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TAXONOMY OF THE PLETHODONTID SALAMANDER GENUS *ENSATINA*

DAVID B. WAKE[1] AND CHRISTOPHER J. SCHNEIDER[2]

[1]*Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720-3160, USA*
[2]*Department of Zoology, University of Queensland, Brisbane, Queensland, 4072, Australia*

ABSTRACT: Highton (1998) argued that published data warrant a taxonomic revision of the *Ensatina* complex. The complex comprises units that have varying degrees of phenetic and phylogenetic differentiation, but morphological/coloration, protein, and mtDNA data sets are less concordant than Highton believed. We employ different criteria to discover species than did Highton. His proposed species do not fulfill our criteria. Several are not diagnosable, nor do they have identity as evolutionarily independent lineages or as genetically cohesive units. Furthermore, he misinterpreted Stebbins' (1949) conception of a ring species, which was an evolutionary and biogeographic hypothesis. As observed as long ago as Stebbins' original work, taxonomic resolution of the complex is neither simple nor will a changed taxonomy solve the biological problems identified. The biological complexity of *Ensatina* argues against a simple taxonomic resolution, because the evolutionary realities of diversification in old and persistent complexes require compromises if Linnean taxonomies are to be used. We prefer a taxonomy that clarifies the evolutionary relationships among the components and that highlights, rather than obscures, the complex interactions of the past and present. Accordingly, while we recognize that a new taxonomy may be required when studies in progress are concluded, for the present we recommend continued recognition of the *Ensatina* complex as a single polytypic taxonomic species.

Key words: *Ensatina eschscholtzii*; Plethodontid salamanders; Species concepts; Taxonomy; Ring species; Biogeography; Phylogenetics

BASED on the now classical work of Stebbins (1949), *Ensatina* is currently recognized as being a polytypic species or species complex, with seven named taxa, typically treated as subspecies (Frost, 1985; Stebbins, 1985). These taxa show varying degrees of morphological differentiation, and the different morphs generally occupy geographically contiguous areas around the Central Valley of California. Since 1949, an ever increasing variety of biological interactions has been recorded as taking place between parapatric and sympatric groups involved in secondary contacts (Brown, 1974; Jackman and Wake, 1984; Wake, 1997; Wake et al., 1989). Limited gene exchange between genetically and morphologically differentiated populations in some contact zones has led some authors to suggest that the taxonomy of the *Ensatina* complex should be revised to recognize additional species (Frost and Hillis, 1990). Highton (1998) examined published data and suggested recognizing multiple species based on the clustering of populations into groups separated by genetic distances that he considers typical of species in related genera and vertebrates generally. Current taxonomic conventions are inadequate to convey the complexity of relationships and interactions within the complex, and Highton's attempt suffers from these and other considerations, magnified by his lack of knowledge of unpublished data available to us. Intensive field and laboratory studies of the complex are underway, and we feel that the current taxonomy of the complex is best left undisturbed until current studies (e.g., Wake, 1997) are completed. A new taxonomy eventually may be in order, and a paper from this laboratory (Graybeal, 1995) suggested one possible arrangement. It is our view that a revised taxonomy should be consistent with the evolutionary relationships within the complex and should reflect the complexity of relationships and interactions. We do not have sufficient understanding of these relationships to warrant changing taxonomy at this time. Here we examine some of the criticisms that have been made of the ring

species concept as applied to *Ensatina*, comment on Highton's (1998) proposed taxonomy, and make recommendations based on unpublished results and work in progress. For purposes of communication, we refer to Highton's groups (his putative species) by the Roman numerals that he used (Highton, 1998). Our most general response to Highton's re-evaluation is that interactions within the complex at different levels, from local populations to nominal subspecies, are complex and are not made more understandable by his taxonomic proposals. In our opinion, the methods espoused by Highton either obscure or ignore some of the complexity that makes the *Ensatina* complex so challenging, and accordingly they do not represent satisfactory solutions to perceived problems. Furthermore, Highton largely ignored the phylogenetic analyses that were crucial to our interpretation of the evolutionary history of the *Ensatina* complex.

Ring Species and Species Complexes

The perfect demonstration of a ring species would be a continuous circular sequence of differentiating populations that meet and overlap without mating at one point in the ring (Mayr, 1942:180). No example that we know meets this requirement, although it remains an ideal against which claims of ring species are measured. Stebbins (1949) realized that *Ensatina* did not meet this standard, for the populations were not continuously distributed, but what impressed him was the intergradation that occurred where geographically adjacent subspecies met and the general impression of a ring-like distribution, with overlap among the evidently most derived (i.e., dissimilar) members. Stebbins professed doubts concerning the appropriate taxonomy for the complex, but in the framework of the then prevalent Biological Species Concept he was influenced by evidence of intergradation and hybridization at various points in the range of the complex and thus opted for a polytypic species. As we understand it, and we thank R. Stebbins for extensive discussion on this point, Stebbins' conception of *Ensatina* as a ring species involved both evolutionary and historical biogeographical hypotheses. He viewed color variation in the complex as a stepped cline, with extensive regions showing morphological stability joined by broad areas of intergradation of traits around the ring. Important to the ring species interpretation, Stebbins believed that differentiation in *Ensatina* had occurred as the species expanded southward from the northern part of its range along two main axes defined by mesic habitats along the coast and interior mountains. The adaptive divergence of the populations as they spread southward led to highly differentiated populations coming into secondary contact with little or no hybridization despite apparent continuity across the top of the ring. In contrast to these relatively dramatic secondary contacts, interactions between adjacent forms along the axes were characterized by intergradation of characters, some changing relatively abruptly and others gradually. This biogeographical and evolutionary interpretation of *Ensatina* as a ring species forms the basis for the continued used of the term. With the advantage of years of additional study and large quantities of genetic data, we remain impressed with the degree to which Stebbins' main biogeographic hypothesis has resisted refutation. For example, phylogenetic treatments of mtDNA cytochrome *b* gene sequences and allozymic data give general support to the hypothesis (contra Highton, 1998), especially to Stebbins' idea that the general pattern of movement had been from north to south (Jackman and Wake, 1994, Moritz et al., 1992).

We have repeatedly referred to *Ensatina* as a "complex" (Jackman and Wake, 1994, Moritz et al., 1992, Wake, 1992, 1997; Wake et al., 1989). That requires further study, and have stated "what we are seeking, before disrupting the current long-stable taxonomy, is some evidence of monophyly for the separate segments of the ring and of a hierarchical structure within the complex" (Wake, 1992:172). That is, we are trying to recover the evolutionary history of this complex; once we have reached a satisfactory level of understanding, taxonomic revision may be desir-

able if we can find an alternative that better reflects this history and the hierarchical phylogenetic structure among components of the complex than is achieved by the present taxonomy. While some may find the present taxonomy unsuitable, our dilemma is that we find any alternative to be more unsuitable. The complex is a geographically and genetically differentiated monophyletic group, and the current taxonomy recognizes that fact. The subspecific taxa vary greatly in degree of diagnosability and in the degree to which they are differentiated, morphologically and genetically. Subspecific designation nonetheless serves the useful purpose of tagging clusters of populations that at least share some distinctive color patterns and geographic distributions. Certainly by now (starting with Wake and Yanev, 1986) the scientific community knows that the evolutionary history of *Ensatina* is complicated and taxonomically challenging, and when this exchange is published, even field guides will be constrained to take notice of the controversy.

