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EVOLUTIONARY AND HISTORICAL ANALYSIS OF PROTEIN VARIATION IN THE BLOTCHED FORMS OF SALAMANDERS OF THE *ENSATINA* COMPLEX (AMPHIBIA: PLETHODONTIDAE)

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Abstract.—Geographic variation in 23 to 29 protein-encoding genetic loci was examined in 48 populations of the *Ensatina* complex, a "ring species" distributed around the Central Valley of California. The samples span two critical links in the chain of morphologically distinct units: the transition from the unblotched to blotched color pattern types in the vicinity of Lassen Peak, northeastern California, and a geographic gap in the range of the complex in the San Gabriel Mountains, southern California. A general pattern of isolation by distance with a regular buildup of genetic distance correlated with increases in geographic distance characterizes the populations studied, with the exception of a little-differentiated group of populations in the northern Sierra Nevada; this region is postulated to be a zone of genetic reticulation characterized by relatively high gene flow. An adaptively significant color pattern is thought to have spread into the northern Sierra Nevada from the south, but protein variants have been introduced both from the north and the south. Genetic distances across the San Gabriel Mountain gap match expectations from the pattern of buildup of genetic distance as a function of geographic distance elsewhere in the complex. A phylogenetic analysis of the protein data supports the reticulation hypothesis; whereas the southernmost populations currently do constitute a monophyletic assemblage, an "extinction experiment" demonstrates that the distinction could be the result of the recent extinction of populations in a present gap in our sampling. The *Ensatina* complex appears to be a dynamic entity representing several stages in the evolution of species. It is a ring species, and whereas various taxonomic arrangements are possible, no taxonomic changes are proposed.

Key words.—Biogeography, *Ensatina eschsholtzii*, extinction, gene flow, multidimensional scaling, phylogenetics, proteins, ring species, speciation, taxonomy.

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Salamanders of the *Ensatina* complex form the best known and most extensively studied ring species—geographically differentiated populations distributed in a circle, with species-level differentiation and sympatry where the circle is closed. These fully terrestrial, direct-developing, lungless salamanders occur in relatively mesic parts of the Pacific Coastal region of North America, from southern British Columbia to northern Baja California. Groups of populations are strongly differentiated in color and pattern, especially in California. Two main pattern classes exist, the blotched forms, which occur in the Sierra Nevada and various mountain ranges in southern California, and the unblotched forms, which occur throughout the rest of the range. The current taxonomy (Stebbins 1949) recognizes a single species, *Ensatina eschscholtzii*, and seven subspecies (blotched: *platensis, croceater,* and *klauberi*; unblotched: *eschscholtzii, oregonensis, picta,* and *xanthoptica*) (fig. 1). Stebbins' concept of a polytypic ring species was based on perceived primary intergradation between the subspecies where they met, with the exception of two instances of postulated secondary contact. Ancestors were thought to have migrated from northern California to the south in two descending limbs on both sides of the Central Valley of California. Dobzhansky (1958) added the hypothesis that gene flow via a long and circuitous route around the central valley of California was the reason speciation was incomplete. In southern California, where *klauberi* and *eschscholtzii* meet in a secondary contact, gene flow is sharply restricted or absent (Brown 1974; Wake et al. 1986, 1989). Gene flow also is sharply restricted in another zone of secondary contact involving *platensis* and *xanthoptica* in the foothills of the central Sierra Nevada (Wake et al. 1989). Because there is sympatry of two markedly different forms in these two areas, the *Ensatina* complex is a double-ring species. At approximately the halfway point in the ring, in the central Sierra

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Nevada, more hybridization occurs than at the full-ring level, where no hybridization is found at the southernmost point of sympatry (Wake et al. 1986, 1989).

This is one of a series of papers (Wake and Yanev 1986; Wake et al. 1986, 1989; Moritz et al. 1992) that reexamines the ring species concept as it applies to *Ensatina*. In this paper, we examine protein variation in 48 populations, including all of the blotched forms and their northern relatives.

Central to the concept of *Ensatina* as a ring species is the primary intergradation of the unblotched *oregonensis* populations at the head of the Sacramento Valley with the blotched *platensis* populations of the northern Sierra Nevada, the southern Cascades, and intervening mountains (fig. 1). In the absence of this zone of intergradation, the blotched and unblotched forms would be considered separate species. In general, *platensis* is an upland form and *oregonensis* occurs at lower elevations. Stebbins (1949) reported a very broad zone of intergradation, extending from Jackson County, Oregon, in the north, to Trinity County, California, in the west, and as far to the east and south as eastern Shasta County, California. A gap appeared in Stebbins' sampling, with the northernmost specimens of *platensis* coming from the vicinity of Mineral, south of Lassen Peak in Tehama County, California (near population 16, fig. 1), approximately 60 km to the southeast of the last intergrade population (northeast of population 10, fig. 1). This gap region is a primary focus of attention in our present study.

Stebbins (1949) recognized some additional "weak links" in the "chain" of subspecies that formed the ring species. One is the approximately 180-km distribution gap (known informally as "Bob's Gap") between *croceater* in the Tehachapi mountains and intergrade populations between *croceater* and *klauberi* in the northern San Bernardino mountains (between populations 35–39 and 40, fig. 1). Like other workers (e.g., Stebbins 1949; Schoenherr 1976), we have failed to find blotched salamanders in the intervening San Gabriel mountains, although we and Stebbins (pers. comm. 1993), suspect that populations remain undiscovered on the north-facing slopes of these mountains. Schoenherr (1976) reports a sighting of a blotched salamander in the San Gabriel mountains. We have given special attention to interpretation of data from populations on both sides of this apparent gap.

