
PROBLEMS WITH SPECIES:
PATTERNS AND PROCESSES OF
SPECIES FORMATION
IN SALAMANDERS¹

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ABSTRACT

How many species one recognizes within a given taxon remains a difficult question, especially when morphology is relatively stable or when clinal variation is present, thus complicating diagnosis. I accept the general lineage concept of species, and my goal is to recognize historically distinct evolutionary lineages that are likely to remain distinct. Here I analyze this task with respect to patterns of species formation in two genera of plethodontid salamanders in California. *Ensatina* is a ring species complex surrounding the Central Valley of California. At present it is a single species with seven subspecies that are linked by apparent clinal variation in intergrade zones, but there are also some narrow hybrid zones where morphologically and ecologically differentiated forms interact. In contrast, *Batrachoseps*, which has much the same distribution, has about 20 species in California, most occurring in sympatry with *Ensatina*. Divergence in the two taxa is based on two fundamentally different phenomena, and yet there are some common themes. Adaptive divergence in coloration is the dominant theme in *Ensatina*, whereas differentiation is largely perceived at the molecular level in *Batrachoseps*. Yet both have evolved in the same region and have been affected by many of the same climatic and earth historical phenomena. Within the *Ensatina* complex, different adaptations related to predator avoidance have evolved. Coloration has diverged in different directions in coastal and inland populations, even though genetic interactions continue to take place. Where coastal populations meet other coastal populations, ecologically and morphologically similar populations merge genetically, even if well differentiated in molecular traits. In contrast, where the ring is crossed and where ecologically and morphologically differentiated populations meet, they hybridize narrowly or are sympatric and behave as if they are species. Within the ring-like distribution, clinal patterns of variation occur. The current polytypic taxonomy is retained, even though it is problematic, because alternatives are even less appropriate. In contrast, where genetically differentiated populations of *Batrachoseps* meet they typically do not merge. Instead, they replace one another spatially, in part because they are so similar ecologically. Apparently the periods of isolation were sufficiently long that even in the absence of adaptive divergence there has been divergence of isolating mechanisms. Analysis of patterns of genetic differentiation in allozymes and mtDNA in relation to the geological history of California is used to generate biogeographic scenarios to help explain the contrast between *Batrachoseps* and *Ensatina*.

Key words: *Batrachoseps*, California paleogeography, *Ensatina*, historical biogeography, phylogeography, salamanders, species formation.

Every generation of biologists is destined to deal with species problems, sometimes repeatedly, or, in my case, continuously throughout my career. I described my first new species in 1962, based on a single specimen from Colombia that had been collected in the nineteenth century and assigned to a species that was thought, mistakenly, to range throughout Middle America and into South America (Brame & Wake, 1962). That species has so far stood the test of time, although even today it is known from fewer than 10 specimens. The decision to describe the species was based solely on anatomy and color pattern. The vast majority of all known species of

salamanders is based on such criteria. When molecular methods became accessible to systematists, roughly 35 years ago, many surprises resulted. Kay Yanev, the first to use such methods in my lab, found that allozymes uncovered different patterns than did morphology in the Slender Salamanders (*Batrachoseps* Bonaparte, 1839) of California (Yanev, 1978). Several nominal species were species complexes, and a number of undescribed species were identified (Yanev, 1978). James Hanken, also in my lab, made similar observations on tropical salamanders of the genus *Thorius* Cope, 1869 (Hanken, 1980). Richard Highton was among the first systematists to use molecular

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³ I dedicate this paper to R. C. Stebbins, who remains an enthusiast for *Ensatina*, on the occasion of his 91st birthday, and to the memory of Arden H. Brame Jr. II (1934–2004), who first introduced me to *Batrachoseps* and its diversity.

methods in comprehensive geographic sampling; soon he uncovered levels of differentiation that were previously unimaginable (e.g., Highton, 1989). Before the main impact of the new molecular systematics revolution, the first modern complete catalog of amphibians reported 356 salamander species (Frost, 1985). The number of salamanders has increased since by 56% (the most recent count is 556, AmphibiaWeb, 2006). These are surprising increases for a group of relatively well-known terrestrial vertebrates, especially given that many of the new species reflect new discoveries. However, the new descriptions also highlight a modern species problem that is not unique to salamanders, but common to most taxa—how to translate molecular findings into taxonomy.

At the same time as new laboratory methodologies were drawing attention to species problems, new phylogenetic methods were having a major impact. In particular, methods ranging from mainly phenetic numerical taxonomy to cladistics were causing systematists to rethink species concepts and criteria (e.g., Cracraft, 1989, 1997; Mayden, 1997). As genetic analyses became ever more sophisticated, renewed focus on species was one result (e.g., Templeton, 1989; Coyne & Orr, 2004; Hey, 2001).

Given the sheer volume of recent publication on species concepts and on the species problem in general, it is a bit surprising that de Queiroz (1998, 2000, 2005) concluded that no fundamental change in our species concept has taken place and that such a change is unnecessary. I agree with this perspective. There are indeed many ongoing debates about species, but these are fundamentally about criteria and the debate is mainly joined by proponents of different kinds of data or more explicit methodologies than used in the past.

Here I examine patterns of species formation in Californian salamanders that display contrasting patterns. My goal is to highlight the connection of pattern and process in the formation of species. I am especially interested in comparisons of tree-based and character-based methods of delimiting species and what it is that we have delimited using these methods. In addition, I explore whether species recognition and delimitation is an exercise in recovering history or in predicting the likely course of further evolution.

I accept the general lineage concept of species that has been developed by Mayden (1997) and de Queiroz (1998, 2005). Species are historical lineages of metapopulations, or, more precisely, they are segments of metapopulation lineages; all other considerations are secondary to this primary principle. In any given instance, one might rely on any of a number of secondary species concepts as criteria to delineate

what species are recognized. The critically important point with respect to the general concept is that there are no *necessary* attributes of species; rather, there are different lines of evidence that can be used concerning separation of lineages and these are all contingent. The pragmatic matter of delimiting species remains, however, and many methods have been proposed to deal with the issue. All are problematic (reviewed by Sites & Marshall, 2004). Furthermore, even adopting the concept framed by de Queiroz does not mean that different taxonomists will reach the same conclusions because criteria can be interpreted in different ways. I suspect that de Queiroz (based on de Queiroz, 2005) would recognize more species than I would in most instances, because of his emphasis on detection of initial splits.

PATTERN AND PROCESS IN SPECIES FORMATION

Classifications of modes of species formation may be pattern-based or process-based. By pattern I refer in particular to geographic units inferred to be independently evolving lineage segments, i.e., species. Criteria vary greatly, from some relative measure of degree of divergence to tree-based approaches. The simplest way to explain such patterns is vicariance plus something else, such as differential selection or even haphazard changes in gene frequencies. By process I refer specifically to adaptive processes that are critically important to the establishment of independent lineages. Although an enormous amount of literature deals with species formation, I focus here on adaptive divergence and nonadaptive radiation. These are alternative ways that lineages diversify. The main problem that has been identified with adaptive divergence is the establishment of genetic isolation between the adaptively diverging populations when they are not allopatric. The main problem with nonadaptive radiation is the historic one of determining the status of geographically isolated but phylogenetically related parts of lineages, the classical problem with allopatry.

