure model predicts that the coastal and inland clades will be independent and derived from a northern ancestor, and our Bayesian topology, as well as the vast majority (99.92%) of the topologies present in the 95% credible set of trees, are consistent with these criteria (cf. Figs 2a & 4a). We conclude that our data most strongly support the southern closure model of Stebbins (1949).

Mitochondrial DNA haplotype clades and patterns of nuclear differentiation

The current study builds on that of Moritz *et al.* (1992) by identifying several new clades, by resolving much phylogeographical structure within previously known clades, and by advancing our understanding of the geographical distribution of mtDNA haplotype lineages throughout the *Ensatina* complex (Fig. 3). Two new, allopatric lineages were found in *oregonensis* [1], for example, as were new lineages within *xanthoptica*, *eschscholtzii*, northern *platensis* and southern *platensis* (Figs 3 & 4a). In northern California, four separate clades of *oregonensis* ([1–4]) were found, all of which possess substantial phylogeographical structure of their own (Figs 3 & 4a). In addition, a clade representing the subspecies *picta*, and some haplotypes of unclear phylogenetic affinity, are found in northern California. Finally, striking levels of diversity were found in the San Francisco Bay area, including seven supported haplotype lineages within *oregonensis* (see Kuchta *et al.*, 2009 for a detailed consideration of patterns of diversity in central coastal California).

The phylogenetic complexity revealed by our analysis of mtDNA haplotypes is in accordance with early work on patterns of geographical variation in allozymes, which also disclosed notable levels of genetic differentiation (Wake & Yanev, 1986; Jackman & Wake, 1994). For example, Wake & Yanev (1986) examined 19 populations throughout the *Ensatina* complex and found that most populations were separated by large Nei's (1978) genetic distances (D), in excess of 0.4 in some comparisons. When we map the results of Wake & Yanev (1986) onto the distribution of haplotype lineages recovered in the current study, we find that where Nei's genetic distances are high, populations represented by separate mtDNA lineages are being compared. For example, Nei's D between three populations in northern California ranges from 0.11 to 0.29 (Fig. 5a). Using the terminology of the current study, this corresponds to comparisons between *picta*, *oregonensis* [1] and *oregonensis* [4]. On the other hand, in the few instances in which Nei's D is relatively low, comparisons are between populations within single mtDNA haplotype lineages. For instance, Nei's D between populations of *eschscholtzii* in Monterey and Santa Barbara County is only 0.03, and both populations are within the range of the northern mtDNA haplotype lineage of *eschscholtzii*.