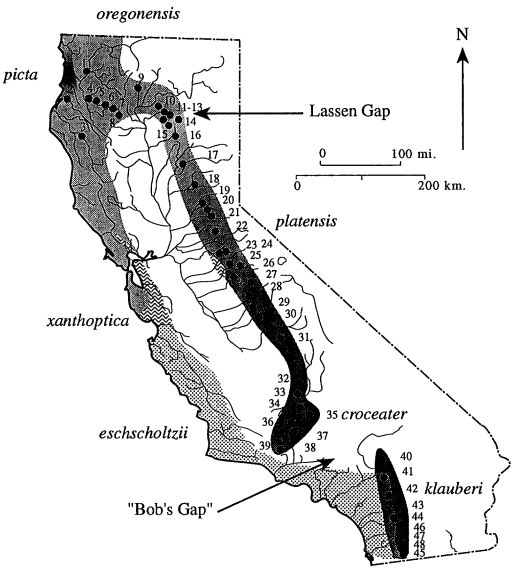

FIG. 1. Map of California showing the location of the 48 populations sampled in this study (see table 1 for details). The ranges of the seven subspecies of *Ensatina eschscholtzii* in California are indicated. Two important distributional gaps, the Lassen Gap in northeastern California, and Bob's Gap, in southern California, are also indicated (see text for details).

Frost and Hillis (1990), in the context of a discussion of species concepts, ignored the possibility of intergradation between adjacent subspecies in the ring and focused instead on the existence of sympatry. They argued that at least two species, *klauberi* and everything else (which would take the name *eschscholtzii*), should be recognized. However, the *platensis-xanthoptica* hybrid zone in the central Sierra Nevada also involves units that interact as if they are species (Wake et al. 1989). Following the logic of Frost and Hillis, the *Ensatina* complex could be divided further taxonomically, as we discuss later in this paper. The existence of the mid-Sierran hybrid zone means that the region of Bob's Gap is less critical to the ring species concept than formerly seemed to be the case; for even without the zone of sympatry in southern California, there is a secondary ringlike interaction in the complex.

Wake and Yanev (1986) showed that levels of protein differentiation within the *Ensatina* complex were higher than one expects within species of salamanders. Recently, Moritz et al. (1992) showed that high levels of differentiation also exist within the complex in relatively long sequences of the mitochondrial gene cytochrome

B. Phylogenetic analysis of the sequence data supported the main historical biogeographic hypothesis of Stebbins (1949). Moritz et al. found evidence in their data for the monophyly of *klauberi* but not for *platensis*. In this paper, we concentrate attention on the three blotched forms, their interactions with each other, their interaction with unblotched forms at the northern end of their range, and implications of our findings and previously published data for species concepts and taxonomy.

For ease of communication, and because the subspecies recognized by Stebbins (1949) are "candidate" species, we refer to subsets of our 48 population sample by trinomials. It is difficult to segregate *picta* from *oregonensis* on morphological grounds, and we decided to identify only one population as *picta*. In contrast, a sharp morphological distinction appears in the region of Lassen Peak between the unblotched *oregonensis* and the blotched *platensis*; thus, we do not assign any of these populations as intergrades. It is more difficult to separate *platensis* from *croceater*; we have used a combination of geographic and coloration criteria. We call a group of southern populations *klauberi*, although the two northernmost of these (our populations 40 and 41) were considered by Stebbins (1949) to be *croceater-klauberi* intergrades.

Materials and Methods

We examined samples of *Ensatina* collected from populations occurring mainly in inland and montane regions of California (fig. 1, table 1). In many of these areas, salamanders are difficult to find and thus we have been limited to relatively small samples. Our main analysis uses 48 samples ranging in size from 4 to 23 specimens, but we have obtained useful information from smaller samples taken from geographically important populations. In particular, samples of one or two were used to pinpoint a genetic break in the Lassen Peak area and to confirm patterns of isolation by distance from other localities throughout the range. Starch-gel electrophoresis was used to examine protein variation in the samples, following the methods of Wake and Yanev (1986). Freshly sacrificed specimens were dissected, and tissue samples (usually liver and intestine) were stored at −76°C until used. Carcasses were preserved as voucher specimens in the collections of the Museum of Vertebrate Zoology. Aqueous mixed homogenates of the tissues were assayed using standard horizontal starch-gel electrophoresis and histochemical staining procedures (Ayala et al. 1972; Harris and Hopkinson 1976; Selander et al. 1971; table 2). Variants are designated alphabetically, with "a" being the fastest migrant. Polymorphism is based on all observed variants and heterozygotes were recorded from direct counts.

The electrophoretic survey was conducted in three stages. We have combined these and numbered the samples consecutively from north to south. The first stage focused on the southern parts of the range and included samples of southern *platensis, croceater,* and *klauberi* (populations 31–48, excluding 33). We refer to this study in the text as study 1 (table 3). The second stage included samples 19–33. The final stage included populations 1–20. The overlapping samples permitted direct comparison and allowed us to combine the results for 23 proteins. We examined five additional proteins for populations 1–20, and one additional protein for populations 19–33. The combined investigations of populations 1–33 are called study 2 in the text (table 4). The two sample sizes indicated for populations 31 and 32 (table 1) are those used in studies 1 and 2, respectively.

Ensatina displays great allozymic polymorphism (Wake and Yanev 1986), and this fact makes it difficult to be certain, with limited material, that all of the low-frequency variants have been correctly homologized. We did not use exactly the same specimens for populations 31 and 32 in the two studies, and thus we report the results of the separate investigations in table 4. Because of the high degree of polymorphism encountered, users of the data in tables 3 and 4 are cautioned that it has been impossible to integrate completely the first and second studies. Thus, in those instances in which all variants failed to appear in populations 31 and 32, we assumed that the common variants are homologues. The impact of this assumption on our results is minimal.

Genetic distances were calculated using the methods of Nei (1972, 1978) with the BIOSYS-1 program (Swofford and Selander 1981). We use the Nei distances because we are dealing with populations considered conspecific and to facilitate comparisons with prior studies of the genus. Multidimensional scaling of genetic distances was calculated using NTSYS version 1.5 (Rohlf 1989).