ADAPTIVE DIVERGENCE

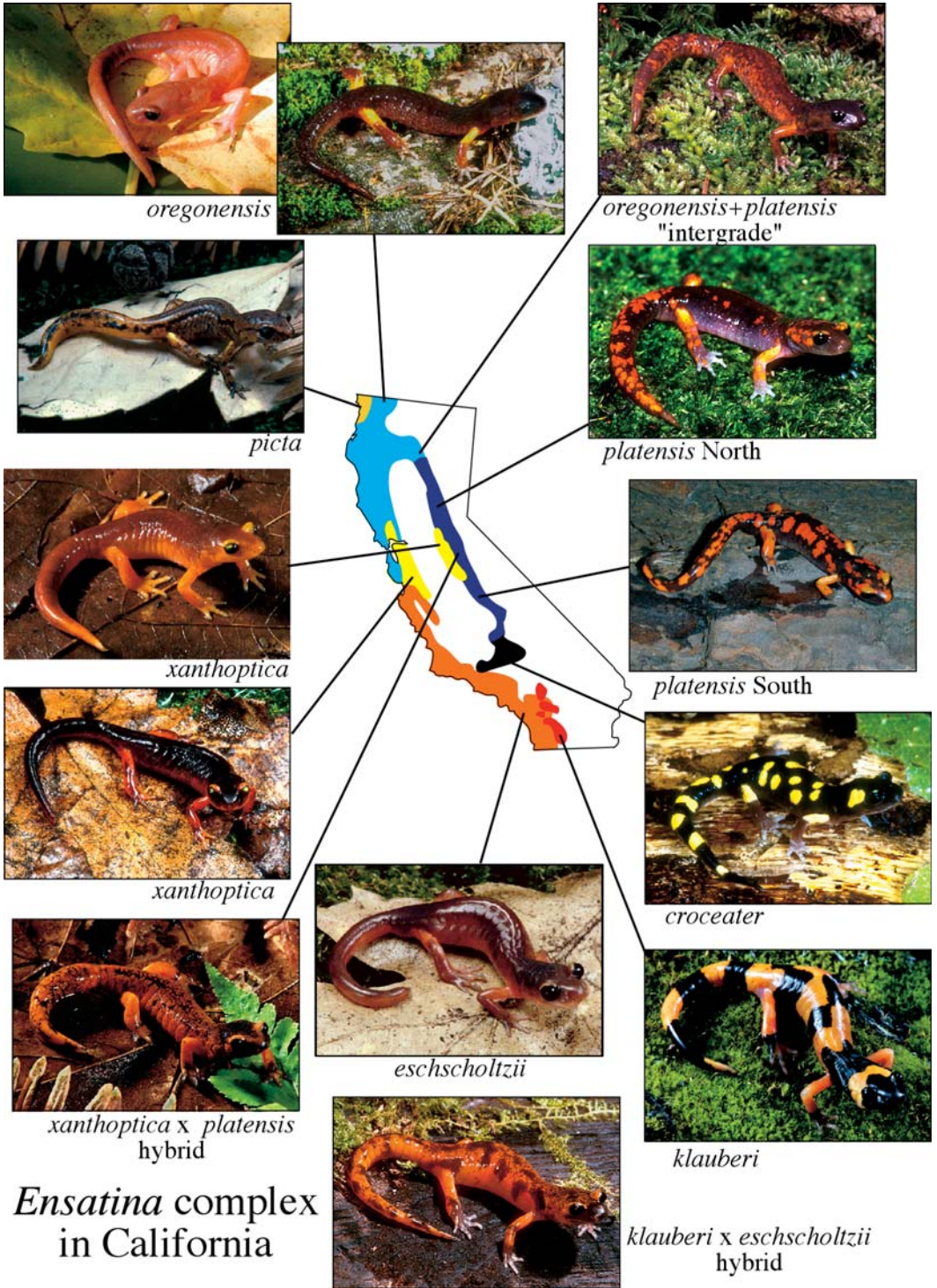
Papers by Bush (e.g., Bush, 1969) and a book by Endler (1977) are the starting points for my own interest in adaptive divergence (as opposed to the historical allopatry arguments) as a factor in species formation. Bush emphasized adaptive divergence in sympatry. Endler was more interested in geographic variation, in particular clines in characters or gene frequencies, and appearance of steps in clines, which might be preludes to species formation; his emphasis was parapatry or alloparapatry.

My studies of adaptive divergence have focused on the plethodontid salamander *Ensatina* Gray, 1850, which displays geographical differentiation interpreted as clinal differentiation and adaptive divergence on the way to species formation (Stebbins, 1949); it remains problematic whether subsets of the lineage have achieved independence and how many species warrant recognition. *Ensatina* is a ring species complex, a taxonomically challenging group of populations spread from British Columbia to Baja California, generally west of the Cascade-Sierra Nevada mountain system. These are terrestrial salamanders that never enter water, and they never gather in assemblages. They display direct development (lecitotrophy) in which embryos form inside the egg capsules and hatch as miniatures of the adult. *Ensatina* is relatively long-lived (ca. 8 years), sedentary, philopatric, and shows little dispersal (Stebbins, 1954; Staub et al., 1995). Prior to Stebbins (1949) four species were recognized, and although some originally had been described as members of the genus *Plethodon*, *Ensatina* long has been recognized as monophyletic (e.g., Dunn, 1926). Three of the species, *Ensatina croceater* (Cope, 1868), *E. klauberi* Dunn, 1929, and *E. platensis* (Jiménez de la Espada, 1875), were boldly marked (spotted or blotched) salamanders associated with forested regions from inner montane areas from Mount Lassen, California, southward to San Diego County and northern Baja California. A more widespread species, *E. eschscholtzii* Gray, 1850, was more uniformly colored, lacking spots or blotches, and was distributed along the coast of California and inland as well, to the Cascade Mountains in Oregon, Washington, and British Columbia. This form is associated with woodlands in the north, but in central and southern California it frequently occurs in more open habitats such as coastal sage scrub and chaparral.

The conclusions of Stebbins's (1949) groundbreaking study were revolutionary. He postulated that the four, then-recognized, species were part of a single lineage that had originated in the north and had expanded its range southward, adaptively diverging in different directions in the coastal and inland mountains. Stebbins recognized seven subspecies (*Ensatina eschscholtzii eschscholtzii*, *E. e. croceater*, *E. e. klauberi*, *E. e. oregonensis* (Girard, 1856), *E. e.*

picta Wood, 1940, *E. e. platensis*, *E. e. xanthoptica* Stebbins, 1949; Fig. 1). He envisioned an ancestor having the traits of *picta*, restricted now to the ancient forests of southwestern Oregon and extreme northwestern California. This form has a somewhat variegated color pattern, and in it Stebbins envisioned the potential of all of the patterns found elsewhere. Each subspecies was diagnosed on the basis of its color pattern and some subtle morphological differences, although *oregonensis* was something of a default and had highly variable color patterns. From a *picta*-like ancestor Stebbins postulated southward migrations accompanied by divergent adaptation. In the Sierra Nevada the forms became restricted to montane areas, usually with closed canopy forests, and these were thought to show progressive stages in the development of disruptive coloration, from intergrades in the far north to spotted red and brown *platensis*, to more vivid yellow and black *croceater* in the Tehachapi Mountains, finally culminating in the boldly blotched *klauberi* in the far south (Fig. 1). Along the coast the generalized *oregonensis* is variable within and among populations in coloration, displaying generally cryptic coloration, but not disruptive in pattern. As populations became increasingly associated with more open habitats, a mimicry relationship developed with the dangerously poisonous newts (*Taricha* Gray, 1850). Mimicry is most highly developed in *xanthoptica*, mainly found in the inner coast ranges east of San Francisco Bay (Kuchta, 2005). These aposematically colored animals display vivid yellow and orange coloration that contrasts sharply with the disruptive patterns of the blotched inland forms. Adaptive divergence was accelerated as a result of these alternative adaptations, each different from the ancestral condition. When the contrasting *eschscholtzii* and *klauberi* contact each other at the southern extent of their ranges, they look and act like different species (Fig. 1), although they often hybridize. Critical to Stebbins's interpretation was a "transvalley leak," a relatively recent invasion of the coastal *xanthoptica* into the foothills of the Sierra Nevada, where it came into contact with *platensis* and the two hybridized. The ring was formed by the intergrading populations of *picta*, *oregonensis*, and *platensis* at the northern end of the Sacramento Valley.