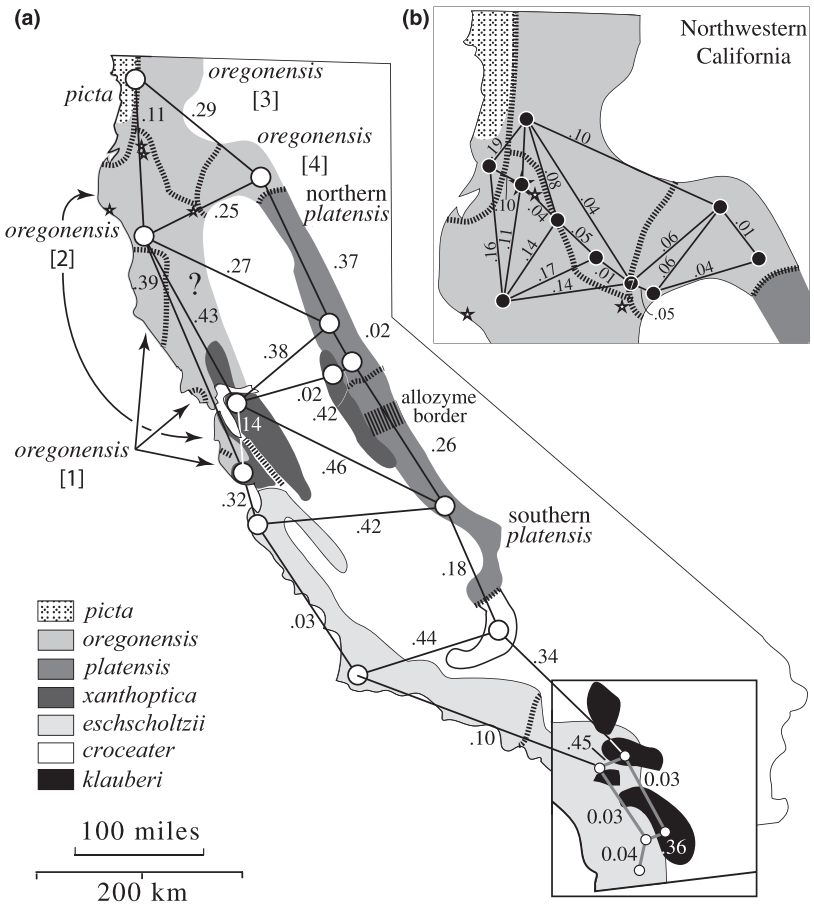

Figure 5 Map illustrating the relationships between previous allozyme studies and the mtDNA clades recovered in the current study. Subspecies are differentially shaded. Within subspecies, the distributions of major mtDNA clades are designated with thick dashed lines. (a) Nei's (1978) genetic distances between samples as reported by Wake & Yanev (1986). The allozymic intergradation zone between northern and southern *platensis* is also shown (Jackman & Wake, 1994). The stars show the location of the northern haplotypes, which are of unclear phylogenetic affinity (Fig. 4a). The question mark in northern California points out an area that we have not sampled for mtDNA, thus we are not confident regarding its phylogenetic affinity. (b) Inset showing an expanded view of northern California. Points show populations studied by Jackman & Wake (1994) (population 7 is labelled because it is referred to specifically in the text); numbers are Nei's (1978) genetic distances between samples.

Given the high levels of phylogeographical structure and the strong pattern of isolation by distance in the *Ensatina* complex (Jackman & Wake, 1994; Kuchta *et al.*, 2009), it is not surprising that the large genetic distances documented by Wake & Yanev (1986) are associated with separate mtDNA clades, because Wake & Yanev (1986) had widely spaced samples (a strategy that made sense given that little was known about genetic diversity in the *Ensatina* complex at the time). Denser sampling is needed to examine patterns of concordance and discordance between allozyme and mtDNA markers (Wake & Schneider, 1998). A first step in this direction can be obtained by examining divergence among populations in northern California, where Jackman & Wake (1994) investigated allozymic differentiation among populations from north western California (*picta*) across the northern end of the Central Valley (*oregonensis* [2–4]) to the northern limit of the Sierra Nevada region (*oregonensis* [4]) (Fig. 5b). They found that Nei's *D* was generally high, ranging up to 0.17 between nearest neighbours within the subspecies *oregonensis*, and isolation by distance characterized the transect. Figure 5b shows how the patterns of allozymic differentiation reported by Jackman & Wake (1994) relate to mtDNA phylogeographical diversity. We see that the allozyme sampling spans four unrelated mtDNA haplotype lineages, suggesting that the high levels of allozyme differentiation are in part a consequence of comparing discrete phylogeographical units. In most instances, however, only a small number of populations (1–4) were sampled for allozymes within the range of each mtDNA lineage, severely hampering comparisons of patterns of variation within vs. between lineages (Fig. 5b). Nonetheless, there is some evidence of admixture where lineages contact one another, as population 7 of Jackman & Wake (1994) was found to contain two sympatric haplotypes belonging to divergent mtDNA lineages (Fig. 5b). In the current study, these haplotypes are assigned to population 7 (*oregonensis* [3]) and population 23 (*oregonensis* [4] Fig. 3). Interestingly, this locality is unremarkable for allozymes, with low Nei's *D* (≤ 0.06) to nearby populations of *oregonensis* [3] and *oregonensis* [4].

Where lineages around the ring meet, such as among lineages of *oregonensis* in northern California, it is necessary to distinguish among the various kinds of evolutionary dynamics that might occur, such as localized hybridization, introgression, or genetic merger. The ring species scenario requires that secondary contacts within the ring are not characterized by reproductive isolation, whereas secondary contacts between the coastal and inland arms must exhibit species-level divergence. In this spirit of assessing contact zone dynamics (Jockusch & Wake, 2002; Alexandrino *et al.*, 2005; Kuchta, 2007), a more rigorous assessment of the association between mtDNA haplotype lineages and patterns of allozymic diversity throughout the *Ensatina* complex has recently been undertaken (Pereira & Wake, in press). Theoretical models suggest

that separate mtDNA clades may evolve *in situ* when populations exhibit isolation by distance (cladogenesis without allopatry; Irwin, 2002). If this model is correct (see Templeton, 2004), the *in situ* evolution of discrete mtDNA clades may be an important factor within the *Ensatina* complex, given the low dispersal abilities of *Ensatina* salamanders (Staub *et al.*, 1995) and the strong patterns of isolation by distance that have been documented (Jackman & Wake, 1994; Kuchta *et al.*, 2009).