Phylogenetic analysis of the protein data was conducted using PAUP 3.0s (Swofford 1991). Proteins (loci) were treated as partially ordered

characters; the gain or loss of a variant (allele) was counted as a single step using step matrices for each locus (e.g., state "a" to state "ab" is one step; state "a" to state "b" is two steps). The logic is that mutational, migrational, and stochastic gains and losses are likely to proceed via polymorphism in the same manner. The analysis is based on 19 phylogenetically informative loci having from 5 to 11 states. To simplify the analysis and make it tractable, variants with frequencies less than 10% in a given population were ignored. A heuristic search was used to find many trees. Unblotched populations 1–13 were used as outgroups. For a particular unrooted tree, any rooting gives trees of the same length. Branch lengths were calculated using MacClade (version 3.01, Maddison and Maddison 1992).

An "extinction experiment" was conducted to determine the effect on the phylogenetic analysis of the sudden disappearance of a group of populations over a geographic distance equivalent to the largest geographic gap in our sampling (which is also the largest geographic gap in the range of *Ensatina,* called Bob's Gap in this paper, fig. 1). Following elimination of a group of mid-Sierran populations, phylogenetic analysis was repeated on the remaining samples.

To link studies one and two for the phylogenetic analysis, the most common variants encountered in populations 31 and 32 in the separate studies were considered homologous. In cases of ambiguity, study two took precedence.

Results

Patterns of Allele Distribution.—The proteins surveyed show substantial variation within and among the populations studied (tables 3, 4). Patterns of allele replacement and sharing are complicated. Each protein variant has a unique distribution among the populations sampled. However, high-frequency variants usually are shared among geographically contiguous populations.

The northernmost *klauberi* (populations 40, 41) and the southernmost *croceater* (populations 37–39), on both sides of "Bob's Gap," differ completely for four proteins (*ICDH-1, MDH-1, LDH-2,* and *GPI*), but northern or southern alleles for some of these are found in more southerly and more northerly populations, respectively, away from the borders of the gap. Alleles for other proteins cross this gap (e.g., *Ada-2, Acon-1*).

No variants uniquely characterize *platensis* as a whole, although several have distinctly northern [e.g., *Acon-1* (f), *Aat-2* (b)] or southern [*Ldh-2* (f), *Acon-1* (d), *ADH-1* (a), *Pgdh* (i), *Ada-1* (e)] distributions within the taxon (fig. 2). *Ldh-2* (d) spans the *oregonensis-platensis* border, and *Icdh-1* (c) is present throughout all *platensis* and *croceater* populations (as well as in some populations of the other subspecies sampled).

Where *platensis* and *oregonensis* meet, we find substantial differentiation. The northernmost *platensis* (population 14), has unique variants, some present in high frequency. Comparing populations 15, 16, and 17 (northern *platensis*) with populations 8–13 (eastern *oregonensis*), we find one fixed difference in *Acon-2,* whereas *Ada-2, Icdh-1,* and *Pep-B* show substantial but not fixed differentiation. Although no variants are unique to *oregonensis, Ada-2* (e), *Acon-1* (b), *Icdh-1* (a), and *Ldh-2* (b) are widespread and common and generally absent or rare elsewhere. Although our single sample of *picta* (population 2) has a low genetic distance to nearby populations of *oregonensis,* it contains five unique variants [*Pgdh* (h), *Ldh-2* (a), *Iddh* (f), *Aat-2* (d), and *Pep-D* (f)].

Patterns of Genetic Distance.—Genetic distances (tables 5, 6) range from near zero to as great as 0.544–0.642 (maximum values in the two separate studies). We did not combine the studies to measure genetic distances across the full range of the 48 populations, but previous work by Wake and Yanev (1986) recorded genetic distances on the order of 0.6 between *klauberi* and *oregonensis.*

Genetic distances from the single sample of *picta* (population 2) to nearby samples (populations 1, 3, and 4) that Stebbins (1949) considered to be either intergrades or *oregonensis* range from 0.113–0.199. In contrast, genetic distances among populations of *oregonensis* range from about 0.020, for geographically contiguous samples (populations 9 and 10; 10 and 13), to 0.301 between samples from the western and eastern (populations 3 and 12) extremes of the range in northern California. Larger genetic distances exist between *picta* and *oregonensis* (five comparisons exceed $D = 0.3$) than between any populations of *oregonensis,* but *picta* is also the westernmost sample studied. Because geographic distance correlates with genetic distance throughout most of the range of the genus and in particular across northern California (fig. 3), this level of differentiation is about what is expected for the geographic distances involved.

TABLE 1. Collecting localities, population designations, and sample sizes. Each population is assigned to a subspecies of *Ensantina eschscholtzii,* abbreviated as follows: c, *croceater*; i, *picta*; k, *klauberi*; o, *oregonensis*; p, *platensis.*