Figure 1. *Ensatina* that are found in California (all except the first mentioned photographed in that state). Diverse color patterns of the subspecies of *Ensatina*. Clockwise from upper left: *Ensatina eschscholtzii oregonensis* from Kittitas Co., Washington (photo by D. B. Wake); *E. e. oregonensis* from Sonoma Co. (C. W. Brown); intergrade between *E. e. oregonensis* and *E. e. platensis* from Shasta Co. (C. W. Brown); northern *E. e. platensis* from Calaveras Co. (C. W. Brown); southern *E. e. platensis* from Tulare Co. (D. B. Wake); *E. e. croceater* from Kern Co. (C. W. Brown); *E. e. klauberi* from San Diego Co. (C. W. Brown); *E. e. eschscholtzii* from San Diego Co. (C. W. Brown); at bottom, a presumptive F₁ hybrid between *E. e. klauberi* and *E. e. eschscholtzii* from San Diego Co. (C. W. Brown); a hybrid backcross between *E. e. xanthoptica* and *E. e. platensis* from Calaveras Co. (C. W. Brown); *E. e. xanthoptica* from Sonoma Co. (C. W. Brown); *E. e. xanthoptica* from Calaveras Co. (C. W. Brown); *E. e. picta* from Del Norte Co. (C. W. Brown).



Subsequent research lent some support to the hypothesis of Stebbins, but added complexity. Although some hybridization occurs in southern California, there is much more in the Sierra Nevada (Brown, 1974). At the southern-most point of contact in San Diego County, no current or past hybridization has been detected (Wake et al., 1986). Many evolutionists picked up on this example as one illustrating stages in a gradual process of species formation driven by adaptive divergence. *Ensatina* became a textbook example. Dobzhansky (1958) extended the hypothesis by envisioning gene flow throughout the ring retarding species formation and leading to the persistence of hybridization in the center of the ring and in the south. Stebbins (1949) had not discussed gene flow; he was thinking not of a continuous process but one in which there was fragmentation, isolation, and divergence, and then recontact. In other words, his view had elements of both vicariance and adaptive divergence. It was his biogeographical scenario that led him to consider it a ring species (Stebbins, pers. comm.).

When genetic methods became available I initiated studies of *Ensatina*, expecting it to be a complex of several to many species. Indeed, we found extensive genetic differentiation in allozymes (Wake & Yanev, 1986), but thought our sampling density too coarse to detect species borders (for a contrary view, see Highton, 1998). Differentiation was greater at the south end of the distribution than in the region across the upper end of Sacramento Valley, and *xanthoptica* and *platensis* were less differentiated from each other than were *eschscholtzii* and *klauberi*, as would be predicted by the Stebbins model. Many of the genetic distances between adjacent samples were relatively high, and we suspected that finer sampling was necessary to find either species borders or the gradual genetic transitions as expected under an isolation by distance scenario. We could readily reject Dobzhansky's hypothesis of ongoing gene flow throughout the complex. In general, the coastal forms clustered with the widespread *oregonensis*, and the blotched and spotted forms in the south clustered together in phenetic analyses of the allozyme data. However, the blotched *platensis* was highly differentiated, with northern populations clustering with the unblotched coastal forms and the single southern population analyzed clustering with *croceater* and *klauberi*.

In order to provide further resolution we expanded research to many populations extending from *picta* in northwestern California to *klauberi* in the south (Jackman & Wake, 1994). The populations resolved into three general clusters, within which patterns of isolation by distance were detected (Jackman & Wake, 1994). A northern cluster included what Stebbins

(1949) had labeled *picta*, *picta/oregonensis* intergrades, *oregonensis*, and *oregonensis/platensis* intergrades. A second cluster included only *platensis* from the northern and central Sierra Nevada, and the final cluster included *platensis* from the southern Sierra Nevada as well as *croceater* and *klauberi*. Northern *platensis* was closer to populations in the northern cluster than to southern *platensis*, but a population from Wagner Ridge, west of Yosemite National Park, was intermediate between the two groups.

We investigated these patterns in more detail using the mitochondrial cytochrome *b* gene (Moritz et al., 1992). As expected, by examining distributions of haplotype clades, sharp borders between groups of populations were found, notably between northern and southern *platensis*. We also found a sharp break between northern *platensis* and *oregonensis/platensis* intergrades. The scale still seemed too coarse, and subsequently we expanded the sampling to saturate the California range of the genus, including nearly 400 samples (Kuchta et al., in prep.). Based on several different analyses we found it convenient to recognize 11 clusters of haplotypes (Fig. 2) based on patterns of phylogenetic relationships and geographic distribution. Most of these are well supported statistically, but some (e.g., B) appear to be paraphyletic and others (e.g., E) include members of two currently recognized subspecies. The northern (postulated ancestral forms) *picta* and *oregonensis* comprise six of these groups. Again, northern and southern *platensis* are differentiated, with southern populations clustering tightly with *croceater* (Fig. 2); there are three clades in this cluster, each with strong support.

More detailed study of the Sierra Nevada contact zone focused on the hybrid zone between *platensis* and *xanthoptica* (Wake et al., 1989). This transition is sharp, involving 8 fixed or nearly fixed allozymic markers that change in only a few hundred meters. The hybrid zones have been stable for about 40 years (first recorded by Brown, 1974), and may have narrowed over a 20-year period (Alexandrino et al., 2005). The distinction between the brightly colored, lowland *xanthoptica* and the cryptic, upland *platensis* occurs in an ecotone between lower elevation open oak-pine forest and chaparral and higher elevation closed canopy mixed conifer forest. Few F_1 hybrids are found, but there are many backcrosses. Because parental types are syntopic, they would be considered distinct species by most taxonomists were it not for the pattern in the remainder of the complex. The distribution of *xanthoptica* extends for about 100 km in the foothills of the Sierra Nevada. These populations are little differentiated from coastal populations of *xanthoptica* in allozymes and mtDNA, as well as coloration. Hybridization has only been studied in