Ring species and taxonomy

We have focused on the biogeography of the *Ensatina* complex, but there is also a taxonomic dimension to consider. Critics (e.g. Highton, 1998) have argued that the *Ensatina* complex is not special or unusual, but that it is instead comprised of independently evolving species. According to this view, there is no ring species, just inappropriate taxonomy. Highton (1998) thinks recognition of many species – at least 11 – is warranted, using criteria he has developed for species in the plethodontid genus *Plethodon*. Less extreme taxonomic revisions have been proposed. For example, Frost & Hillis (1990) suggested recognizing *klauberi* as a full species because of its allopatric distribution in southern California (Fig. 1a); they also thought the remainder of the complex needs further revision. Graybeal (1995) offered a suggestion for recognizing four species. Highton (1998) argued that no single species could possibly contain so much genetic diversity as that recorded in *Ensatina* (and he did not consider the results published by Wake, 1997). Wake & Schneider (1998) countered by reviewing much of the complexity in *Ensatina*, including numerous instances of discordance among morphological, allozymic and mtDNA sets, and argued that Highton (1998) was using a phenetic (as opposed to phylogenetic) methodology that artificially sharpened the borders between units that lack evidence of genetic and evolutionary independence. The pattern of haplotype clade distributions presented in this paper portrays a patchwork of exclusive geographical ranges. However, it is simplistic to consider these haplotype clades to be full species, despite the parapatric nature of the ranges and the near absence of sympatry except in contact zones. There are discordances between patterns in the mtDNA clades and patterns based on allozymes and coloration (Wake & Schneider, 1998). The stage is now set for in-depth analyses of the regions of discordance, which will entail the use of multiple molecular markers (e.g. projects in progress by T. Devitt and R. Pereira). Pending results of such studies, we continue to accept the taxonomy of Stebbins (1949) as the best available alternative. Of the seven recognized subspecies, *xanthoptica*, *eschscholtzii*, *klauberi* and *croceater* are potentially genealogical entities, monophyletic or nearly so with respect to all three kinds of data. Both *oregonensis* and *platensis* are recognizable as originally diagnosed by Stebbins (1949), but each is di- to polyphyletic with respect to mtDNA, and they are sufficiently complex with respect to allozymes that it seems safe to assume that neither is a genealogical unit. The subspecies category is controversial; there is no general agreement that subspecies must or should be monophyletic. Instead, their utility is to provide labels for phenotypically recognizable, geographically discrete segments of complexes of species that remain under study (Wake & Schneider, 1998; Manier, 2004; Mulcahy, 2008). Examples in addition to *Ensatina* among salamanders include the *Ambystoma tigrinum*, *Salamandra salamandra* and *Bolitoglossa franklini* complexes (Wake & Lynch, 1982; Shaffer & McKnight, 1996; Steinfartz *et al.*, 2000). Until definitive evidence for the evolutionary independence of components of the *Ensatina* complex warrants a taxonomic revision (using diverse criteria within the framework of the general lineage species concept; de Queiroz, 1998), we recommend continuation of the now familiar and utilitarian taxonomy first proposed by Stebbins (1949). We prefer to direct attention towards what the complex has to teach us about the diversification process, as well as the limits of the species category itself.

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REFERENCES

Alexandrino, J., Baird, S.J.E., Lawson, L., Macey, J.R., Moritz, C. & Wake, D.B. (2005) Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. *Evolution*, **59,** 1334–1347.

Atwater, T. (1970) Implications of plate tectonics for the Cenozoic tectonic evolution of western North America. *Geological Society of America Bulletin*, **81,** 3513–3536.