Population	Sub-species	Sample size	Locality
1. Ishi Pishi Road	o	10	1.0 miles S of Somes Bar, Humboldt Co., Calif.
2. Arcata	i	10	Jolly Giant Creek, Humboldt Co., Calif.
3. Alderpoint	o	10	Humboldt Co., Calif.
4. Salyer	o	23	Rest area 2.5 miles SE of Salyer on Hwy. 299, Trinity Co., Calif.
5. Little French Creek	o	9	Milepost 23.3, W of Big Bar, Trinity Co., Calif.
6. Helena	o	4	E Fork Rd., N of Hwy. 299 at Helena, Trinity Co., Calif.
7. Oregon Mountain	o	8	E of Oregon Mountain summit on Hwy. 299, W of Weaverville, Trinity Co., Calif.
8. Buckhorn Summit	o	11	E of Buckhorn summit, on Hwy. 299, Trinity Co., Calif.
9. Hazel Creek	o	13	S slope of Hazel Creek. 0.8 miles by air SE of the Sacramento River, Shasta Co., Calif.
10. Ingot	o	8	6.2 miles NE of Ingot on Hwy. 299, Shasta Co., Calif.
11. Oak Run	o	4	Junction of Oak Run to Fern Rd. and Phillips Rd., Shasta Co., Calif.
12. Whitmore	o	4	6 miles E-NE of Whitmore on Tamarack Rd., Shasta Co., Calif.
13. Bear Creek	o	9	S fork of Bear Creek, 0.9 miles SE of Ponderosa Way, (NW corner section 30, T31N, R1E), Shasta Co., Calif.
14. Viola	p	9	1.2 to 2.2 miles E-NE of Viola on Hwy. 44, Shasta Co., Calif.
15. Bluff Springs	p	9	Ponderosa Way, 1.8 miles S of Forward Rd., (NW sect. 1. T29N, R1E), Tehama Co., Calif.
16. Potato Patch Campground	p	9	On Hwy. 36, Tehama Co., Calif.
17. Feather River	p	13	Milsap Bar Rd. 2.2 miles N of Bald Rock Rd., Butte Co., Calif.
18. Bald Mountain	p	8	Bald Mountain Douglas-Fir Transect, 5.0 miles N (by air) of Nevada City, Nevada Co., Calif.
19. Yankee Jim	p	11	2.5 miles W-NW of Foresthal on Yankee Jim Rd., Placer Co., Calif.
20. Georgetown	p	8	5.3 miles E of Georgetown on Wentworth Springs Rd., El Dorado Co., Calif.
21. Blodgett	p	6	Blodgett experimental forest, 15.3 miles E of Georgetown, El Dorado Co., Calif.
22. Panther Creek	p	10	West Panther Creek, 15.8 miles NE (by Hwy. 88) and 1.8 miles SE (by road) of Pioneer, Amador Co., Calif.
23. Camp Connell	p	10	Highway 4, 1.5 miles by road from Camp Connell post office, (SW sect. 31, T6N, R16E), Calaveras Co., Calif.
24. Holcomb Creek	p	7	3.7 miles N-NW (by air) of Arnold, Calaveras Co., Calif.
25. Tuolumne	p	5	Basin Creek Rd., 4.8 miles E-NE of Tuolumne, (NW sect. 30, T2N, R17E), Tuolumne Co., Calif.
26. Tuolumne UB	p	5	Basin Creek Rd., 9.5 miles E-NE of Tuolumne, (extreme NW sect. 31, T2N, R17E), Tuolumne Co., Calif.

TABLE 1. Continued.

Population	Sub-species	Sample size	Locality
27. Wagner Ridge	p	6	Wagner ridge pine transect, 5.5 miles (by air) of Coulterville, Mariposa Co., Calif.
28. Westfall	p	5	Westfall campground on Hwy. 41, Madera Co., Calif.
29. Southfork	p	4	5.0 miles E-SE of Southfork, (NE sect. 25, T8S, R23E), Madera Co., Calif.
30. Auberry	p	4	Jose Basin Rd., 8.0 miles NE of Auberry, (SE sect. 20, T9S, R24E), Fresno Co., Calif.
31. Hartland	p	10/5	Vicinity of Hartland, Tulare Co., Calif.
32. Sugarloaf	p	9/5	Posey Rd., 6.5 miles E-NE of the junction with Jack Ranch Rd., Tulare Co., Calif.
33. Kern River	p	10	Along Greenhorn Creek, N of Hwy 178, Kern River Canyon, (NW sect. 19, T27S, R32E), Kern Co., Calif.
34. Breckenridge	c	9	Breckenridge Rd., 0.8–1.0 miles W of Pine Saddle, west slope of Breckenridge Mountain. 5000 ft. elev., Kern Co., Calif.
35. Piute Mountains	c	4	0.5–1.0 miles S of Piute Peak at Brown Meadow, Kern Co., Calif.
36. Cummings Valley	c	6	Cummings Valley Rd., 2.5 miles SW of the junction with Highway 202, Kern Co., Calif.
37. Clear Creek	c	3	Hart Flat, 3 miles from Highway 58, Kern County, Calif.
38. Tejon Ranch	c	10	Dryfield Rd., 4.5 to 4.8 miles (by road) E of Interstate 5 (E of Castaic Lake), Tejon Ranch, Kern Co., Calif.
39. Little Mutau Creek	c	4	Little Mutau Creek, S of Alamo Mine, 1.2 air miles W-NE of McDonald Peak, 6850 ft. elev., Ventura Co., Calif.
40. Crystal Creek	k	10	Lucerne Valley, N side of the San Bernardino Mountains, (SE sect. 14, T3N, R1W), San Bernardino Co., Calif.
41. Sawmill Creek	k	3	Junction of W and N branches of Sawmill Canyon, (center sect. 26, T1S, R1E), San Bernardino Co., Calif.
42. San Jacinto	k	7	Vicinity of Fuller Mill Creek along Hwy. 243, Riverside Co., Calif.
43. Santa Rosa Mountains	k	10	4.9 miles southeast of Hwy. 74 on Santa Rosa Mountain Rd., (NW sect. 28, T7S, R5E), Riverside Co., Calif.
44. Palomar	k	14	East side of Cedar Creek above Hwy. S-7, Jeff Valley, (NW sect. 24, T10S, R1E), San Diego Co., Calif.
45. Camp Wolahi	k	28	S side of Boulder Creek near Colby Spring at Camp Wolahi, Cuyamaca Mountains, San Diego Co., Calif.
46. Juch Canyon	k	9	Wynola Rd., 1.2 miles E of Hwy 78 and 79, (extreme NW sect. 36, R3E, T12S), San Diego Co., Calif.
47. Engineer's Road	k	6	3.5 miles (by road) W of Hwy. 79, (W central sect. 30, T13S, R3E), San Diego Co., Calif.
48. Heise County Park	k	9	4.6 miles SW of Hwy. 78 and 79, W of Julian, San Diego Co., Calif.

TABLE 2. Proteins studied and buffer systems used for starch-gel electrophoresis. Buffer systems as in Selander et al. (1971). Abbreviations: A, Tris-citrate II (pH 8.0); B, Tris-citrate II (pH 8.0) with NADP in gel; C, Poulik (pH 8.7); D, LiOH (pH 8.2); E, PGI Phosphate (pH 7.1).