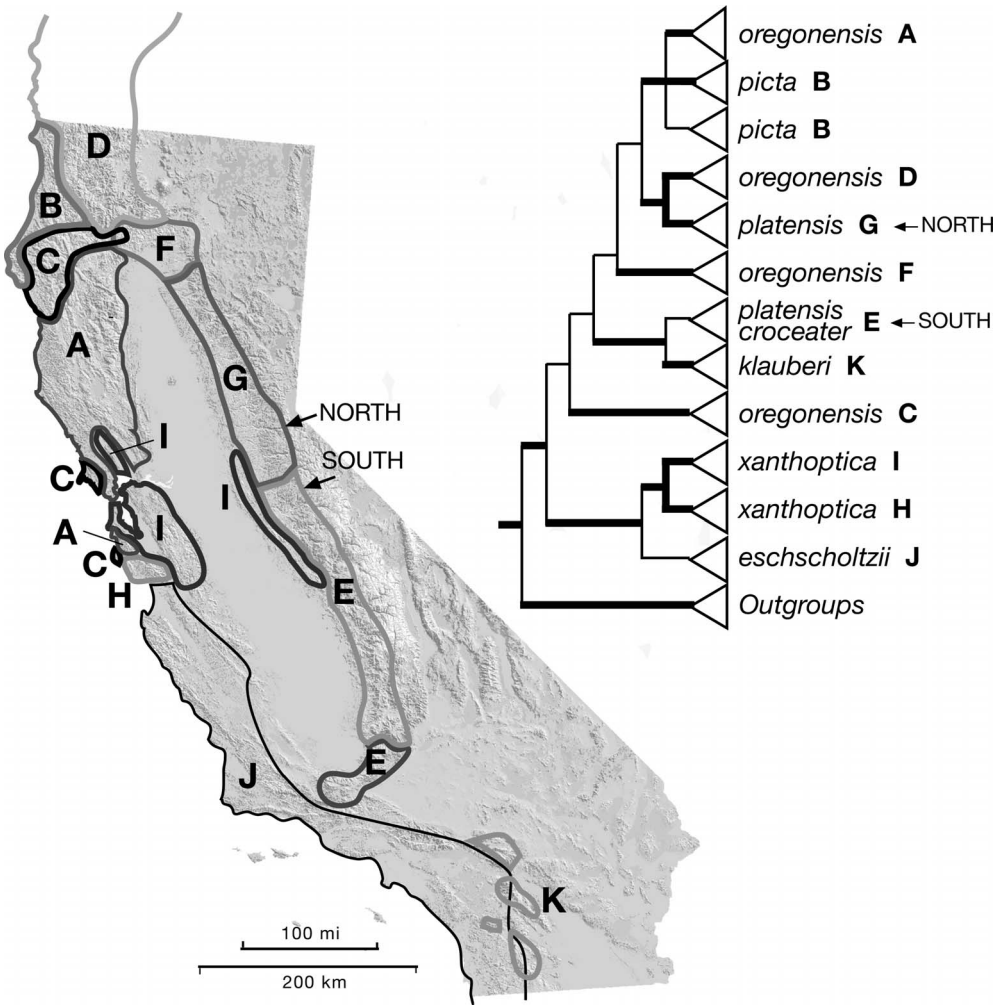


Figure 2. *Ensatina* in California. Left. Distribution of haplotype clades identified using the mitochondrial DNA gene cytochrome b. Right. Phylogram of Bayesian analysis of cytochrome sequences for nearly 400 samples of *Ensatina* from throughout the range of the genus. This is a preliminary analysis from a study in progress (Kuchta et al., in prep.). Clades with posterior probability of support at levels from 95%–100% are shown with thick lines. Some of the groups are not clearly clades, but they consist of relatively closely allied haplotypes that are geographically contiguous. The terminal groups are labeled and keyed to the map.

detail in the north (Alexandrino et al., 2005), but hybrid zones are also known to occur between southern *platensis* and *xanthoptica*. In contrast, where northern and southern *platensis* interact there is no evident difference in coloration or habitat preference. The genetic distance (Nei, 1972) between “pure” northern and southern *platensis* is 0.26–0.30, with major frequency differences in three loci. The genetic transition as determined from allozymes takes place over about 300 km, but a major transition in haplotypes takes place near the southern end of this region, about 75 km south of the major zone of allozymic transition (Wake & Schneider, 1998). Extraordinary variation in color pattern is found in Yosemite Valley. This is where

the allozyme transition first is detected, and I believe that the variation may be a consequence of the merger of two genetically differentiated populations.

Jackman and Wake (1994) presented a possible scenario to account for the historical biogeography of *Ensatina* in the Sierra Nevada. They envisioned an early southward migration and differentiation of spotted and blotched salamanders, then geographic isolation as a result of a gap in the distribution that developed midway along the Sierra Nevada. Subsequently the northern Sierra Nevada was occupied by precursors of northern *platensis*, which in turn became isolated from *oregonensis* by repeated volcanism and glaciation in the region of present-day Mt. Lassen, in

northeastern California. Northern and southern *platensis* remained separated, probably by glaciation concentrated in the deep valleys of the central Sierra Nevada associated with the present-day Tuolumne, Merced, and San Joaquin river canyons, until late Pleistocene, when the two groups met and merged genetically. A selective sweep is responsible for rapid northward movement of the strongly adaptive blotched color pattern of southern *platensis* (Wake & Schneider, 1998). This pattern is postulated to have spread to the northern end of the range of the northern *platensis* haplotype group, in the Mt. Lassen area. Stebbins (1949) thought that populations in northeastern California were intergrades between *oregonensis* and northern *platensis*. An alternative interpretation is that strongly marked (but not blotched) salamanders in this area that differ from northern *platensis* in both allozymes and mtDNA might be an indication that southern *platensis* color alleles have only recently reached this area (e.g., Fig. 1), where they are introgressing into *oregonensis*.

The combination of allozymic and haplotype information led Highton (1998) to conclude that there is a broad hybrid zone separating northern and southern *platensis*, which he considered to be separate (but unnamed) species. He was silent with respect to coloration. However, in my view, the zone of interaction is far too broad (at least 75 km) to be considered a hybrid zone. I consider a hybrid zone to be a site either where two different parental forms co-occur and form hybrid individuals, or where two different parental forms are separated by a distance equivalent to a few, on the order of tens, of home ranges, with the intervening area occupied by hybrids and perhaps several generational backcrosses. I have argued elsewhere (Wake, 1997) that any of several suggestions for taxonomic change are problematic, and have retained the taxonomy of Stebbins (1949) until compelling evidence of species borders around (as contrasted with across) the ring-like distribution of *Ensatina* is found. When morphologically and ecologically similar forms meet they merge genetically, across haplotype clade borders, which remain as markers of past distributional limits. In contrast, when morphologically and ecologically differentiated forms meet they hybridize, in ecotones in which both parental forms are present. No indication of post-mating isolation is found. The hybrids and backcrosses are hypothesized to be at an adaptive disadvantage to either parental class, and we have measured extraordinary levels of selection against them (ca. 46%–75%) (Alexandrino et al., 2005). The classic explanation by Stebbins (1949) that intergradation occurs in the north, hybridization in the region of the transvalley leak in the middle, and sympatry in

the south is correct, but the intergradation in the north may be secondary rather than primary, and sympatry with no hybridization is found in only one of four hybrid zones in the south. While sympatry with no hybridization is found at one site in the extreme south, hybridization is documented for three other *klauberi* isolates (Wake et al., 1986).

The situation on the coast is far from simple. There are two separate haplotype groups associated with distinctive allozymic-based clusters of populations within *xanthoptica*, one on the southern San Francisco Peninsula and the other widely distributed to the north and east of San Francisco Bay, including in the Sierran foothills. There are complex interactions where *oregonensis* (with two haplotype groups in the region) meets *xanthoptica* north and south of San Francisco Bay (Fig. 2; Wake, 1997). Furthermore, *eschsoltzii* is more differentiated (with respect to haplotypes) than would have been anticipated from the early allozyme study, with distinctive northern and southern geographic segments that form only a weakly supported possible clade.

These considerations led me to propose an historical biogeographic hypothesis for the complex, approximately 5 million years before present, which postulates a widely distributed *oregonensis/platensis* precursor in the north, an isolated precursor of northern *platensis* in the northern Sierran region, and a precursor of southern *platensis/croceater/klauberi* in the southern Sierran region (Wake, 1997). The Central Valley of California was at this time an inland extension of the Pacific Ocean, and the precursors of *xanthoptica/eschsoltzii* may have originated on an archipelago (cf. *Batrachoseps* scenario, below). How they got to the archipelago is uncertain, but because the land associated with the Salinian Block originated in the south, Parks (2000) postulated that an early movement of *oregonensis*-like salamanders southward gave rise both to northern *platensis* and to the precursors of *xanthoptica/eschsoltzii*. Subsequent orogenic as well as plate movements led to the assembly of populations of *Ensatina* into the current form of a ring. Whereas *klauberi* has usually been envisioned as well-nested within the complex, this alternative view (shown in Fig. 2, although the branching near the base of the complex is not well supported) is that *xanthoptica* + *eschsoltzii*, which form a distinctive phylogeographic unit, might form a clade that is sister to everything else, even including the diverse populational groups identified as *oregonensis* or *picta*. Some of Stebbins's subspecies are historical units that can be diagnosed by morphology and molecular characters (*klauberi*, *xanthoptica*, *eschsoltzii*); others are either nonmonophyletic amalgams (*platensis*) or incompletely differentiated

but adaptively diverged groups (southern *platensis-croceater*, *picta-oregonensis*). Finally, *oregonensis* is an undiagnosable grouping of differentiated lineages that branched independently (Fig. 2).