Brown, C.W. (1974) Hybridization among the subspecies of the plethodontid salamander *Ensatina eschscholtzii*. *University of California Publications in Zoology*, **98,** 1–57.

Burnham, K. (2005) Point Lobos to Point Reyes: evidence of approximately 180 km offset of the San Gregorio and northern San Andreas faults. *Cenozoic deformation in the central Coast Ranges, California* (ed. by C.H. Steven and J.D. Cooper), pp. 1–29. Society for Sedimentary Geology, Tulsa, OK.

Calsbeek, R., Thompson, J.N. & Richardson, J.E. (2003) Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology*, **12,** 1021–1029.

Chatzimanolis, S. & Caterino, M.S. (2007) Toward a better understanding of the 'Transverse Range break': lineage diversification in southern California. *Evolution*, **61,** 2127–2141.

Chippindale, P.T., Bonett, R.M., Baldwin, A.S. & Wiens, J.J. (2004) Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution*, **58,** 2809–2822.

Dobzhansky, T. (1958) Species after Darwin. *A century of Darwin* (ed. by S.A. Barnett), pp. 19–55. Harvard University Press, Cambridge, MA.

Dupré, W.R. (1990) Quaternary geology of the Monterey Bay region, California. *Geology and tectonics of the central California Coast Region, San Francisco to Monterey* (ed. by R.E. Garrison), pp. 185–191. US Geological Survey, Menlo Park, CA.

Feldman, C.R. & Spicer, G.S. (2006) Comparative phylogeography of woodland reptiles in California: repeated patterns of cladogenesis and population expansion. *Molecular Ecology*, **15,** 2201–2222.

Frost, D.R. & Hillis, D.M. (1990) Species in concept and practice: herpetological applications. *Herpetologica*, **46,** 87–104.

Futuyma, D.J. (1998) *Evolutionary biology*, 3rd edn. Sinauer Associates, Sunderland, MA.

Graybeal, A. (1995) Naming species. *Systematic Biology*, **44,** 237–250.

Hall, C.A.J. (2002) Nearshore marine paleoclimate regions, increasing zoogeographic provinciality, molluscan extinctions, and paleoshorelines, California: late Oligocene (27 Ma) to late Pliocene (2.5 Ma). *Geological Society of America, Special Paper*, **357,** v-489.

Highton, R. (1998) Is *Ensatina eschscholtzii* a ring-species? *Herpetologica*, **54,** 254–278.

Holman, J.A. (2006) *Fossil salamanders of North America*. Indiana University Press, Bloomington, IN.

Huelsenbeck, J.P. & Rannala, B. (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology*, **53,** 904–913.

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17,** 754–755.

Irwin, D.E. (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution*, **56,** 2383–2394.

Irwin, D.E., Irwin, J.H. & Price, T.D. (2001) Ring species as bridges between microevolution and speciation. *Genetica*, **112/113,** 223–243.

Jackman, T.R. & Wake, D.B. (1994) Evolutionary and historical analysis of protein variation in the blotched forms of salamanders of the *Ensatina* complex (Amphibia: Plethodontidae). *Evolution*, **48,** 876–897.

Jockusch, E.L. & Wake, D.B. (2002) Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. *Biological Journal of the Linnaean Society*, **76,** 361–391.

Kuchta, S.R. (2005) Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific newts. *Copeia*, **2005,** 265–271.

Kuchta, S.R. (2007) Contact zones and species limits: hybridization between lineages of the California newt, *Taricha torosa*, in the southern Sierra Nevada. *Herpetologica*, **63,** 332–350.

Kuchta, S.R. & Tan, A.M. (2005) Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*. *Molecular Ecology*, **14,** 225–244.

Kuchta, S.R. & Tan, A.M. (2006) Lineage diversification on an evolving landscape: phylogeography of the California newt, *Taricha torosa* (Caudata: Salamandridae). *Biological Journal of the Linnaean Society*, **89,** 213–239.

Kuchta, S.R., Krakauer, A.H. & Sinervo, B. (2008) Why does the Yellow-eyed ensatina have yellow eyes? Batesian mimicry of Pacific newts (genus *Taricha*) by the salamander *Ensatina eschscholtzii xanthoptica*. *Evolution*, **62,** 984–990.