While *Ensatina* is unique among salamanders in the apparent ring-like nature of its distribution, it is not unique in being genetically, phylogenetically, and evolutionarily complex. Herpetologists are aware of other complexes of taxa at and around the species level that have proven to be taxonomically difficult. The *tigrinum* complex of *Ambystoma* is made up of taxa of varied taxonomic status (Shaffer and McKnight, 1996); as a whole, this complex is less differentiated genetically than *Ensatina*. The diverse taxa presently recognized as members of the *Salamandra salamandra* complex are another assemblage with great differentiation, including alternative reproductive modes, that challenges any simple taxonomic resolution (Alcobendas et al., 1996). The *ochrophaeus* and *fuscus* complexes of *Desmognathus* are other examples of complicated systems that challenge conventional taxonomic resolution (Tilley, 1997; Tilley and Mahoney, 1996; Titus and Larson, 1996). Highton (1989, 1995) proposed a dramatic new taxonomy for the *glutinosus* group of *Plethodon*, and he has shown that many of the new taxa have complicated interactions with neighboring taxa; at least one of the species in this complex is thought to be of hybrid origin. These and other complexes (e.g., *Batrachoseps*, currently under revision by workers in Wake's laboratory) challenge Linnean taxonomy, and we predict that any taxonomic resolution will prove to be controversial and unstable. We believe that some of these complexes, *Ensatina* among them, are best labeled as complexes so that workers will be alerted to controversies and uncertainties.

Species Concepts and Criteria

Highton (1998) advocated the biological species concept (Highton, 1990) but also took some notice of other perspectives. Based on his experience with eastern *Plethodon*, in the absence of sympatry, he believed that a genetic distance of approximately 0.15 marks species boundaries. That is, "this amount of genetic divergence represented the only level that unified geographically contiguous morphologically and genetically similar groups" (Highton, 1990:118; see also Highton, 1998). He cited evidence from workers on other groups that this approximate level corresponds to species borders as currently recognized. Why this should be the case is unclear; Highton believed that genetic distance is primarily, if not exclusively, a product of time since divergence of populations being compared. High genetic distances are not found routinely within otherwise uncontested species, and in general we agree that most species are expected to show relative genetic uniformity. However, we believe that genetic distances averaged over large numbers of populations that have complicated patterns of interconnection poorly reveal evolutionary history. More important for us than the quantification of genetic differentiation is the particular genetic changes that have occurred and how these influence interactions between geographically adjacent populations. Highton's contention that genetic distances within species must be below a certain level is questionable because this result depends entirely on how groups of populations are identified initially. His contention that low

mtDNA variation is necessarily characteristic of species is equally misleading (see below). To some degree, therefore, the *Ensatina* controversy centers on the issue of whether this complex is a special case that shows how reinitiation of genetic interaction following isolation and divergence can play a role in inhibiting completion of species formation, as we contend.

Ensatina as a lineage is old (Larson et al., 1981; Maxson et al., 1979), and we have long known it to be composed of a complicated group of populations, many of which are well differentiated genetically (Wake and Yanev, 1986). While the degree of allozymic differentiation within the complex is relatively great, we are not convinced that an average level of genetic differentiation should be accepted as indicative of "species-level" genetic differentiation. Nor do we find justification for a "threshold" level (approximate Nei $D < 0.15$) of divergence, above which species formation is indicated. There are many examples of allozymic differentiation in excess of that cited by Highton as indicative of species-level differentiation found in taxa currently recognized as species, both within Caudata (*Bolitoglossa meliana* and *B. franklini*: Wake and Lynch, 1982; *Thorius macdougalli*, *T. arboreus*, and *T. boreas*: Hanken and Wake, 1994; *Bolitoglossa macrinii*: Papenfuss et al., 1983; *Pseudoeurycea leprosa*: Lynch et al., 1983; *Hydromantes shastae*: Wake et al., 1978; *Cynops pyrrhogaster*: Hayashi and Matsui, 1988; *Salamandra salamandra*: Alcobendas et al., 1996; *Triturus italicus*: Ragghianti and Wake, 1986; *Rhyacotriton cascadae*, *R. kezeri*, and *R. variegatus*: Good and Wake, 1992; *Plethodon kentucki*: Highton and MacGregor, 1983; *Aneides flavipunctatus*: Larson, 1980; *Desmognathus carolinensis*, *D. ocoee*, and *D. orestes*: Tilley, 1997, Tilley and Mahoney, 1996) as well as within other vertebrate taxa (e.g., *Bufo japonicus*: Kawamura et al., 1990; *Hyla regilla*: Case et al., 1975; *Rana tagoi*: Nishioka et al., 1987; *Rana limnocharis*: Nishioka and Sumida, 1990; *Rana brevipoda*: Nishioka et al., 1992; *Rana nigromaculata*: Nishioka et al., 1992; *Rana rugosa*: Nishioka et al., 1993; *Rana japonica*: Sumida and Nishioka, 1994; *Thomomys bottae*: Patton and Smith, 1990). Note that published studies of 24 of the species listed post-date the summary of Thorpe (1982), cited by Highton in support of his favored level of *D* for species designation, and that most of these involve relatively comprehensive studies with large samples of many populations, in contrast to many of those cited by Thorpe. Some of these taxa may justifiably be broken into more finely defined species in the future, but we suspect that adoption of Highton's criteria and methods would lead to a taxonomy that would treat some nonindependent groups of populations as separate species. It would, of course, lead to dramatic changes in the taxonomy of salamanders and frogs around the world.

The controversy concerning taxonomy of the *Ensatina* complex is grounded not so much in different species concepts as in the application of criteria that translate concept into taxonomy. Most workers are interested in recognizing discrete and "permanent" pieces of an evolutionary continuum (de Queiroz, 1997). At issue is what constitutes evidence of permanence and individuality. We have an evolutionary species concept that is close to that of Frost and Hillis (1990), but we recognize that others who share this concept might recognize different numbers of species in the *Ensatina* complex, from one to many. In recognizing species, we aim to identify genetically cohesive groups of populations that are evolutionarily independent, as gauged by several different criteria, such as continuity of patterns of genic exchange (e.g., de Queiroz and Good, 1997; Good and Wake, 1992, 1993; Tilley and Mahoney, 1996), and degree of admixture following recontact of groups of populations previously isolated by geographic barriers. In most cases, biological and evolutionary species will be concordant. In this respect, we endorse Ghiselin's definition: "Biological species are populations within which there is, but between which there is not, sufficient cohesive capacity to preclude indefinite divergence" (Ghiselin, 1997:99). We accept that with allopatric and parapatric groups of populations, species status

is necessarily uncertain (covered in depth for general cases by Avise, 1994; Avise and Wollenberg, 1997, Endler, 1977). Thus we should not expect an orderly traditional taxonomy for groups with complicated patterns of differentiation and interaction, such as occur in the *Ensatina* complex.

A fundamental difference between Highton and us is the discovery process used to initially identify species taxa. As we understand his approach, Highton clusters populations on the basis of genetic similarity, seeking discontinuities in the general vicinity of $D = 0.15$, which he considers to be an empirically validated level of interspecific differentiation. We characterize this approach as phenetic, in that overall genetic similarity and amount of difference from other presumptive taxa are the criteria for species membership. To then reach a taxonomic conclusion following this discovery process, Highton determines if population clusters are geographically contiguous, if they can be separated from neighboring clusters (often calling intermediate populations "hybrids"), and whether there is sympatry with representatives of other genetically defined clusters. In contrast, we first seek to identify groups of populations that show evidence of an independent evolutionary history using a variety of criteria (i.e., genetic distances, number of fixed allozyme differences, mtDNA sequence, morphological and ecological differences). An expectation exists that within such candidate species there will be evidence of recent or past gene flow among populations, and that populations will be geographically cohesive. We then examine interactions between groups of populations to determine the integrity of the groups and their relative permanence (i.e., the likelihood that they will retain their integrity in the face of ongoing genetic exchange). Finally, we establish the diagnosability of the candidate species, expecting diagnosability to emerge if the criteria that we use to identify evolutionarily independent groups have been met. Species should be diagnosed by discrete characters, not by such elusive and changeable features as relative gene frequencies.