Ensatina illustrates a complicated relationship between adaptive divergence (as exemplified by the evolution of aposematic coloration in association with mimicry in the coastal populations, and cryptic coloration in the inner montane populations) and vicariant events (for example, the differentiation of northern and southern *platensis*). While our understanding of the complex has changed greatly since the work of Stebbins (1949), the basic point that this is a case in which adaptive divergence has been dominant still holds. This understanding does not help us to establish a better taxonomy, and with the present data, no alternative seems more appropriate than maintaining what we now have, a polytypic species. Establishing taxonomic species within the complex creates new problems. For example, *platensis* is readily diagnosable on morphological grounds, but not with either DNA (diphyletic) or proteins, and *croceater*, also diagnosable on morphological grounds is nested within southern *platensis* in the DNA tree. I conclude that this is a rare instance in which subspecies are helpful. They reflect what is in essence the shallowest (most recent) time depth, related to current adaptive antipredator strategies. At an intermediate time level are the genetic interchanges associated with population-level mergers around the ring, revealed by allozymes. Finally, the deepest level is reflected in the exclusive distributions of the haplotype groups recognized in Figure 2, all of which have discrete geographic limits and may reflect, in part, allopatric episodes at different times in the past.

NONADAPTIVE RADIATION

The Slender Salamanders, genus *Batrachoseps*, have a geographic range in California that is similar to that of *Ensatina* (Fig. 3), but in other respects they are a sharp contrast to *Ensatina*. Species of *Batrachoseps* occur in syntopy with *Ensatina* at most sites of range overlap, including a vast array of habitats ranging from temperate rain forest in northwestern California, to relatively high elevations (ca. 2000 m) in the forests of the Sierra Nevada, to chaparral and coastal sage scrub along the southern coast. Like *Ensatina*, these are strictly terrestrial salamanders that lay direct-developing eggs on land. They are sedentary, with home ranges that are even smaller than those of *Ensatina*; there is little evidence of dispersal.

A detailed morphological analysis of *Batrachoseps* (Hendrickson, 1954) recorded extensive geographic

variation and concluded that only a single species, *B. attenuatus* (Eschscholtz, 1833), was represented in California. A related northern species, *B. wrightorum* (Bishop, 1937), was found in the northern Cascade Mountains of Oregon. Hendrickson's conclusions were controversial because his two subspecies of *B. attenuatus* occurred in complete sympatry on Santa Cruz Island, off the coast of southern California. This unusual taxonomy resulted from his envisioning a complex biogeographic scenario in which the island was invaded by two somewhat differentiated populations, one from the north and the other from the south, establishing sympatry that formed from a ring-like pattern of differentiation (he was strongly influenced by the work of his adviser, R. C. Stebbins, on *Ensatina*).

Subsequent research revealed many species borders, and currently 20 species of *Batrachoseps* are recognized. Only one, *B. wrightorum*, is not found in California. Analysis of allozymic and mtDNA data sets identifies six major clades, all found in California. Species belonging to different clades often are so similar morphologically that they cannot be discriminated without molecular analysis. The three members of the subgenus *Plethopsis* Bishop, 1937, the sister taxon of subgenus *Batrachoseps*, differ from the latter in some osteological traits, and while most species of subgenus *Batrachoseps* are more slender than those of *Plethopsis*, one (*B. stebbinsi* Brame & Murray, 1968) closely resembles species of *Plethopsis*. At the molecular level, however, *B. stebbinsi* is sharply diverged from *Plethopsis*, differing both in allozymes (more than 10 fixed differences in conservatively evolving proteins) and in mtDNA sequences (Wake et al., 2002).

Along the coast of California clades replace one another geographically (Figs. 3, 4). The *attenuatus* clade in the north is replaced by the *pacificus* clade in the central coastal region, and it in turn is replaced by the *nigriventris* clade to the south, which finally is replaced by another member of the *pacificus* clade in the far south. Ecological transitions between the borders of the group are almost imperceptible, and the species represented are similar in morphology, including coloration, and ecology. In the central coastal region, the *pacificus* clade is represented by four morphologically similar species that once again replace one another from north to south. Furthermore, in southern California a geographical replacement pattern is found within the *pacificus* clade, starting with one species on the northern Channel Islands and a second on the southern Channel Islands and adjacent mainland. The most southerly member of the *pacificus* clade, *B. major* Camp, 1915, has several clearly distinguished haplotype clades within it, and

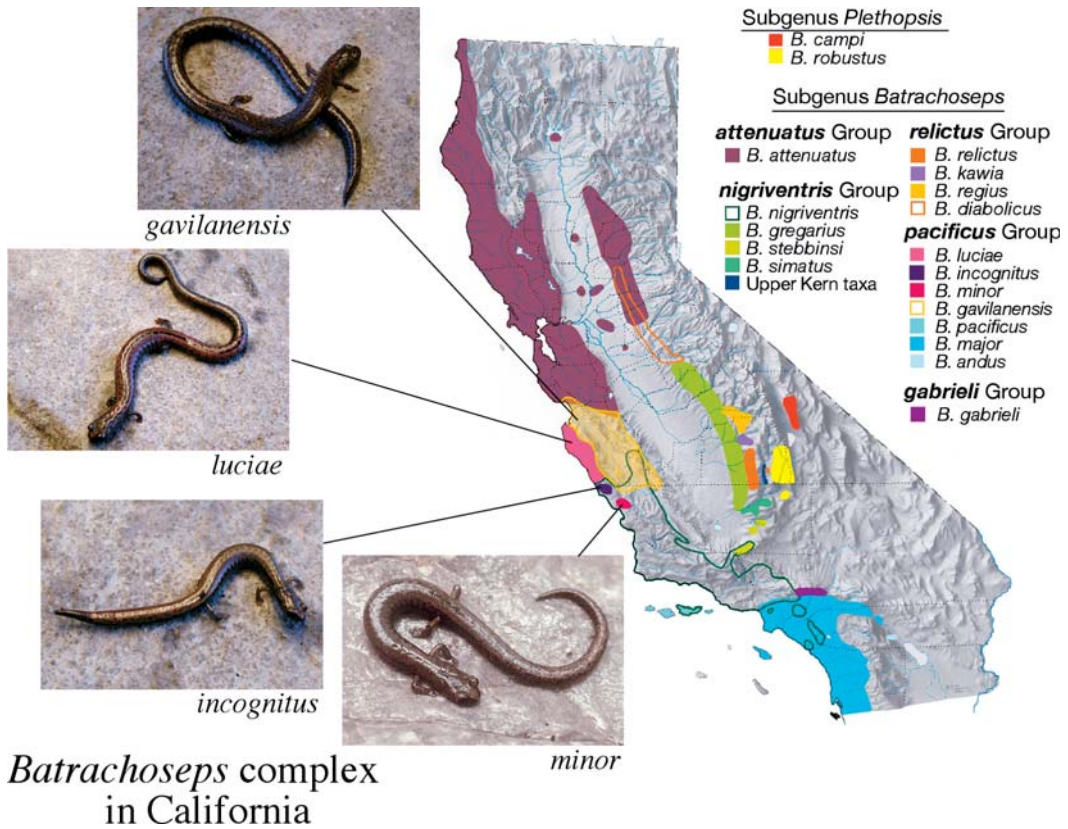


Figure 3. Distribution of the species of *Batrachoseps* in California. The photographs are of specimens of the four species comprising the central coastal cluster of the *pacificus* clade (photos by M. García-París & D. B. Wake). Authorities for species where not provided in the text: *B. campi* Marlow, Brode & Wake, 1979, *B. robustus* Wake, Yanev & Hansen, 2002, *B. kawia* Jockusch, Wake & Yanev, 1998, *B. regius* Jockusch, Wake & Yanev, 1998.

again they replace one another from north to south (Wake & Jockusch, 2000), this pattern apparently continuing into Baja California (unpublished).