Enzyme	Enzyme commision no.	Locus	Buffer system
Aconitate hydrase (2 loci)	4.2.1.3	*Acon-1, 2*	B
Adenosine deaminase (2 loci)	3.5.4.4	*Ada-1, 2*	E
Alcohol dehydrogenase (2 loci)	1.1.1.1	*Adh-1, 2*	E
Aspartate aminotransferase	2.6.1.1	*Aat-1, 2*	C,D
Dipeptidase	3.4.13.11	*LA*	D
General protein	—	*GP-1*	C
Glutamate dehydrogenase	1.4.1.2	*Gtdh*	A
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pdh*	A
Glucose dehydrogenase	1.1.1.47	*Gdh*	A
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gapdh*	B
Glucosephosephate isomerase	5.3.1.9	*Gpi*	E
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pdh*	F
3-hydroxyacyl CoA dehydrogenase	1.1.1.35	*Hadh*	E
L-Iditol dehydrogenase	1.1.1.14	*Iddh*	A
Isocitrate dehydrogenase (2 loci)	1.1.1.42	*Icdh-1, 2*	B
L-lactate dehydrogenase (2 loci)	1.1.1.27	*Ldh-1, 2*	C
Malate dehydrogenase	1.1.1.44	*Mdh-1, 2*	A
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	A
Phosphoglucomutase	5.4.2.2	*Pgm*	A
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	B
Peptidase-B	3.4.11.4	*Pep-B*	D
Peptidase-D	3.4.13.9	*Pep-D*	D
Superoxide dismutase	1.15.1.1	*Sod*	D

No geographic areas of genetic uniformity exist for *oregonensis*. Small groups of populations (e.g., 6–9) have maximal genetic distances less than 0.1, but in general there is substantial regional differentiation (fig. 3). The smallest genetic distance between *oregonensis* and *platensis* is between two of the geographically most remote populations ($D = 0.158$, 1 to 20), not between geographically close populations as would be expected from a simple model of steady geographic expansion from the north to the south. Several other comparisons are less than 0.2 (e.g., 8 compared with 18; 9 with 19). Wake and Yanev (1986) recorded similarly low genetic distances between populations in the central Sierra Nevada and northwestern California.

Within *platensis* (populations 15–33) genetic distances can be as high as 0.484. When the unusual (see above) population from near Lassen Peak (14) is included, the greatest genetic distance rises to 0.567. An extensive area in the northern Sierra Nevada, represented by populations 15 through 26 (we refer to this group of populations, plus 14, as northern *platensis* in this paper), is relatively uniform genetically, with no genetic distance exceeding 0.1. From population 26 southward (extending continuously as far as population 39, and discontinuously through population 48, see below), genetic distance builds mainly as a function of geographic distance, although the smallest genetic distances are not always between populations that are the closest geographically. Populations of *platensis* south of population 26 (27–33) have no special identity as a genetic unit, but for the purposes of this paper we refer to them as southern *platensis*.

If we treat populations 33 and 34 as intergrades between *platensis* and *croceater* (based on color pattern only, following Stebbins 1949), the smallest genetic distance between "pure" *platensis* (32) and "pure" *croceater* (35) is only 0.041. Within *croceater* (35–39) the maximum genetic distance is 0.108 (37–39). The minimal genetic distance between *croceater* and *klauberi* is 0.362 (38–41), whereas the maximal is 0.544 (39–47). Within *klauberi* (40–48), the largest genetic distance is 0.160 (40–47), and genetic distance builds as a function of geographic distance (fig. 4).

Phylogenetic Analysis.—Phylogenetic analysis (heuristic search) of the protein data found 1892 unrooted trees of equal length (219 steps). The shape of the frequency distribution of tree lengths was approximated by computing the lengths of 10,000 randomly chosen trees (using PAUP 3.0s)

TABLE 3. Study 1 (see the text for details). Protein variation. When populations show no variation, they are indicated as fixed.