The contrasting results of these two different approaches is seen most vividly with respect to our treatments of populations that Stebbins (1949) assigned to the subspecies *platensis*. Highton (1998) found two phenetic (based on allozymic data) clusters of populations, with one population being intermediate between them. Accordingly, he treated the intermediate population as a hybrid between two distinct species taxa. In contrast, we find neither of his putative species to be evolutionarily independent by several criteria, neither is diagnosable by discrete characters, and we dispute designation of the population in question as a hybrid. This particular case is crucial to understanding our divergent perspectives on *Ensatina*, for if one accepts Highton's approach, then his proposed taxonomy largely makes sense, whereas if one accepts our approach and interpretation, there are no diagnosable historical units within the complex that warrant recognition as species taxa, although some come close (see below).

In our opinion, Highton's focus on genetic distance between groups of populations was extreme. Genetic distances are useful, but mainly in providing information about genetic structuring of species and species complexes with respect to geography, and perhaps to provide perspective on relative time of separation of populations or groups of populations. Highton's emphasis on genetic distance among population clusters suggests that he has adopted an approximation of what might be termed a metronomic species concept, so that time alone, with accumulation of largely random genetic changes, is sufficient to produce species differences that are predictable at a certain knowable level. In contrast, of greater concern to us than mean genetic distances among groups of populations are the evolutionary relationships among populations that can be inferred from molecular data, and the interactions that take place at the borders of genetically cohesive groups. It is these interactions that give evidence of degree of evolutionary independence.

Subspecies and Intergradation

Stebbins (1949) recognized a single polytypic species within *Ensatina*, comprised of seven subspecies, with broad zones of intergradation. These taxa, while recognizable by coloration, are heterogeneous genetically. While *eschscholtzii* displays some degree of genetic cohesion, there is progressively less in *klauberi* and *xanthoptica*. Neither *picta* nor *croceater* is diagnosable allozymically, and we believe that the apparent distinctiveness of these taxa based on mtDNA sequences is largely sampling artifact. Both *platensis* and *oregonensis* are deeply differentiated internally, with respect to both allozymes and mtDNA. We believe that the former is composed of once separated components that have rejoined. Of these named taxa, *oregonensis* is unique in apparently being a basal (ancestral) group, retaining a generalized color pattern recognized mainly by default and having deeply divergent mtDNA sequences. However, there appears to be allozymic continuity throughout the taxon (extending seamlessly into *picta*). These issues are considered in detail below. While we continue to use this familiar taxonomy, ongoing genetic analyses have given greater definition to the limits of distribution and to zones of intergradation between some of the nominal subspecies. We have therefore assigned populations from areas that Stebbins identified as intergrade zones to subspecies consistent with our genetic analyses. Accordingly, our intergrade and admixture zones typically are far smaller than presented in Stebbins (1949), and some such zones (as in the central Sierra Nevada, see below) were not known to Stebbins.

Cases in point are the allozymic border between the subspecies *platensis* and *oregonensis*, which has been narrowed to a zone of a few kilometers north and west of Lassen Peak, and between *platensis* and *croceater* to the Kern River Canyon (Jackman and Wake, 1994). Boundaries have recently been clarified for the interactions between *oregonensis* and *xanthoptica* north and south of San Francisco Bay and between *xanthoptica* and *eschscholtzii* in the vicinity of Monterey Bay (Wake, 1997). Extensive unpublished studies (Wake and associates) have failed to find allozymic or mtDNA sequence evidence of differentiation that corresponds to a boundary between *picta* and *oregonensis*. Stebbins (1949:434) acknowledged difficulty in assigning individuals to one or the other of these subspecies, or as intergrades. We recognize Stebbins' analysis of color variation as an independent analysis of complex and undefined genetic factors associated with color variation, and it is an important part of the overall analysis of variation in this genus, especially because most of the color patterns have clear geographic delineation. We do not demand complete concordance between DNA sequences, allozymic variants, and color pattern, but we would expect more than is evident if putative species are permanent, independent lineages.

Secondary Contacts and Hybrid Zones

Highton's concept of a hybrid zone (Highton, 1989, 1995) is different from ours. When previously separated populations rejoin, there is a continuum of possible interactions ranging from reestablishment of gene flow leading to panmixia, to no gene flow leading eventually to sympatry. Between these extremes are an array of possibilities, from persistence of some degree of genetic distinctiveness, despite genetic introgression, to hybridization, to parapatry. For us, hybridization should involve the possibility of different kinds of organisms (i.e., organisms drawn from distinct gene pools) meeting, mating, and producing offspring. We believe that this is the general view that biologists have of hybridization (e.g., Harrison, 1993:5). The hybrid zones between blotched and unblotched forms of the *Ensatina* complex conform to this conception. However, the zones of intergradation of genetic traits within the blotched and unblotched forms that Highton (1998) characterized as hybrid zones do not. In fact, some of his hybrid zones are extraordinarily broad. Under any circumstances, it is most appropriate to measure the width of hybrid

zones in terms of dispersal distances of organisms rather than in terms of absolute measurements such as kilometers. Barton and Hewitt (1989) emphasized the spatial and temporal nature of hybrid zones. They surveyed >170 hybrid zones and found that most are narrow relative to the range of the species, usually <50 times the standard deviation of the geographic distance between parents and offspring. For *Ensatina*, such distance is likely to be on the scale of tens of meters or less, rather than hundreds or thousands of meters; Staub et al. (1995) found that mean cumulative movements of adults in a marked population studied over a period of nearly 2000 days averaged 31.2 m in males and 23.3 m in females.

Many of what Highton interprets as hybrid zones in *Ensatina* (and in some *Plethodon*: Highton, 1995) are viewed by us as zones of admixture of populations following secondary contact. When the interacting populations breed more or less freely, we use the term "admixture" to denote the process (e.g., Bowcock et al., 1991). Highton's approach implicitly assumes that zones of secondary contact between populations separated by D_N on the order of 0.15 or greater become reinforced in time and lead inexorably to species formation (although Highton did cite one instance in *Plethodon* of an entire species of possibly hybrid origin: Highton, 1989, 1995). We have published evidence that populations in secondary contact (representing contact between some of Highton's putative species) have lower genetic distances than those more distant from the contact zone (Jackman and Wake, 1994; Wake, 1997), which is what would be expected if genetic interactions have been renewed between groups of populations that have been temporarily separated.

There is diversity among herpetologists as to how hybrid zones are recognized and interpreted. While we adhere to the designation by Barton and Hewitt (1989) that envisions hybrid zones as relatively narrow, Highton (1995, 1998) obviously did not, although what criteria he did use remain unclear. Shaffer and McKnight (1996) noted that there is a hybrid zone from 50–100 km wide in the *Ambystoma tigrinum* complex. However, Routman (1993) showed that within this zone hybridization is an apparently localized phenomenon. Shaffer and McKnight recognized the two interacting units as species, arguing (as did Routman) that away from the zone the units maintained their historical integrity and independence; however, Shaffer and McKnight clearly stated that they supported the phylogenetic species concept of Cracraft (1989), whereas neither we nor Highton (1998) support that concept. If we did use criteria such as those used by Shaffer and McKnight and endorsed by Cracraft, many more species in the *Ensatina* complex than those recommended by Highton would have to be recognized (see discussion below and information in Wake, 1997).