These patterns raise questions concerning species delimitation and circumscription. Because *Batrachoseps attenuatus* (*attenuatus* clade) occurs in sympatry with *B. gavilanensis* Jockusch, Yanev & Wake, 2001 (*pacificus* clade) without evidence of interbreeding and with great genetic divergence between them (D as defined in Nei, 1972, ca. 1.6), two species are present, even though they cannot be distinguished in the field. Other instances of sympatry led Brame and Murray (1968) to undertake a progressive revisionary study that was a prelude to subsequent research. Brame and Murray recognized two species in southern California (*B. major*, on the mainland and southern Pacific Islands, and *B. pacificus* Cope, 1865, on the northern Channel Islands), which they separated from *B. attenuatus* on morphological grounds. They also described three species from the southern Sierra Nevada (*B. stebbinsi*, *B. simatus* Brame & Murray, 1968, and *B. relictus* Brame & Murray, 1968). The curious distribu-

tion of *B. relictus* (Sierra Nevada, central Coastal California, Santa Cruz Island, and mountains of Baja California) stimulated Yanev (1978, 1980) to conduct an extensive study of allozymes. She found unexpectedly large divergences and high levels of variation within the taxa she recognized. Yanev found that *B. attenuatus* was restricted to the region north of Monterey Bay. In the central coastal region, populations that Brame and Murray considered to be *B. attenuatus* were assigned to *B. pacificus* (an unnamed semispecies, now *B. gavilanensis*). Populations from more southerly parts of the central coastal region identified as *B. relictus* by Brame and Murray were considered by Yanev to constitute another unnamed semispecies of *B. pacificus* (now three species: *B. luciae* Jockusch, Yanev & Wake, 2001, *B. incognitus* Jockusch, Yanev & Wake, 2001, and *B. minor* Jockusch, Yanev & Wake, 2001). Brame and Murray's *B. major* and *B. pacificus* were also considered semispecies by Yanev and treated taxonomically as subspecies of *B. pacificus*. Yanev had a narrower perspective on *B. relictus* (restricted by Yanev to the

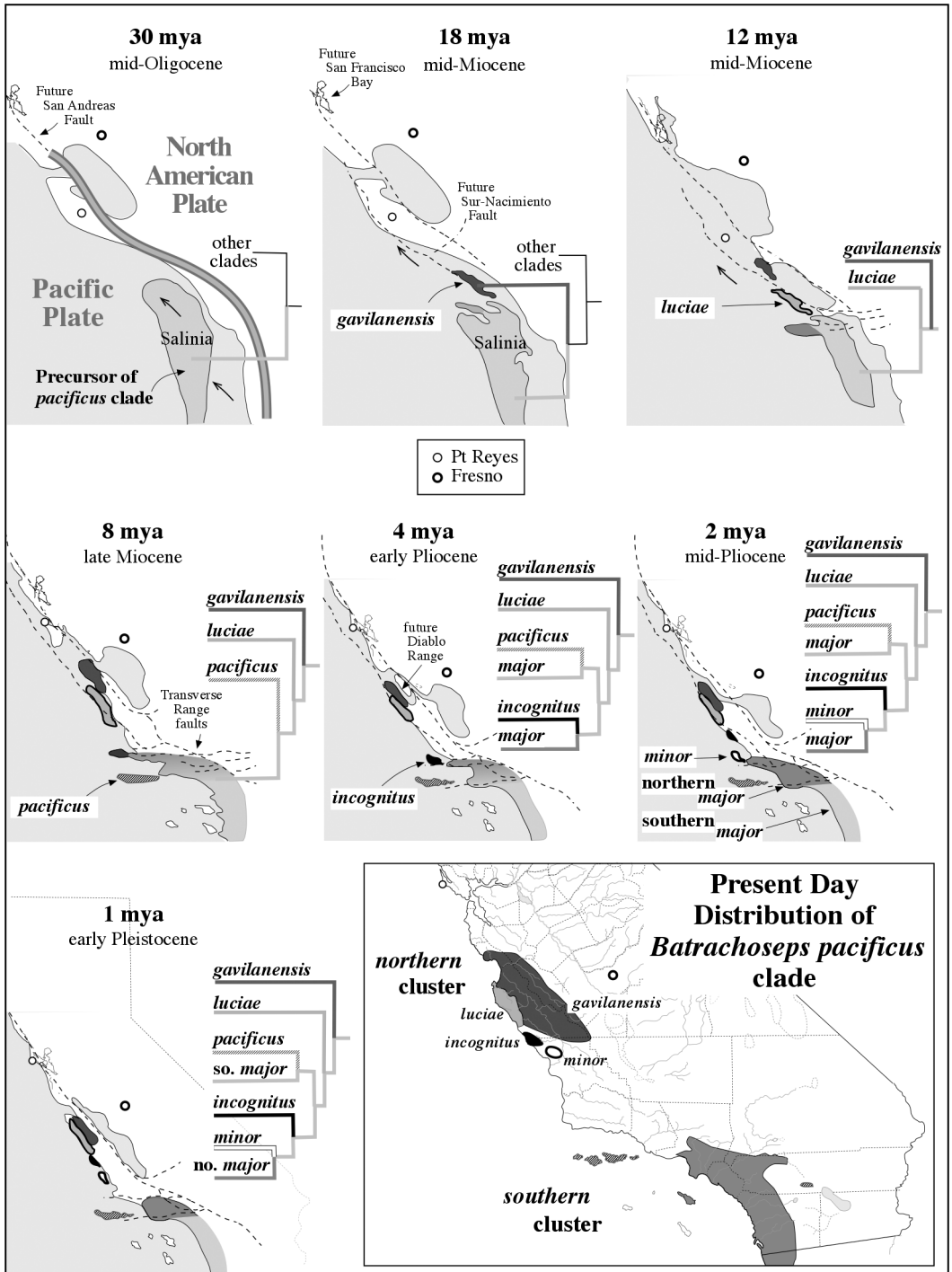


Figure 4. Historical biogeography of the *Batrachoseps pacificus* clade in southern and central California. This scenario is based on geological reconstructions by Hall (2002) and the phylogenetic hypotheses of Jockusch et al. (2002) and Jockusch and Wake (2002).

southern Sierra Nevada), considered to be a semispecies within the *pacificus* complex, and she reduced it, too, to subspecific status. The populations from the mountains of Baja California were considered to represent a final unnamed semispecies of *B. pacificus*. Populations on Santa Cruz Island, assigned to *B. relictus* by Brame and Murray, were assigned by Yanev to a resurrected species, *B. nigriventris* Cope, 1869. In many ways Yanev's recognition of *B. nigriventris* was her most important contribution. This widespread species occurred in sympatry with other species of *Batrachoseps* in central and southern coastal California, on Santa Cruz Island, and in the southern Sierra Nevada. It turned out to be the key in unraveling the complex history of the genus, which is more complicated than even Brame and Murray had conceived. Of special interest was the complex pattern of range overlap with no sign of hybridization or genetic leakage of morphologically cryptic species in the Inner Coast Range in central California, where *B. attenuatus* and the current *B. gavilanensis* were narrowly sympatric in the north, and *B. gavilanensis* and *B. nigriventris* were narrowly sympatric in the south. In coastal central California the geographic range of *B. attenuatus* overlaps that of *B. gavilanensis*, whose range abuts that of *B. luciae*. Parapatry, but as yet with no range overlap, occurs further south between *B. luciae* and *B. incognitus*, and *B. incognitus* and *B. minor* (Figs. 3, 4). However the range of *B. nigriventris* overlaps that of both *B. incognitus* and *B. minor*, with local sympatry, and the northwestern-most range limit of *B. nigriventris* is within a few km of the southern-most range of *B. luciae*.