Kuchta, S.R., Parks, D. & Wake, D.B. (2009) Pronounced phylogeographic structure on a small spatial scale: geomorphological evolution and lineage history in the salamander ring species *Ensatina eschscholtzii* in central coastal California. *Molecular Phylogenetics and Evolution*, **50,** 240–255.

Lapointe, F.J. & Rissler, L.J. (2005) Congruence, consensus, and the comparative phylogeography of codistributed species in California. *The American Naturalist*, **166,** 290–299.

Larson, A., Wake, D.B., Maxson, L.R. & Highton, R. (1981) A molecular phylogenetic perspective on the origins of morphological novelties in the salamanders of the tribe Plethodontini (Amphibia, Plethodontidae). *Evolution*, **35,** 405–422.

Maddison, W.P. & Maddison, D.R. (2006) *Mesquite: a modular system for evolutionary analysis. Version 2.01*. Available at: http://mesquiteproject.org [last accessed August 2008].

Manier, M.K. (2004) Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubrideae): beyond the subspecies debate. *Biological Journal of the Linnaean Society*, **83,** 65–85.

Martens, J. & Päckert, M. (2007) Ring species – do they exist in birds? *Zoologischer Anzeiger*, **246,** 315–324.

Maxson, L.R., Highton, R. & Wake, D.B. (1979) Albumin differentiation and its phylogenetic implications in the plethodontid salamander genera *Plethodon* and *Ensatina*. *Copeia*, **1979,** 502–508.

Mayr, E. (1942) *Systematics and the origin of species*. Columbia University Press, New York.

Mayr, E. (1963) *Animal species and evolution*. Belknap Press, Cambridge, MA.

Moritz, C., Schneider, C.J. & Wake, D.B. (1992) Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology*, **41,** 273–291.

Mueller, R.L., Macey, J.R., Jaekel, M., Wake, D.B. & Boore, J.L. (2004) Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proceedings of the National Academy of Sciences USA*, **101,** 13820–13825.

Mulcahy, D.G. (2008) Phylogeography and species boundaries of the western North American nightsnake (*Hypsiglena*

torquata): revisiting the subspecies concept. *Molecular Phylogenetics and Evolution*, **46,** 1095–1115.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **23,** 341–369.

Nylander, J.A.A. (2004) *MrModeltest ver. 2*. Program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Parks, D.S. (2000) *Phylogeography, historical distribution, migration, and species boundaries in the salamander* Ensatina eschscholtzii *as measured with mitochondrial DNA sequences*. PhD Thesis, University of California, Berkeley, CA.

Pereira, R. & Wade, D.B. (in press) Genetic leakage after adaptive and non-adaptive divergence in the *Ensatina eschscholtzii* ring species. *Evolution*.

de Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. *Endless forms: species and speciation* (ed. by D.J. Howard and S.H. Berlocher), pp. 57–75. Oxford University Press, New York.

Rich, K.A., Thompson, J.N. & Fernandez, C.C. (2008) Diverse historical processes shape deep phylogeographical divergence in the pollinating seed parasite *Greya politella*. *Molecular Ecology*, **17,** 2430–2448.

Ridley, M. (1996) *Evolution*, 2nd edn. Blackwell Science, Oxford.

Rissler, L.J., Hijmans, R.J., Graham, C.H., Moritz, C. & Wake, D.B. (2006) Phylogeographic lineages and species comparisons in conservation analyses: a case study of California herpetofauna. *The American Naturalist*, **167,** 655–666.

Sarna-Wojcicki, A.M., Meyer, C.E., Bowman, H.R., Hall, N.T., Russell, P.C., Woodward, M.J. & Slate, J.L. (1985) Correlation of the Rockland ash bed, a 400,000-year-old stratigraphic marker in northern California and western Nevada and implications for middle Pleistocene paleogeography of central California. *Quaternary Research*, **23,** 236–257.

Shaffer, H.B. & McKnight, M.L. (1996) The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander (*Ambystoma tigrinum*) (Amphibia: Caudata) complex. *Evolution*, **50,** 417–433.

Sims, J.D. (1993) Chronology of displacement on the San Andreas fault in central California: evidence from reversed positions of exotic rock bodies near Parkfield, California. *The San Andreas fault system: displacement, palinspastic reconstruction, and geologic evolution* (ed. by R.E. Powell, R.J. Weldon and J.C. Matti), pp. 231–256. Geological Society of America, Boulder, CO.