Protein	Fixed populations	Polymorphic populations
Sod	a(31), b(36, 38, 39–48)	34(a = .75, b = .25), 35(a = .50, b = .50), 37(a = .83, b = .17)
Icdh-1	c(32, 34–39), a(40–42, 45–48)	31(b = .20, c = .80), 43(a = .85, c = .15)
Icdh-2	a(31, 32, 34–42, 44, 47, 48)	42(a = .64, c = .36), 45(a = .94, b = .06)
Pgdh	a(32, 40–48), c(36, 37)	31(a = .85, b = .15), 34(a = .50, c = .50), 35(a = .38, c = .62), 38(a = .10, c = .90), 39(a = .25, c = .75)
Acon-1	b(31, 32, 34–46, 48)	47(a = .02, b = .98)
Acon-2	a(31, 32, 34–44, 46–48)	45(a = .89, b = .11)
Pgm	a(32, 35–38, 40–44, 46–48)	31(a = .80, b = .15, d = .05), 34(a = .78, b = .78, c = .11), 39(a = .50, d = .50), 45(a = .89, d = .11)
Mdh-1	a(32, 35–38), d(40, 41, 43, 45, 46)	31(a = .85, b = .05, e = .10), 34(a = .83, f = .17), 39(a = .63, f = .37), 42(d = .93, g = .07), 44(c = .17, d = .83), 47(c = .18, d = .82), 48(c = .08, d = .92)
Mdh-2	a(31, 32, 34–48)	None
Mpi	a(31, 34–37, 39–44)	32(a = .94, b = .06), 38(a = .95, b = .05), 45(a = .67, b = .33), 46(a = .94, b = .06), 47(a = .95, b = .05), 48(a = .50, b = .50)
Iddh	a(32, 34–45, 47, 48)	31(a = .85, b = .15), 46(a = .72, b = .28)
Ldh-1	a(31, 32, 34–40)	42(a = .43, b = .57), 43(a = .40, b = .55, c = .05), 44(a = .71, b = .29), 45(a = .56, b = .44), 46(a = .63, b = .37), 47(a = .45, b = .55), 48(a = .33, b = .67)
Ldh-2	a(31, 32, 35–39), c(40, 41, 44–46, 48)	34(a = .89, c = .11), 42(c = .93, d = .07), 43(b = .15, c = .85), 47(b = .04, c = .96)
Aat-1	c(35–39), a(41, 42, 44–46)	31(a = .35, c = .55, e = .10), 32(a = .06, c = .94), 34(a = .33, c = .61, e = .06), 40(a = .40, b = .60), 43(a = .90, b = .10), 47(a = .96, c = .04), 48(a = .92, c = .08)
Aat-2	c(31, 32, 34–43)	44(a = .46, c = .54), 45(a = .17, c = .83), 46(a = .18, b = .06, c = .75), 47(a = .27, b = .07, c = .64), 48(a = .17, b = .17, c = .66)
LA	a(35, 39, 40, 42)	31(a = .95, c = .05), 32(a = .89, b = .11), 34(a = .50, b = .50), 36(a = .60, b = .40), 37(a = .67, b = .33), 38(a = .88, b = .12), 41(a = .50, b = .50), 43(a = .90, b = .10), 44(a = .89, b = .11), 45(a = .72, b = .28), 46(a = .50, b = .50), 47(a = .43, b = .57), 48(a = .83, b = .17)
Pep-D	a(38–43), b(48)	31(a = .95, b = .05), 32(a = .94, b = .06), 34(a = .83, b = .17), 35(a = .88, b = .12), 36(a = .75, b = .25), 37(a = .67, b = .33), 44(a = .32, b = .68), 45(a = .11, b = .89), 46(a = .22, b = .79), 47(a = .23, b = .73, c = .04)
Pep-B	None	31(c = .80, d = .20), 32(a = .28, b = .06, c = .67), 34(a = .50, c = .50), 35(a = .50, c = .50), 36(a = .42, c = .58), 37(a = .33, b = .67), 38(a = .56, c = .44), 39(a = .88, c = .12), 40(a = .45, c = .55), 41(a = .67, c = .33), 42(a = .36, e = .14, c = .50), 43(a = .35, c = .65), 44(a = .14, c = .86), 45(a = .56, c = .44), 46(a = .22, b = .11, c = .67), 47(a = .16, b = .05, c = .79), 48(a = .08, b = .08, c = .84)
Ada-1	a(37, 40, 41, 43)	31(a = .75, c = .25), 32(a = .61, c = .33, d = .06), 34(a = .72, b = .11, c = .17), 35(a = .50, b = .37, c = .13), 36(a = .42, b = .58), 38(a = .85, b = .10, c = .05), 39(a = .63, c = .37), 42(a = .93, c = .07), 44(a = .64, c = .36), 45(a = .94, c = .06), 46(c = .67, c = .33), 47(a = .77, c = .23), 48(a = .75, c = .25)
Ada-2	a(31, 34, 35), b(44–48)	32(a = .83, b = .17), 36(a = .25, b = .75), 37(a = .17, b = .83), 38(a = .55, b = .45), 39(a = .75, b = .25), 40(a = .45, b = .55), 41(a = .50, b = .50), 42(a = .50, b = .50), 43(a = 45, b = .55)
Adh-1	a(36–39, 44), c(40)	31(a = .95, d = .05), 32(a = .78, e = .22), 34(a = .90, d = .05, e = .05), 35(a = .88, e = .12), 41(a = .33, c = .67), 42(a = .57, c = .43), 43(a = .90, c = .10), 45(a = .94, c = .06), 46(a = .94, b = .06), 47(a = .82, b = .11, c = .17), 48(a = .58, b = .25, c = .17)
Gpi	a(31, 34–39), b(40-48)	32(a = .94, b = .06)
G3pdh	a(34–41, 44, 45)	31(a = .90, b = .10), 32(a = .83, b = .17), 42(a = .79, c = .21), 43(a = .50, c = .50), 46(a = .81, c = .19), 47(a = .93, c = .07), 48(a = .92, c = .08)

TABLE 4. Study 2 (see text for details). Protein variation as in table 3.