The addition of mitochondrial gene sequences and the reinterpretation of unpublished data gathered by Yanev (1978), as well as recent discoveries in the field, led to further taxonomic revision (e.g., Marlow et al., 1979; Wake, 1996; Jockusch et al., 1998, 2001; Wake et al., 2003), summarized by Jockusch and Wake (2002). The distribution of species and the inferred pattern of species formation is what Gittenberger (1991) termed nonadaptive radiation, phylogenetic diversification not accompanied by adaptation into distinctively different ecological niches (see also Wiens, 2004, who refers to ecological niche conservatism). The result is a group of allopatric or parapatric species, with the parapatric forms isolated geographically because of inferred competitive interactions at borders of species ranges. The levels of genetic divergence are sufficient to preclude interbreeding or hybridization, and the ecological similarities are sufficient to maintain largely exclusive distributions.

A general feature of the nonadaptive radiation in *Batrachoseps* is the relatively limited sympatry. The

only sympatry between two members of any one of the six major clades is between *B. nigriventris* and *B. stebbinsi*, which co-occur in the Tehachapi Mountains at the southern end of the Central Valley. These species display morphological and ecological divergence, unusual among close relatives in this genus. The smaller and more slender *B. nigriventris* is a habitat generalist, found in woodlands under small to large cover objects and using retreats in the soil. The substantially larger and more robust *B. stebbinsi* is most frequently found in rocky soil and small talus slopes. These two species, which are not sister taxa, may have undergone character displacement, and if so it is unique in the genus.

There are some important exceptions to the general rule in *Batrachoseps* that species either replace one another without hybridizing or are sympatric. In southern California, where population density is low, populations of "northern" and "southern" *B. major* that are morphologically identical have mtDNA (*cyt b*) genes that are about 9% diverged and are not sister (Wake & Jockusch, 2000). In fact, mtDNA of southern *B. major* is more closely related to *B. pacificus*, a more robust species that is morphologically distinct, than to northern *B. major* (Fig. 4). However, northern and southern populations are only slightly differentiated in proteins, and Wake and Jockusch argue that when they meet secondarily and interact genetically, alleles from northern populations spread over southern populations, but the interactions have been insufficient as yet to dislodge the maternally inherited southern mitochondrial genes. This situation is reminiscent of *Ensatina e. platensis* in the central and northern Sierra Nevada (see above).

The apparently nonadaptive radiation of *Batrachoseps* results from the complicated geological history of California (Yanev, 1980; Jockusch et al., 2001). The first split, an ancient one, was between members of subgenus *Plethopsis* and subgenus *Batrachoseps*. Today *Plethopsis* is located peripheral to *Batrachoseps*, to the north and east of the main range. Within subgenus *Batrachoseps* the first split segregated *B. attenuatus* from everything else. The *attenuatus* clade, with a single but differentiated species, is distributed mainly northeast of the San Andreas Fault zone and north of the historically significant embayment of the Central Valley in the vicinity of present-day Monterey Bay. The *relictus* clade, perhaps the next to branch phylogenetically, is restricted to the Sierra Nevada and western adjacent lowlands. It displays a distinct pattern of regional displacement, with substantially more divergence than within the *attenuatus* clade. Four species are recognized, each occupying an exclusive geographic range. The northern species, *B. diabolicus* Jockusch, Wake &

Yanev, 1998, split from the remaining species in the vicinity of the mid-Sierran glacial region, a region in which there are north-south splits in diverse taxa. The remaining clades, *gabrieli*, *nigriventris*, and *pacificus*, are largely southern, with *gabrieli* isolated in the southern transverse ranges, *nigriventris* concentrated in the southern Sierra Nevada, Tehachapi Mountains, and some more southerly and westerly extensions, and *pacificus* located in coastal regions, extending from northern Baja California to just north of Monterey Bay, south and west of the San Andreas Fault zone.

The only clade that crosses the San Andreas Fault zone to any degree of significance is *nigriventris*, and in particular *Batrachoseps nigriventris*, a species that has violated the range exclusiveness so characteristic of the genus to establish populations sympatric with members of the *pacificus* clade along the central and southern California coast and on the Channel Islands. This taxon displays greater ecological breadth than any other member of the genus and likely represents an extensive and relatively recent range expansion from its origin, inferred to be near the southern end of the range of its sister taxon, *B. gregarius* Jockusch, Wake & Yanev, 1998, in the vicinity of the Kern River, where so much of the evolution of the *nigriventris* and *relictus* clades has been centered.

The *pacificus* group has been more affected by the massive land movements that have taken place west and south of the San Andreas Fault zone than any of the other *Batrachoseps*. The scenario developed by Jockusch et al. (2001), based on their mtDNA phylogeny, envisioned an origin of the lineage in southern California. A new geological reconstruction of coastal California (Hall, 2002) has enabled a further development of the scenario (Fig. 4). The first split separated the *pacificus* clade from a *nigriventris* or *gabrieli* ancestral sister taxon, with the *pacificus* clade associated with the Pacific geological plate in the paleoterrane known to geologists as Salinia. This may have occurred as long ago as about 30 million years. Since Oligocene, extensive land movements have transported pieces of the continental crust associated with Salinia more than 160 km to the northwest. The first split within the *pacificus* clade was associated with early stages of the northwestward movement and fragmentation of Salinia. Today the northernmost member of the *pacificus* clade, *B. gabilanensis*, is the only species to have penetrated territory north of the ancient Central Valley embayment, in the vicinity of present-day Monterey Bay. Its penetration northward, probably a relatively recent event, has established a zone of sympatry with *B. attenuatus*. As one would expect for a relatively old species, *B. gabilanensis* shows the greatest degree of protein

and mtDNA diversity within the northern cluster of extant species in the *pacificus* clade.

The next species to split (Fig. 4) was present-day *Batrachoseps luciae*, which now occurs on a plate fragment (Jockusch et al., 2001). After the first two splits our phylogeny is less robust. What is clear is that each of the central coastal species is sister not to its nearest geographic neighbor but to some complex of populations to the south. Thus, *B. pacificus* appears to have been the next to move north, and out to sea, on the precursor terrane that gave rise to the northern Channel Islands, becoming isolated from populations on the mainland. Progressively, *B. incognitus* and *B. minor* were transported northward, leaving a complicated *B. major* in the south that is made up of several distinctive phylogeographic units, essentially left over from episodes of isolation. The northern phylogeographic unit within *B. major* either has or is in the process of extending its range south and east, where it appears to be merging genetically with populations characterized by non-sister mtDNA haplotype clades, morphology, or both. For example the problematic *B. aridus* Brame, 1970, variously recognized as a subspecies of *B. major* or as a full species, is closely related to a southern phylogeographic unit of *B. major*.

The *pacificus* clade displays at a fine scale what I consider to be a long-sustained pattern of nonadaptive radiation in *Batrachoseps*. An ancient lineage fragmented again and again, with a resulting patchwork-quilt pattern of lineage segments, i.e., species, that generally show "tight stitching," close parapatry with no hybridization. Some restricted sympatry occurs between members of different major clades, which in the cases of *B. nigriventris* and *B. gregarius* is more widespread and involves more than one other taxon.