The term intergradation was used by Stebbins (1949) to refer to the gradual change in mean distribution of traits from one population to the next near the borders of subspecies. Mayr (1942) referred to the practical difficulties in taxonomic studies of geographic variation and the fact that frequently traits varied independently. Subspecies ranges were demarcated by concordance of traits, and where this concordance failed an intergrade zone was recognized. Stebbins mapped very large geographic areas as such intergrade zones, based mainly on his analysis of color variation. In our genetic work, we have discovered some genetic borders within intergrade zones. In some instances (e.g., Wake, 1997) these borders are sufficiently distinct that we have been able to identify genes that have introgressed across these borders. This introgression results from the interbreeding of members of genetically distinct populations with the result that alleles of one penetrate into the geographic range of the other. We have documented hybridization with virtually no introgression between distinct color morphs of the *Ensatina* complex in the Sierra Nevada and southern California (Wake et al., 1989), introgression between genetically distinct groups of populations within one of Stebbins' (1949) zones of intergradation (Wake, 1997), and admixture between two

less distinct groups of populations within one of Stebbins' subspecies (*platensis*).

Secondary contacts within the *Ensatina* complex are well documented. In the foothills of the Sierra Nevada, there is a zone >150 km in length where four secondary contacts, each with hybridization, occur (Brown, 1974; Wake et al., 1989). Jackman and Wake (1994) argued that there were two other zones of secondary contact in the Sierra Nevada–Cascade region. One of these is between northern populations of what they identified as "southern *platensis*" and southern populations of their "northern *platensis*", and displays admixture rather than hybridization. The other is between the northernmost populations of northern *platensis* and the most inland (southeastern) populations of *oregonensis*, but here there is a small geographic gap in distribution and past contacts must be inferred. Recently additional zones of secondary contact, again with admixture and introgression rather than hybridization, were found in the San Francisco Bay region (Wake, 1997). There are four documented areas of secondary contact in southern California, three showing hybridization and one featuring sympatry without hybridization (Brown, 1974; Wake et al., 1986, 1989). There is an ecological dimension to the interactions taking place upon secondary contact. In the seven sites where there is narrow hybridization (that is, where F_1's and backcrosses can be recognized within the geographically restricted, localized extended population, but virtually no introgression occurs in neighboring populations) and the one site where there is sympatry with no evidence of past or present hybridization, the interactions are between unblotched, low-elevation, coastal forms that do well in scrub, chaparral, and open woodland situations, and blotched, high-elevation, inland populations that favor heavily wooded situations, often with closed canopies. In contrast, where secondary contacts involve groups of populations that are not ecologically differentiated and only slightly if at all differentiated in terms of coloration and morphology, admixture or less frequently introgression, not hybridization, occur. Thus, around the ring admixture and introgression prevail, and only when the ring is crossed (*xanthoptica* in the Sierra Nevada) or closed (*eschscholtzii* and *klauberi* in southern California) does true hybridization and, or, sympatry occur, as Stebbins' (1949) original model predicted.

Genetic Continuity, Discontinuity, and Sampling Gaps

Genetic discontinuity in the *Ensatina* complex is apparent among isolated allopatric populations but is also found in some areas where populations are continuous. There is a buildup of genetic distance with geographic distance in some parts of the complex that we interpret as a manifestation of reduction and loss of gene flow in the face of selective and neutral factors leading local populations to differentiate. The small movements characteristic of plethodontids in general, and of *Ensatina* in particular (Staub et al., 1995), make this phenomenon probable. The tendency for the geographic range to break up into temporarily isolated units which later rejoin also contributes to this phenomenon. We have demonstrated a correlation of increasing genetic differentiation with increasing geographic distance across the northern end of the Central Valley of California (Jackman and Wake, 1994: their Fig. 3), and for populations in the southern Sierra Nevada and Tehachapi Mountains (Jackman and Wake, 1994: their Fig. 4). We have argued that these patterns may extend to discontinuous populations across currently unoccupied (or unsampled) regions, as in the case of *klauberi* relative to southern *platensis-croceater* (Bob's Gap, see below), and of *klauberi* (*klauberi-croceater* intergrades and *klauberi*, following the taxonomy of Stebbins, 1949). That is, if the patterns seen within *klauberi* or within *platensis-croceater* had also existed within Bob's Gap, then the pattern evident in comparing *klauberi* to *platensis-croceater* could reasonably be interpreted as resulting simply from the elimination of the populations once present in Bob's Gap. Accordingly, the genetic distinction between *platensis-croceater* and *klauberi* could largely be the

result of recent extinction of intermediates, or even of our failure to locate elusive populations in this area that remains very difficult to access.

The second kind of discontinuity occurs between genetically divergent groups in secondary contact where there is limited hybridization. Such discontinuity is found at several places around the ring; we discuss the taxonomic implications of these interactions below. Perhaps more importantly, some secondary contacts have resulted in significant introgression of genetic markers and of elements of the color pattern as well, so much so that we regard these areas as evidence of the merging of once differentiated populations. In what follows, we show how Highton's approach to averaging genetic distances among groups of populations, and his treatment of intermediate populations as "hybrids", is misleading with regard to the degree of genetic distinctiveness and discontinuity among populations.

Sierra Nevada

Jackman and Wake (1994) identified northern and southern groups within *platensis*, but they found no reason to differentiate between southern *platensis* and *croceater*, based on allozymic data. They hypothesized that there was an asymmetry in the zone of contact between northern and southern *platensis*, with genes for color (apparently of high selective value) having moved far to the north, to the northern end of the range of the blotched populations, while allozymes had moved a shorter distance, with variation among the loci in degree of northward penetration. Earlier, Moritz et al. (1992) showed that southern *platensis* and *croceater* sequences of mitochondrial DNA formed a clade, as sister groups, and were in turn a sister group to the clade formed by their populations of *klauberi*. Northern *platensis* had a set of unique cyt *b* haplotypes that differed from southern *platensis* by 12.2–14.0%.

Highton (1998) would recognize three taxonomic species in this region, his groups VIII (our northern *platensis*), IX (southern *platensis*) and X (*croceater*). This decision well illustrates the differences in our approaches to the species question. The critical factor in Highton's analysis was his decision that population 27 (Wagner Ridge) of Jackman and Wake (1994) is a hybrid (using his definition of hybrid, not ours) between groups VIII and IX. This decision is not based on the presence of hybrid individuals, which are not found, but on his interpretation that the population shows intermediate frequencies for five allozymic loci. He accordingly eliminated this population from his analysis, creating a gap that he considered to be of species level in magnitude—D_N = 0.18 between populations 25 and 28 on either side of Wagner Ridge, while D_N = 0.10–0.11 from population 27 (Wagner Ridge) to either of the two neighboring populations. Jackman and Wake (1994) considered the allelic composition of population 27, as well as populations to the north and south, to be evidence of admixture between once separated northern and southern *platensis*. Highton (1998) was impressed that the distribution of *D*-values within parts of the *Ensatina* complex in this region is multimodal (his Fig. 2), but such a pattern is predicted by Jackman and Wake's (1994) finding. We have some new data that are relevant to the controversy and report them here.