Protein	Fixed populations	Polymorphic populations
Pgdh	e(12–15, 17–20, 25, 26)	1(e = .96, f = .04), 2(b = .10, e = .60, h = .30), 3(e = .85, f = .15), 4(b = .07, c = .15, e = .76, f = .02), 5(c = .13, e = .87), 6(b = .06, c = .31, e = .63), 7(c = .25, e = .75), 8(c = .05, e = .75, f = .20), 9(e = .89, f = .11), 10(c = .06, e = .94), 11(c = .17, e = .83), 16(a = .17, e = .83), 21(e = .95, f = .045), 22(e = .90, g = .10), 23(e = .95, g = .05), 24(e = .93, g = .07), 27(e = .67, i = .33), 28(d = .80, i = .20), 29(d = .25, i = .75), 30(d = .75, i = .25), 31(d = .90, i = .10), 32(d = .40, i = .60), 33(d = .90, e = .10)
Icdh-1	a(6–8, 10–13), e(14–17, 19, 22–27, 29, 30, 32, 33)	1(a = .41, c = .50, e = .09), 2(a = .20, c = .80), 3(e = .43, e = .57), 4(a = .59, b = .14, c = .27), 5(a = .94, c = .06), 9(a = .77, c = .23), 18(c = .93, d = .07), 20(a = .25, c = .75), 21(a = .04, e = .92, d = .04), 28(c = .90, d = .10), 31(c = .70, a = .30)
Icdh-2	b(1–15, 18, 20–30, 32, 33)	16(a = .08, b = .92), 17(a = .15, b = .85), 19(a = .05, b = .95), 31(b = .90, c = .10)
Acon-1	b(6–13), f(14–16, 22–26), d(28–33)	1(b = .50, e = .45, g = .05), 2(b = .15, e = .85), 3(b = .10, e = .30, g = .60), 4(a = .04, b = .83, e = .13), 5(a = .13, b = .87), 17(d = .09, f = .91), 18(d = .20, f = .80), 19(d = .25, f = .75), 20(d = .25, f = .75), 21(d = .10, f = .90), 27(e = .75, f = .25)
Acon-2	c(2, 5–9, 11–22, 24–32), b(33)	1(c = .86, d = .14), 3(a = .30, e = .70), 4(c = .91, d = .09), 10(c = .83, d = .17), 23(c = .95, e = .05)
Ldh-1	b(1–19, 25, 27, 28, 32, 33)	20(a = .12, b = .88), 21(a = .12, b = .88), 22(a = .05, b = .95), 23(b = .95, d = .05), 24(b = .86, d = .14), 26(b = .70, d = .30), 29(b = .75, c = .25), 30(b = .75, c = .25), 31(b = .60, c = .40)
Ldh-2	b(3, 5), d(10–16, 18–21), f(30–33)	1(b = .77, d = .23), 2(a = .05, b = .65, e = .10, d = .15, f = .05), 4(b = .93, c = .07), 6(b = .36, d = .64), 7(b = .87, c = .13), 8(b = .10, d = .90), 9(b = .07, d = .93), 17(d = .91, e = .09), 22(d = .75, f = .25), 23(d = .85, f = .15), 24(d = .36, f = .64), 25(d = .60, f = .40), 26(d = .30, f = .70), 27(d = .08, f = .92), 28(d = .20, f = .80), 29(d = .25, f = .75)
Gpi	c(1–21, 26–28, 30, 31, 33)	22(a = .11, c = .72, e = .17), 23(c = .95, e = .05), 24(c = .93, e = .07), 25(c = .80, d = .20), 29(c = .50, e = .50), 32(b = .10, e = .90)
Ada-1	c(2–7, 9–13, 18–20, 22, 23, 26)	1(c = .96, d = .04), 8(a = .15, c = .85), 14(a = .71, c = .29), 15(a = .17, c = .83), 16(a = .63, c = .37), 17(a = .82, c = .18), 21(a = .04, c = .96), 24(b = .14, c = .86), 25(b = .10, c = .90), 27(c = .42, e = .58), 28(c = .80, e = .20), 29(c = .75, e = .25), 30(c = .63, e = .37), 31(c = .70, e = .30), 32(b = .20, c = .50, e = .30), 33(c = .70, e = .30)
Ada-2	e(6–13), d(14), b(15, 20–26, 29, 31), a(33)	1(d = .18, e = .82), 2(d = .93, e = .07), 3(d = .12, f = .88), 4(d = .94, e = .06), 5(d = .37, e = .63), 16(b = .50, e = .50), 17(b = .90, c = .10), 18(b = .79, c = .21), 19(b = .85, c = .10, e = .05), 27(b = .67, c = .33), 28(b = .90, c = .10), 30(b = .88, c = .12)
Adh-1	c(3, 8, 11, 12, 14, 16–18, 20–22, 26)	1(c = .91, e = .09), 2(c = .65, e = .35), 4(c = .88, e = .12), 5(c = .81, e = .19), 6(c = .94, e = .06), 7(c = .88, e = .13), 9(c = .50, e = .50), 10(c = .33, d = .33, e = .33), 13(b = .25, c = .75), 15(c = .90, d = .10), 19(c = .95, d = .05), 23(c = .80, d = .10), 24(c = .79, d = .14, h = .07), 25(a = .10, c = .80, h = .10), 27(a = .25, c = .75), 28(a = .70, c = .30), 29(a = .75, c = .25), 30(a = .88, c = .12), 31(a = .90, f = .10), 32(a = .70, f = .30), 33(a = .65, f = .35)
Adh-2	c(5, 8, 18, 20, 22–30, 32, 33)	1(c = .91, d = .09), 2(c = .88, d = .12), 3(c = .86, d = .14), 4(b = .04, c = .96), 6(c = .83, d = .17), 7(c = .67, d = .33), 9(c = .17, d = .83), 10(c = .50, d = .50), 11(a = .17, c = .50, d = .33), 12(b = .75, d = .25), 13(c = .20, d = .80), 14(c = .17, e = .83), 15(a = .37, c = .25, d = .38), 16(c = .50, d = .50), 17(c = .85, d = .15), 19(b = .29, c = .21, d = .50), 21(c = .86, d = .14), 31(c = .90, d = .10)
Mdh-1	b(2–30, 32, 33)	1(b = .77, c = .23), 31(a = .10, b = .90)
Mdh-2	d(6, 8–21, 25, 26), c(28–31, 33)	1(b = .41, d = .59), 2(b = .05, d = .95), 3(b = .10, d = .90), 4(b = .37, d = .63), 5(b = .31, d = .69), 7(b = .25, d = .75), 22(c = .05, d = .95), 23(c = .05, d = .95), 24(c = .07, d = .93), 27(c = .58, d = .42), 32(a = .10, c = .90)

TABLE 4. Continued.