Staub, N.L., Brown, C.W. & Wake, D.B. (1995) Patterns of growth and movements in a population of *Ensatina eschscholtzii platensis* (Caudata: Plethodontidae) in the Sierra Nevada, California. *Journal of Herpetology*, **29,** 593–599.

Stebbins, R.C. (1949) Speciation in salamanders of the plethodontid genus *Ensatina*. *University of California Publications in Zoology*, **48,** 377–526.

Stebbins, R.C. (1957) Intraspecific sympatry in the lungless salamander *Ensatina eschscholtzii*. *Evolution*, **11,** 265–270.

Stebbins, R.C. (2003) *A field guide to western reptiles and amphibians*, 3rd edn. Houghton Mifflin, Boston, MA.

Steinfartz, S., Veith, M. & Tautz, D. (2000) Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of central Europe from distinct source populations of *Salamandra salamandra*. *Molecular Ecology*, **9,** 397–410.

Templeton, A.R. (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology*, **13,** 789–809.

Thorne, J.A. & Kishino, H. (2002) Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51,** 689–702.

Thorne, J.A., Kishino, H. & Painter, S. (1998) Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution*, **15,** 1647–1657.

Tihen, J.A. & Wake, D.B. (1981) Vertebrae of plethodontid salamanders from the lower Miocene of Montana. *Journal of Herpetology*, **15,** 35–40.

Venczel, M. & Sanchíz, B. (2005) A fossil plethodontid salamander from the middle Miocene of Slovakia (Caudata, Plethodontidae). *Amphibia–Reptilia*, **26,** 408–411.

Vieites, D.R., Min, M.-S. & Wake, D.B. (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proceedings of the National Academy of Sciences USA*, **104,** 19903–19907.

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

Wake, D.B. (2006) Problems with species: patterns and processes of species formation in salamanders. *Annals of the Missouri Botanical Garden*, **93,** 8–23.

Wake, D.B. & Lynch, J.F. (1982) Evolutionary relationships among Central American salamanders of the *Bolitoglossa franklini* group, with a description of a new species from Guatemala. *Herpetologica*, **38,** 257–272.

Wake, D.B. & Schneider, C.J. (1998) Taxonomy of the plethodontid salamander genus *Ensatina*. *Herpetologica*, **54,** 279–298.

Wake, D.B. & Yanev, K.P. (1986) Geographic variation in allozymes in a 'ring species', the plethodontid salamander *Ensatina eschscholtzii* of western North America. *Evolution*, **40,** 702–715.

Wake, D.B., Yanev, K.P. & Brown, C.W. (1986) Intraspecific sympatry in a 'ring species', the plethodontid salamander *Ensatina eschscholtzii* of southern California. *Evolution*, **40,** 866–868.

Wake, D.B., Yanev, K.P. & Frelow, M.M. (1989) Sympatry and hybridization in a 'ring species': the plethodontid salamander *Ensatina eschscholtzii*. *Speciation and its consequences* (ed. by D. Otte and J.A. Endler), pp. 134–157. Sinauer, Sunderland, MA.

Yanev, K.P. (1980) Biogeography and distribution of three parapatric salamander species in coastal and borderland

California. *The California Islands: Proceedings of a Multi-disciplinary Symposium* (ed. by D.M. Power), pp. 531–550. Santa Barbara Museum of Natural History, Santa Barbara, CA.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Population numbers, collecting localities, Museum of Vertebrate Zoology (MVZ) accession numbers, identifications of sequences used in analyses, and GenBank accession numbers.

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Research in the Museum of Vertebrate Zoology (MVZ) is centred on evolutionary biology from the perspectives of systematics, ecology, behaviour, functional and developmental morphology, population biology, and evolutionary genomics. In addition, the MVZ aims to lead the way in developing and using natural history collections for research, education and problems in biodiversity conservation. S.R.K., D.S.P. and R.L.M. are all former graduate students under D.B.W. Members of the Wake group study how diversity is generated through time and space, with a particular research focus on salamanders. S.R.K., D.S.P. and D.B.W. conceived the ideas and collected the data; S.R.K. and R.L.M. conducted the analyses; the manuscript was written by S.R.K. and D.B.W.

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