Because northern and southern *platensis* differ in mtDNA, we examined the distribution of mtDNA halotypes in populations from the vicinity of Wagner Ridge (population 27 of Jackman and Wake, 1994). All of the new samples are between populations 25 and 28 of Jackman and Wake, and they bracket the Wagner Ridge population (Fig. 1). The populations sampled (Gooseberry Flat, n = 3; Tuolumne, n = 8; Jawbone Ridge, n = 1, and Wagner Ridge itself, n = 1) span the gap between locations known to contain either northern or southern mtDNA (Moritz et al., 1992), including one population studied previously (Gooseberry Flat = "plat Ma" of Moritz et al., 1992). The new samples interdigitate with three large river drainages (from north to south the Tuolumne, the Merced, and the San Joaquin), each of which was formed in part by large glaciers that descended as tongues of ice to below

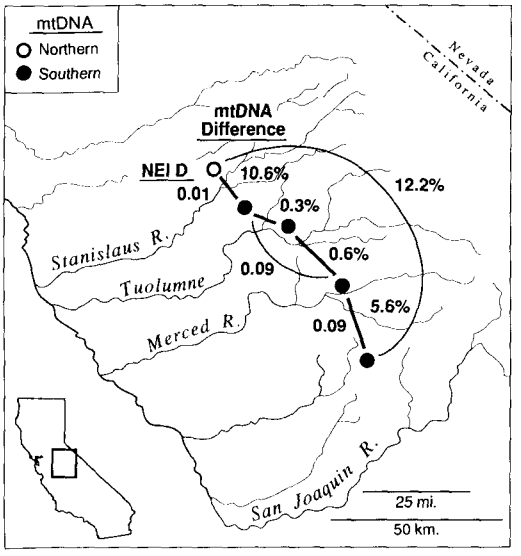

FIG. 1.—Map of a section of the central Sierra Nevada, California showing the genetic distances, to the left, and corrected percent sequence divergence in the mtDNA gene cytochrome *b*, to the right, in geographically adjacent populations of *Ensatina eschscholtzii platensis*. From north to south, the samples are Arnold, Calaveras Co. (plat Ar of Moritz et al, 1992, and population 23 of Jackman and Wake, 1994), Basin Creek Rd. ENE Tuolumne, Tuolumne Co. (population 25 of Jackman and Wake, 1994), Jawbone Ridge, Tuolumne Co., Wagner Ridge, Mariposa Co. (population 27 of Jackman and Wake, 1994), and Gooseberry Flat, Madera Co. (plat MA of Moritz et al., 1992; this last site is very near to population 28 of Jackman and Wake, 1994). The open symbol indicates assignment of the mtDNA haplotypes to a northern lineage, and the closed symbol indicates assignment to a southern lineage, following the phylogenetic analysis of Moritz et al. (1992). Locations of the rivers that have formed the three deepest canyons on the west slope of the Sierra Nevada, aided by large Pleistocene glaciers, are shown, as is the smaller, more northern Stanislaus river. An incomplete sequence from an individual collected near the west entrance of Yosemite National Park, at El Portal is identical to that at Gooseberry Flat. As the type locality of the taxon *sierrae* is Yosemite Valley, if this name should be resurrected for any segment of the *Ensatina* complex it would most likely go with this haplotype group (note, however, that Highton, 1998, considered the only population of this group that has been studied allozymically to be a hybrid).

600 m during the Pleistocene (Hinds, 1952). There were as many as nine Quaternary glaciations, and the Merced and Tuolumne glaciers achieved thicknesses of about 1829 m and 1220 m, respectively (reviewed by Bateman and Wahrhaftig, 1966). The extensive glaciation in the area provides a likely vicariant event (perhaps only the most recent of several in the region) that would have separated northern and southern populations of *platensis* probably in a complex manner, with subunits of each being isolated for varying amounts of time). Following secondary contact, either sympatry or a narrow hybrid zone with restricted gene flow would be expected if populations were behaving as biological species, but neither is found.

We examined the distribution of mitochondrial variation among these populations by first using restriction enzymes that are diagnostic for northern and southern *platensis* mtDNA sequences (a 649 bp fragment) of the cytochrome *b* gene amplified with primers MVZ 15 and 18 (Moritz et al., 1992). We then confirmed the results by sequencing 300 bp from this fragment (see Moritz et al., 1992, for details) for one specimen from each locality.

Amplified products from all individuals showed a restriction pattern characteristic of southern haplotypes of *platensis*. Sequence analysis confirms this conclusion, and phylogenetic analyses unambiguously place these sequences in a clade with the southern *platensis* sequences of Moritz et al. (1992). The sequence from Gooseberry Flat is identical to the "plat Ma" sequence of Moritz et al. (1992). The new sequences differ from the southern *platensis* ("plat Ma" and "plat Ha" localities of Moritz et al., 1992) at from 4.6–6.6% of nucleotide positions, and from northern *platensis* sequences ("plat B1" and "plat Ar" sequences of Moritz et al., 1992) by 9.0–12.5% (all distances are corrected following Brown et al., 1982). The three new sequences form a distinct haplotype group, with a maximal intragroup divergence of 0.9%. Together with the two southern *platensis* and the two *croceater* sequences of Moritz et al. (1992), these sequences form a monophyletic group that is the sister group to the three *klauberi* sequences reported by those authors. The placement of sequences from the new localities in a clade with the northern *platensis* sequences, alone or in any combination, is rejected by both

winning sites and likelihood ratio tests (sequences available from the authors).

A transition from northern to southern mitochondrial types of *platensis* might have been expected near Wagner Ridge (population 27 of Jackman and Wake, 1994). There is a transition in that area, but it is between two distinct mtDNA groups within southern *platensis*. Surprisingly, the transition between northern and southern mtDNA groups of *platensis* occurs well to the north, even north of the three large rivers mentioned previously, between the north and south forks of the relatively small Stanislaus River in a region of near uniformity of allozymes within what is allozymically northern *platensis* (Fig. 1). The allozymic genetic distance (Nei, 1972) between the Camp Connell (population 23 of Jackman and Wake, 1994, and having northern *platensis* mtDNA) and Tuolumne (population 25 of Jackman and Wake, 1994, and having mtDNA of southern *platensis*) samples is only 0.01 (Jackman and Wake, 1994). Genetic distances of 0.10 are found between our Tuolumne and Wagner Ridge (across the Tuolumne River) samples, and of 0.11 between Wagner Ridge and Westfall (across the Merced River). Either of these regions would appear more likely, *a priori*, than the more northern Stanislaus region for a mitochondrial transition zone, given their recent geological and glacial histories which could well have formed barriers to gene flow. Instead, the mitochondrial transition zone is 50–75 km to the north of Wagner Ridge, in a region where there is no associated transition in allozymes (Fig. 1). This discordance in mitochondrial and nuclear DNA markers is not unexpected in areas of secondary contact, where there has been introgression among populations, but what is surprising is the degree to which at least one set of genetic markers has moved. Because we have evidence that mtDNA markers can be highly structured over short geographic distances, we believe that females move little among populations once populations are established, and we have direct evidence that females do move less than males (Staub et al., 1995). If this is the case, the mtDNA transition may reliably mark the location of the secondary contact. This result would imply that southern *platensis* moved north once the glacial barrier disappeared, and that allozymic markers characteristic of the northern populations of *platensis* have introgressed >50 km to the south. Alternatively, secondary contact may have occurred further south, in the vicinity of the Merced or Tuolumne river valleys where the allozymic transition is more apparent, in which case mtDNA markers would have moved >50 km to the north. In either case, the discordant distribution of mtDNA and allozymic markers over such a large area suggests that extensive admixture has occurred and that there is no stable, narrow hybrid zone. In fact, the occurrence of a distinct, albeit southern, haplotype group in the vicinity of Wagner Ridge and neighboring locations suggests that there have been several episodes of separation and differentiation in the region.

Both mtDNA and protein data suggest that populations in the Sierra Nevada have resulted from two ancient, independent colonizations: one producing southern *platensis*, *croceater*, and *klauberi*, and the other resulting in populations living in the present-day northern and central Sierra Nevada. The central portion of the Sierra Nevada is topographically complex, for it is dissected by several of the largest rivers in the region and there are a number of high, semi-isolated ridges that extend to the west from the main mountain range. Populations of *Ensatina* are discontinuously distributed on these ridges at present, but we believe that interaction of northern and southern *platensis* has occurred relatively recently, probably during the Pleistocene. If species formation (using our criteria) was incipient, it has failed and the formerly isolated units are merging. The genetic interaction among populations in this region suggests admixture among populations having relatively complex histories, not a species border. Even though northern *platensis* and southern *platensis-croceater-klauberi* are both monophyletic for mtDNA (Moritz et al., 1992), the reticulation of nuclear markers and the ab-

sence of any morphological differentiation draws into question their evolutionary independence and permanence. Accordingly, we reject the proposal of Highton that northern and southern *platensis* merit status as taxonomic species.