Why the nonadaptive radiation in *Batrachoseps* resulted in a patchwork-quilt pattern of distribution requires closer examination of the history of the central coastal region. The four species of the *pacificus* clade in this region occupy closely abutting geographic ranges, and they are very similar in morphology and ecology (Figs. 3, 4). Two of these, *B. gabilanensis* and *B. luciae*, occupy relatively large areas and have sufficient samples to disclose significant geographic and genetic variation. These two species show extensive geographic variation in both allozymes and mtDNA haplotypes. In fact, the differences within each approach the level of difference between them (Jockusch et al., 2001). Yet, in the border zone where the ranges of the two species abut, no syntopy or genetic interchange is detected. Genetic distances across this border remain high, with some fixed allozymic differences and complete segregation of mtDNA haplotypes, but no ecological differentiation is observed.

The patchwork pattern, which is general in the genus, likely arises from several factors. First, these salamanders are extremely sedentary, with limited dispersal. Second, today's species represent lineage fragments of former species, which themselves were geographically differentiated. Thus species are "born" with geographic variation. Third, species, especially close relatives within a clade, replace one another geographically because they are so similar ecologically that there is what may be called preemptive occupancy of space. That is, the space-holders have relatively large and well-established populations that resist invasion by others that are virtually identical ecologically. Wiens (2004) has discussed such niche conservatism and considers it to be a major factor in the formation of similar species under allopatric conditions. Areas where populations merge with others having non-sister haplotypes typically are ecologically marginal, with populations scattered and small. Under such circumstances, populations may have very low densities, and individual organisms that meet may have few mating opportunities other than with heterospecific individuals.

Sympatry between species is found in two contrasting situations. The first is where there is some ecological divergence, often related to differentiation in the body sizes of the two species. Examples include *Batrachoseps major* with *B. nigriventris*, *B. nigriventris* with *B. stebbinsi*, and *B. nigriventris* with *B. gabrieli* Wake, 1996. The second is in areas where the ranges of species become discontinuous and where populations are highly localized. This is the situation in the generally inhospitable Inner Coast Range, a largely treeless area with exceedingly hot and dry summers and little winter rain. Here broad regional ranges overlap, but little or no local syntopy is found, as in the cases of *B. attenuatus* and *B. gavilanensis* and *B. gavilanensis* and *B. nigriventris*. This is the same ecological situation in which genetic mergers may take place. One can only speculate that the interactors that fail to merge have been separated sufficiently long (as estimated from allozymic genetic distances and degree of haplotype divergence) that genetic isolating mechanisms have arisen incidental to diverse genetic processes during the long period of geographic isolation.

The main feature of a nonadaptive radiation, then, is the general impression of allopatry or parapatry with a lack of ecological or any other kind of divergence other than in molecular traits. In situations like this, where there is little or no hybridization, it may be fruitful to investigate the evolution of isolating mechanisms, which might have arisen incidentally in allopatry but serve now to cause individuals from adjacent populations not to recognize each other as

potential mates. In the case of salamanders, this is most likely to involve mating pheromones (Houck & Arnold, 2003). Postulated pre-mating isolating mechanisms might be incidental, a byproduct of the different genetic histories of lineages since time of divergence. If isolating mechanisms did arise at the borders of two incipient species, it is very difficult to imagine a scenario in which they would spread "backwards" throughout the far-flung populations of each species, which are so genetically heterogeneous. In such situations the evolution of isolating mechanisms seems unlikely to be related directly to the formation of species.

GENERAL DISCUSSION

The contrast between *Batrachoseps* and *Ensatina* is great. Where morphologically and ecologically similar populations of *Ensatina* meet they exchange genes, whereas in *Batrachoseps* they do not. Why does this stark contrast exist? The two genera belong to the same major clade (Plethodontidae), have similar life histories and ecologies, and species of the two are often sympatric. Both are fully terrestrial. However, *Ensatina* is a larger salamander, with better locomotory ability and larger home ranges (compare Stebbins, 1954, and Staub et al., 1995, with Cunningham, 1960, and Hendrickson, 1954). Both genera are phylogenetically isolated, having no close relatives, and likely represent very old lineages (perhaps on the order of 60 million years or more, based on estimates from rates of albumin evolution and degree of divergence in mtDNA and other data, e.g., Larson et al., 1981, 2003; Chippindale et al., 2004; Mueller, 2005; Mueller et al., 2004). *Batrachoseps* is the more internally diverged and differentiated, and is likely the older. Its lower vagility, fidelity to pieces of the planetary crust, and inferred great age may be responsible for the fragmentation of the primordial lineage and what has been mainly a nonadaptive radiation. The processes involved in differentiation of *Batrachoseps* appear to be largely related to factors associated with earth history, especially crustal movements, as first proposed by Yanev (1980). In contrast, *Ensatina* appears to be responsive to challenges from its biological community and has evolved several different antipredator mechanisms, which have concomitant behavioral and ecological consequences. The result is a differentiated, but incompletely fragmented and very widespread, meta-population structure.

Both examples have strong geographic components to their histories. Most species formation in terrestrial vertebrates appears to be geographic in nature, as first generalized by Mayr (1942). Geographic variation

within living terrestrial vertebrate species (as well as many other taxa) in natural populations is nearly ubiquitous (e.g., Avise, 2000). In the taxa I study, there is so much divergence within species that only adaptively significant alleles or allele complexes are likely to spread broadly and rapidly. Accordingly, *Ensatina* has experienced spread of adaptively significant traits, and this has kept the lineage from permanent fragmentation. However, in *Batrachoseps*, where no clearly adaptive traits beyond those general to most species have been identified, divergence and lineage fragmentation has proceeded. To focus attention on the evolution of isolating mechanisms in situations like this is problematic. When long-separated populations that have diverged significantly come into secondary contact they may be sufficiently distinct that they no longer recognize each other as potential mates (e.g., Kozak, 2003). I suspect that this has happened in *Batrachoseps*, where one never finds hybrids, but instances of haplotype parphyly and polyphyly are found, suggesting that secondary contact was followed by genetic merger (cf. Funk & Omland, 2003). Alternatively, offspring produced in such contacts may be adaptively inferior. This is the case in the adaptively different *Ensatina* in the central Sierran and southern California hybrid zones, which are only a few home-range diameters wide. One might study isolating mechanisms in these instances, but genetic isolation likely evolved incidentally during the allopatric divergences, not upon recontact. Perhaps hybrid zone or alloparapatric interactions (Alexandrino et al., 2005) are settings in which the potential reinforcement of isolating mechanisms might be studied (Servedio & Noor, 2003). Geography is the common theme, whether species formation has progressed adaptively or has been incidental to events related to isolating mechanisms.

At what level of lineage segmentation do we recognize and name species? Recently de Queiroz (2005) pointed out that while the process of evolutionary divergence leads to the acquisition of different properties by diverging lineages, taxonomists differ with respect to which particular property they emphasize. He argues that the adoption of different thresholds is the main cause of the "species problem." I have attempted to use the same thresholds for the two examples presented here, but have reached very different conclusions. De Queiroz argues that "all separately evolving metapopulation lineages would be species" regardless of particular attributes, but in the case of *Ensatina*, for example, the issues of separateness and metapopulation limits remain unclear. The likelihood that once separate lineage segments are now genetically merged, or have in the recent past undergone secondary genetic merger, is high. In contrast, in *Batrachoseps* the same issues are more generally resolved.

Biologists will continue to have problems with species. We may all share the same conceptual framework, but it is safe to predict that arguments concerning thresholds and attributes will continue to produce controversy. However, regardless of taxonomic treatment, careful analysis of patterns and processes associated with species formation will inform meaningful discussion and debate.

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