Highton (1998) argued that southern *platensis* and *croceater* are candidate species (his units IX and X). For practical purposes, we use the Kern River as the transition from one to the other, which is consistent with Highton's distinctions (Stebbins, 1949, recognized a broad zone of intergradation between *platensis* and *croceater*, centered on the Kern drainage). On the north side of the river populations have orange–yellow blotches, while on the immediate south side the blotches are yellow to lemon yellow, with occasional orange-yellow individuals (approximately 10%). Samples 33 (southern *platensis*-p) and 34 (*croceater*-c) of Jackman and Wake (1994) bracket the Kern River on the north and south, respectively. These samples were not compared directly (for reasons given by Jackman and Wake 1994). Two other nearby localities, 32 (p), and 34 (c) were compared directly. D_N is 0.10 from 32 to 33 (within p), but only 0.04 from 32 to 34 (from p to c). Thus there is substantially more differentiation within p than between p and c in the vicinity of the contact zone. These facts are obscured by Highton's phenetic approach of averaging genetic distances for groups of populations and comparing these means. As Jackman and Wake (1994: their Fig. 4) showed, there is no evident gap in the pattern of increasing genetic distance with geographic distance within the combined p + c sample. Highton's methodology creates a gap where none exists—among populations that are continuously varying with respect to allozymes.

Lassen Gap Area

This is a region of recent and ongoing habitat change, resulting from volcanic activity and glaciation (and more recently from extensive and repeated logging activity). Populations of *Ensatina* are scattered and at low density. Jackman and Wake (1994) presented as much allozymic detail as is currently available. Genetic distances were large across the small geographic sampling gaps, but they decreased in magnitude as one moves away from the region of potential interaction into areas of more continuous habitat and higher salamander density. They interpreted this pattern as having arisen from repeated extinction and recolonization at the margins of the range of the two populational units involved. Nei's D is 0.2 between two populations at the edge of their respective ranges but falls to 0.17 between more geographically remote populations. This value is less than several interpopulational comparisons within Highton's group II (e.g., the D_N between populations 4 and 12 of Jackman and Wake, 1994, is 0.24), as was clear in the multidimensional scaling analysis presented by Jackman and Wake (1994: their Fig. 7). There is a small genetic gap with little apparent population interaction at present in the vicinity of Lassen Peak, but we do not see it as having the significance given to it by Highton. We acknowledge that the genetic distances measured between *oregonensis* and *platensis* are large by Highton's standards, but we see this as a weak link in the chain of populational units, not as a species border, because we see evidence for admixture among populations with at least as much genetic differentiation in other portions of the complex (see extended discussion in Jackman and Wake, 1994; cf. Wake, 1997).

Northern *platensis* (Highton's group VIII) is a group of populations that is relatively homogeneous allozymically. These populations occur between the Lassen Gap and the Stanislaus River. Because genetic differentiation is so low (maximum D_N about 0.1), it is worth reporting that we have found substantial mtDNA differentiation (unpublished data). Although all mtDNA sequence groups are each other's closest relatives, differentiation between mtDNA haplotype groups within northern *platensis* ranges as high as 6.8% (eight of the 15 comparisons are in excess of 6%). These data draw into question Highton's (1997) argument that species will have low levels of mtDNA differentiation, for here we find high levels despite very little al-

lozymic divergence. There are two problems with Highton's approach to mtDNA comparisons: (1) he insists on using strictly phenetic methods that take into consideration only raw amount of differentiation, and (2) he has a scanty data base. Voluminous but as yet unpublished data from Wake's laboratory will add many instances of much higher levels of mtDNA differentiation even within populations than Highton anticipated within species (e.g., Jockusch, 1996).

North Coastal Region

Highton identified three units as candidate species in this region, which extends from the San Francisco Bay north into southwestern Oregon, his groups II (central *oregonensis*), III (*picta*), and IV (southern *oregonensis*). Our studies remain incomplete, but we here report some data relevant to the issue of whether there is continuity or discontinuity in the region.

Moritz et al. (1992) found a distinctive mtDNA haplotype for *picta* (minimal divergence 7.7% to a population of *oregonensis* with which it consistently clusters in phylogenetic analysis). Jackman and Wake (1994) failed to find a large allozymic genetic distance between *picta* and *oregonensis* to the east (minimal D_N = 0.113 to Salyer, their sample 4). We have partially completed a study of allozymes in many additional populations in the area, and as geographic distances shorten, so do genetic distances, such that *picta* has no allozymic identity. Furthermore, the mitochondrial haplotype of *picta*, while moderately divergent in our original study, is not divergent when additional samples are added (unpublished data). In phylogenetic analysis, it clusters consistently with a group of populations of *oregonensis*: Alderpoint (sample 3 of Jackman and Wake, 1994), Salyer (their sample 4), and Leggett (sample 11 of Wake and Yanev, 1986), as well as several other populations well to the south (3.8–5.8% corrected sequence divergence). There is no reason to raise the taxonomic rank of *picta*, which is of dubious identity as a subspecies even based on coloration.

There is a distinctive set of mtDNA haplotypes within Highton's group IV (oreg Me and oreg Br of Moritz et al., 1992), differentiated from the geographically nearest sample in group II (oreg He) by 11.5–14.4% sequence divergence. We have sampled additional populations within this area for cytochrome *b* sequences, and have also conducted extensive but still incomplete allozymic analyses. Leggett and Alderpoint (in Highton's groups IV and II respectively) have similar mtDNA haplotypes (1.6–1.7% divergence), but these haplotypes differ substantially from that in the oreg Br (Highton's group IV) population (13.9–16.4% sequence divergence). Leggett and oreg Br are separated by about 25 km and there is little allozymic differentiation (D_N = 0.048) between the two localities. There is a larger allozymic genetic distance between two samples within Highton's group IV (D_N = 0.214, populations from Leggett and Barton Gulch, near the coast in central Mendocino County) than between samples from group IV and group III (samples 10 and 11 of Wake and Yanev, 1986, with a D_N of 0.107). There is substantial differentiation of mtDNA haplotypes between oreg He (Highton's group II) and Alderpoint–Leggett (8.6–8.9%), and there is also a relatively large allozymic genetic distance between these samples (D_N = 0.178: Jackman and Wake, 1994). However, the genetic distance is reduced when one examines two geographically intermediate points (maximal D_N between geographically adjacent samples from Alderpoint to oreg He is 0.123: Jackman and Wake, 1994). All of these examples illustrate that there are often large mtDNA differences between populations separated by low allozymic genetic distances, that allozyme and mtDNA differences are not concordant, and that there are no clear breaks between Highton's groups II and IV. Our recent discovery of the sympatry of two very distinctive haplotype groups in a single population also is relevant to Highton's argument that a low level of mtDNA differentiation characterizes species. In a sample a few kilometers east of the "oreg He" of Moritz et al. (1992), within Highton's group II, we find two distinct haplo-

type groups, differing by 8.1%. The second haplotype group is found also to the north and east of this locality (still in Highton's group II). The population (Oregon Mountain) in which both haplotypes are found is population 7 of Jackman and Wake (1994), which is not distinctive allozymically and has $D_N < 0.1$ to the five geographically closest populations sampled. Accordingly, the occurrence of two distinct haplotype groups in an interbreeding population that is not a "hybrid" even by Highton's generous criteria draws into question his entire analysis of mtDNA variation.

Genetic differentiation in this region is influenced, we believe, by the persistence of many, ancient mtDNA lineages. Populations in this region have complicated relationships, with evidence of separation in the past but a great deal of recent mixing. This reticulation shows different patterns with respect to mtDNA haplotypes and allozymes. Highton's proposed species borders do not make sense, for we do not find the concordant patterns of change between independent data sets that one expects at species borders using his own criteria. Again, the phenetic approach of averaging distances among sets of populations is misleading and superficial.

Russian River and San Francisco Bay to Monterey Bay Region

Highton has identified a northern and a southern *xanthoptica* as candidate species (his groups V and VI). A recent analysis was unavailable to Highton but is relevant to the issues under discussion (Wake, 1997). However, even that paper is preliminary, and investigators in Wake's laboratory are currently involved in intensive studies of this area.

At the northern end of this geographic region, in the vicinity of the Russian River, a zone of rapid and largely concordant change is found between *oregonensis* and *xanthoptica*. This North Bay region appears to be a secondary contact, with *xanthoptica* having moved northwestward from the East Bay region relatively recently. The contact zone itself is complicated. There is a large genetic distance across the zone (approximately $D_N = 0.3$) but no evidence of sympatry of two distinct kinds of organisms. Some populations contain introgressed alleles and others cannot be classified, but the pattern is complicated and difficult to summarize briefly (Wake, 1997).

Little information concerning this region was published when Highton (1998) prepared his critique, but the new data show that his proposed classification is inappropriate for this region. On the relatively small San Francisco Peninsula, *Ensatina* is remarkably differentiated (Wake, 1997). Northernmost populations on the peninsula are genetically *oregonensis* (that is, with respect to both allozymic and mtDNA characters they correspond to one of many clusters of populations comprising that heterogeneous assemblage). Within *xanthoptica* on the southern part of the peninsula alone (i.e., within Highton's southern *xanthoptica*), $D_N > 0.3$ is found. The populations comprising this latter group correspond to Highton's group VI, which is based on a single population on the southern part of the peninsula reported by Wake and Yanev (1986). These measures are about equivalent to the largest genetic distances between *xanthoptica* and either *oregonensis* or *eschscholtzii*. Despite this great differentiation within nominal *xanthoptica*, minimal genetic distances between geographic segments of taxa are much less. For example, genetic distances across the Santa Clara Valley, spanning the border between Highton's groups V and VI (both *xanthoptica* according to Wake, 1997), are $D_N = 0.08$. The original comparison between only two populations (13 and 14 of Wake and Yanev, 1986, or V and VI of Highton, 1998) of $D_N = 0.14$ is now seen to be misleadingly high, for as additional samples were added in the intervening area the genetic distance has dropped. The valley is inhospitable for *Ensatina* and now highly modified by human activities (this is the "Silicon Valley"), so we will be unable to close the geographic gap entirely, but we believe that the genetic gap is essentially a geographic sampling problem, based on our experience with genetic variation elsewhere in this complex. We see no

merit in recognizing groups V and VI as separate taxa at this time.

Comparisons between *xanthoptica* and *eschscholtzii* (Highton's groups VI and VII respectively) reveal a similar pattern. These taxa were reported to be separated by $D_N = 0.32$ (Yanev and Wake, 1986), and accordingly it seemed obvious to Highton that these represented different species. However, new studies of genetic differentiation in allozymes illustrate the pitfalls of making such judgements in the absence of sampling in intervening areas (Wake, 1997). Whereas Yanev and Wake had only three populations for the entire region, that is, the East Bay, the San Francisco Peninsula, and the vicinity of Monterey Bay-Pajaro River, we now have over 30. While some of these new populations fill geographic sampling gaps in the earlier study (Wake and Yanev, 1986), other gaps have proven difficult to fill. Using new samples, there is still a high $D_N = 0.31$ between two previously sampled populations representing *eschscholtzii* (topotypic) near Monterey and *xanthoptica* near Santa Cruz (taxonomic assignment here is based on mtDNA; Stebbins, 1949, considered it to be a *xanthoptica-oregonensis* intergrade), essentially identical to the value in the previous study. These samples are separated by about 40 km. However, the genetic distance becomes progressively smaller as the populations sampled become closer geographically, and two local populations appear to be admixed. D_N has dropped to 0.15 as a result of our new sampling, but there remains a zone about 20 km in width that is largely unsampled (habitat along the Pajaro River has been disrupted by agricultural activities and urbanization—this could be a "real" extinction experiment!). These data suggest that D will drop further as additional populations are discovered in the intervening area. We suspect that Highton would dismiss any admixed populations that are found as hybrids, but we emphasize that no hybrid individuals are found.

Comparisons between *xanthoptica* and *oregonensis* on the San Francisco Peninsula reveal that D_N ranges between 0.16 and 0.32, high values but matched in magnitude **within** *xanthoptica* on the same peninsula (Wake, 1997). If we were to use Highton's criteria, there might be four species in this small region alone (none of them sympatric). We believe, instead, that there are geological reasons for postulating periods of isolation and differentiation, followed by subsequent secondary contact and an active reintegration that is still in an early phase.

While *xanthoptica* can be inferred to have an historical identity on the basis of mtDNA phylogenetic interpretations and historical biogeography (Wake, 1997), it has become heterogeneous, displaying great genetic differentiation. The type locality is in the North Bay region, and from the vicinity of the type locality to the East Bay, D_N is about 0.08. D_N between the East Bay and the San Francisco Peninsula is about the same, so despite the great differentiation in the North Bay and on the peninsula, no evident geographic/genetic breaks have yet been found that would justify recognizing two independent species within *xanthoptica*, as Highton proposed.

Group VII (*eschscholtzii*) has distinctive mtDNA haplotypes, similar to those of Highton's groups V and VI (collectively our *xanthoptica*), and it is similar to these in allozymes. Because of the reciprocally monophyletic groups of mtDNA in both *xanthoptica* and *eschscholtzii*, and depending on one's taxonomic philosophy and criteria, group VII could be recognized as a separate species. However, the relationship between the two has become less discrete as data have been added, and we suspect (based on observed introgression and admixture among populations that are separated by much greater genetic distances elsewhere in the complex) that the border between these groups may not be sharp. Certainly this case is less clear-cut than Highton implied.

Bob's Gap

Relatively little can be said concerning what has been the most celebrated controversy historically with respect to *Ensatina*—the discontinuity in the distribution of the blotched forms in the San Gabriel Mountain region of southern California

(spanning the gap between *croceater* and *klauberi*). A large genetic distance is measured across this gap. Jackman and Wake (1994) suggested that the genetic distance might be lessened appreciably if populations could be sampled within the gap region, based on the demonstrated pattern of buildup of genetic distance with geographic distance both north and west, and south and east of the gap. Stebbins (1949) emphasized that populations on the eastern edge of the gap retained color patterns that were more similar to those of *croceater* than to those of more southerly *klauberi*, and we have confirmed his observations. Highton believed that Fig. 4 of Jackman and Wake (1994) inappropriately compared units that he considered to be heterogenous: that is, what he considered to be *klauberi*, *croceater*, and southern *platensis*. We acknowledge that three separate comparisons are shown in that figure and that the two involving comparisons within geographic regions have apparently different slopes and elevations than the comparison between areas. However, the point of the illustration was to show how populations within the gap region might fill the intervening area, assuming that there would be increasing scatter with increasing geographic distance because of the isolated nature of so many populations and the unlikeliness of continuous genetic exchange between adjoining populations. The authors pointed out in the legend to that figure that the points are not independent and no statistical significance was claimed. Intermediate populations may exist (we recently received a report of a sighting of a specimen in Bob's Gap by an apparently reliable observer), or they may only very recently have gone extinct, and this calls into question the independence and permanence of *klauberi*. We continue to search this region, which even today is remote and difficult to access, especially during the brief spring periods of surface salamander activity, for undiscovered populations. We predict, based on the color pattern information, that new populations will be intermediate between those on either end of the Gap.

If one were to recognize *klauberi* as a distinct species, one must contend with the fragmented nature of populations of *klauberi*, the consequent lack of cohesion, and the fact that there is a genetic distance >0.15 from north to south within it (this would be even larger if we used fast-evolving loci, as Highton, 1998, noted in his critique for other parts of the complex). Furthermore, the northern populations more closely resemble *croceater* in coloration than they do the populations of southern *klauberi*. To recognize *klauberi* as a distinct species would be consistent with the mtDNA data, less so with the allozymic data, but not with the information on color pattern; it would not be a cleanly diagnosable unit. However, the fact remains that some populations called *klauberi* and *eschscholtzii* occur in sympatry and are evidently distinct species. If one considers the genetic characteristics of these two taxa alone, relative to each other only, no one would dispute their status as distinct species. It is in the context of their relationships to neighboring populations that the ring species hypothesis was first formulated by Stebbins (1949). If all other members of the *Ensatina* complex but these went extinct, there would be no question that two species are present. It is the existence of the more northern populations, giving perspective to the time-space continuum, that leads to the present taxonomic dilemma.

TAXONOMY

Highton (1998) appropriately referred to the *Ensatina* complex as being comprised of semispecies. The complex has been repeatedly broken into geographic isolates, and there probably have been many rounds of separation, differentiation in allopatry, and recontact (e.g., Wake, 1997). Present-day distributions represent many time levels, with some ancient and some recent secondary contacts extant. It is always problematic to deal with semispecies, although present trends seem to be to recognize them as species. Stebbins' (1949) taxonomy has been retained as a convenience, recognizing that at some point reclassification may be required. Has that time arrived?

Despite much work with the complex in the years since Stebbins' (1949) revision, no subsequent worker has made a formal taxonomic revision, although several have suggested that *klauberi* should be recognized as a distinct species. However, the various populations comprising *klauberi* are geographically isolated from each other, forming at least three units, each of which is distinctive and might qualify as a species using criteria advocated by some proponents of the phylogenetic species concept. Collectively they do not form a cohesive unit. The fact remains that four populations assigned to *klauberi* occur in sympatry with *eschscholtzii*, and those who wish could follow the taxonomy espoused by Frost and Hillis (1990). They would solve the sympatry problem by recognizing *klauberi* as a distinct species-level taxon and leave the remainder as a complex bearing the name *eschscholtzii*. We would have no strong objection to this, and it is a reasonable option for those taxonomists who cannot tolerate sympatric subspecies. It leaves us unsatisfied, however, because of what it leaves behind in the rest of the complex—at least four more areas of sympatry and hybridization, and many zones of secondary contact. A partial resolution would be to recognize *eschscholtzii* and *xanthoptica*, together with *klauberi* (all as defined by mtDNA haplotypes in Moritz et al., 1992), as species-level taxa. This gives the taxonomy suggested by Graybeal (1995). Both of these options would leave unresolved the rest of the complex, to be known as *oregonensis*, which would be heterogeneous with respect to coloration, mtDNA, and allozymes, and have uncertain borders that do not coincide for different markers. One could make this *oregonensis* less heterogeneous by separating a blotched species (northern and southern *platensis*, as well as *croceater*, which would have taxonomic priority) from the unblotched but otherwise undiagnosable *oregonensis*. The resulting taxa would remain heterogeneous and further recognition of taxa would lead to solutions similar to that proposed by Highton (1998), which we have criticized at length. Highton admitted that more study of *oregonensis* (as well as some other segments of the complex) is required before any further taxonomic revision is possible. Given these complications, we recommend continued use of Stebbins' (1949) taxonomy until more resolution is attained.

In a perceptive analysis of species concepts, de Queiroz (1997) analogized the species with the category organism. An important outcome of his treatment is that diverse criteria, such as those used by Highton and by us, are no longer "standards for granting lineages taxonomic status as species", but instead they function as criteria for identifying sequential stages in the existence of species, such as a diagnosable stage, a monophyletic stage, and a reproductively isolated stage. Such a formulation is especially helpful in complexes such as *Ensatina*, where we believe that all of these, and more, stages are represented. The continuity of the evolutionary process evident in the complex leads to a taxonomic conundrum. One can envision a few more extinction events that would apparently advance the sequence and facilitate taxonomic resolution, but in the meantime, we see no option but to continue hypothesis generation and testing using new data

Phenetic and Phylogenetic Approaches in Species-Level Systematics

We apparently have a different world view about species than does Highton. There is historically based genetic structure to species and species complexes, and we expect a continuum from no internal genetic divergence in recently evolved species or those with high vagility, to high genetic distances among populations in widespread species that have low vagility. This elementary point is important, as it is incompatible with the use of a "threshold" criterion (such as $D_N = 0.15$) to recognize species taxa. If there is a pattern of increasing genetic distance with increasing geographic distance, sampling design is critical, for sampling gaps in areas of relatively rapid genetic change (i.e., a steep gradient of buildup of genetic distance with geographic distance) can give the

misleading impression of a species border, especially when phenetic clustering rather than phylogenetic analysis is performed (de Queiroz and Good, 1997). Unfortunately, Highton is committed to phenetic clustering methods, as is evident in his critique. We suspect that many old species will be found to show substantial internal genetic divergence, as has been found in California, not only for *Ensatina* but also in *Batrachoseps* (Yanev, 1980; Yanev and Wake, 1981, unpublished data), *Aneides* (Jackman, 1993; Larson, 1980), *Rhyacotriton* (Good and Wake, 1992), and elsewhere in genera such as *Desmognathus* (Tilley, 1997; Tilley and Mahoney, 1996) and *Salamandra* (Alcobendas et al., 1996). While some of these complexes could be broken up more finely into allopatric units that might be recognized as species, we believe that evolutionary and historical biogeographic patterns would be obscured as a result.

We would like a taxonomy that illuminates, rather than obscures, phylogenetic and biogeographic relationships, and evolutionary dynamics. This is the goal of many other authors as well (e.g., Frost and Hillis, 1990; Graybeal, 1995; Olmstead, 1995). We believe that *Ensatina* is extraordinary in several respects. It is an old, deeply differentiated lineage that still displays, in characters of its color pattern (Stebbins, 1949), in phylogenetics of its mtDNA (Moritz et al., 1992), and in allozyme variation (Jackman and Wake, 1994; Wake, 1997) important components of its evolutionary history. For example, the two derived sets of mtDNA haplotypes identified by Moritz et al. (1992) are associated with *xanthoptica-eschscholtzii* and southern *platensis-croceater-klauberi*, which are the two southern ends of the coastal and inland segments of the ring, as conceived by Stebbins (1949) and (for the inland group) by Jackman and Wake (1994). It is at the southern end of the ring where the completion of a long and complicated process of isolation by distance is manifest in sympatry. Northern parts of the complex, especially those in northwestern California, are envisioned by all of these workers as preserving remnants of ancestral populations (see also Wake and Yanev, 1986). Northwestern California preserves phylogenetically basal mtDNA lineages, displays the highest degree of both intra- and interpopulational genetic diversity, and has populations inferred to contain ancestral components of the color patterns of all of the complex. It is this persistence of ancestral sublineages and populations together with the derived southward extending limbs, as well as evidence of continuity, periodically interrupted, that makes *Ensatina* extraordinary. We will continue to seek a taxonomy that will be maximally informative.

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