Protein	Fixed populations	Polymorphic populations
Iddh	d(1, 6, 7, 10, 12–15, 17–29, 31–33)	2(a = .56, c = .44), 3(d = .95, f = .05), 4(a = .44, d = .30, e = .26), 5(a = .19, c = .19, d = .62), 8(a = .20, d = .80), 9(a = .04, d = .89, e = .07), 11(d = .83, e = .17), 16(d = .62, e = .38), 30(b = .25, d = .75)
Mpi	c(2–14, 16–19, 23, 25–28, 30–32)	1(c = .96, d = .04), 15(c = .80, e = .20), 20(c = .88, e = .12), 21(c = .88, e = .12), 24(c = .93, e = .07), 29(b = .25, c = .75), 33(a = .05, c = .95)
Pgm	b(1, 3, 4, 6–9, 12, 13, 16, 18, 20–30, 32, 33)	2(b = .90, c = .05, e = .05), 5(b = .81, e = .19), 10(b = .94, c = .06), 11(b = .92, c = .08), 14(a = .94, b = .06), 15(a = .64, b = .36), 17(a = .40, b = .60), 19(a = .05, b = .95), 31(b = .90, d = .10)
Aat-1	c(3, 5, 7–20, 24–29)	1(c = .86, e = .14), 2(b = .10, c = .90), 4(b = .15, c = .85), 6(b = .06, c = .94), 21(a = .25, c = .75), 22(a = .15, c = .85), 23(a = .05, c = .95), 30(c = .88, d = .12), 31(a = .20, c = .80), 32(a = .20, c = .80), 33(a = .35, c = .65)
Aat-2	a(1, 5–7, 9, 10, 12–16, 18, 20, 22, 25, 26, 28–33)	2(a = .10, c = .85, d = .05), 3(a = .40, c = .60), 4(a = .52, c = .48), 8(a = .93, c = .07), 11(a = .83, c = .17), 17(a = .95, b = .05), 19(a = .83, b = .17), 21(a = .91, b = .09), 23(a = .95, b = .05), 24(a = .93, b = .07), 27(a = .75, b = .25)
Pep-B	f(11–13), h(16, 18, 19, 25, 26, 29, 30)	1(e = .41, f = .59), 2(e = .10, f = .90), 3(e = .05, f = .70, g = .15, i = .05), 4(e = .11, f = .52, g = .30, i = .07), 5(d = .06, e = .13, f = .31, g = .50), 6(a = .06, f = .81, g = .13), 7(f = .75, g = .25), 8(a = .06, e = .06, f = .44, g = .44), 9(d = .04, f = .96), 10(f = .94, i = .06), 14(h = .86, i = .14), 15(f = .33, h = .67), 17(f = .39, h = .61), 20(f = .13, h = .88), 21(f = .13, h = .71, i = .17), 22(f = .15, h = .85), 23(f = .10, h = .90), 24(f = .07, h = .93), 27(f = .17, h = .83), 28(h = .90, i = .10), 31(c = .10, h = .90), 32(b = .10, f = .20, h = .70), 33(c = .35, h = .65)
LA	d(3, 7, 15, 18–31, 33), e(11, 12)	1(c = .04, d = .82, e = .09, f = .05), 2(c = .11, d = .89), 4(b = .02, c = .02, d = .84, e = .13), 5(d = .94, e = .06), 6(d = .87, e = .13), 8(d = .65, e = .35), 9(b = .11, d = .82, e = .07), 10(b = .17, d = .50, e = .33), 13(d = .38, e = .62), 14(b = .40, d = .60), 16(b = .17, d = .83), 17(d = .80, e = .20), 32(a = .10, d = .90)
G3pdh	c(5–7, 9, 10, 12, 13, 16, 18, 20, 23, 27, 28–30)	1(a = .04, c = .82, d = .14), 2(c = .20, d = .80), 3(c = .83, d = .17), 4(b = .06, c = .78, d = .16), 8(b = .05, c = .95), 11(c = .58, d = .42), 14(c = .94, d = .06), 15(c = .67, d = .33), 17(c = .89, d = .11), 19(c = .89, d = .11), 21(c = .92, d = .08), 22(c = .80, d = .20), 24(c = .86, d = .14), 25(c = .63, d = .37), 26(c = .88, d = .12), 31(c = .80, f = .20), 32(c = .70, e = .30), 33(c = .65, d = .20, e = .15)
Pep-D	b(1, 3–7, 9–14, 17–23, 25, 27–29)	2(b = .80, f = .20), 8(b = .89, d = .11), 15(b = .83, d = .17), 24(b = .93, c = .07), 26(a = .40, b = .40, c = .20), 30(b = .88, e = .12), 31(a = .80, b = .20), 32(b = .50, c = .50), 33(a = .10, b = .90)
*GP-1**	a(1–19)	20(a = .38, b = .62)
*Gtdh**	a(1, 2, 4–15, 17–20)	3(a = .95, b = .05), 16(a = .92, b = .08)
*G6pdh**	b(2, 3, 7, 8, 11–15, 17–19), c(9)	1(a = .14, b = .86), 4(a = .22, b = .58, c = .19), 5(b = .83, c = .17), 6(a = .33, b = .67), 10(b = .80. c = .20), 16(b = .67, c = .33), 20(b = .75, c = .25)
*Gdh**	d(6, 7, 11–13, 16–18), f(14)	1(c = .14, d = .77, e = .09), 2(c = .44, d = .50, e = .06), 3(b = .10, c = .05, d = .50, e = .35), 4(e = .09, d = .91), 5(c = .67, d = .16, e = .17), 8(c = .15, d = .85), 9(d = .50, e = .50), 10(d = .79, e = .21), 15(d = .50, f = .50), 19(d = .95, g = .05), 20(a = .56, d = .33, g = .11)
*Hadh**	c(1, 6–15, 17–19)	2(a = .35, c = .60, d = .05), 3(e = .86, d = .14), 4(a = .30, d = .67, d = .03), 16(a = .08, c = .92), 20(c = .92, d = .08)
Sod†	a(19–32)	33(a = .85. b = .15)

* Loci used for populations 1–20 only.
† Sod used only for populations 19–33.

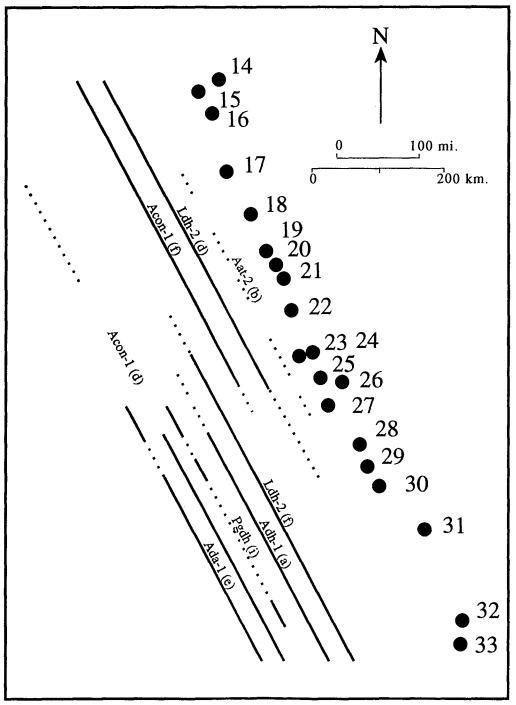

FIG. 2. Distribution of variants for eight proteins among the populations of *platensis,* which are aligned according to their map positions (cf. fig. 1). A solid line indicates a frequency greater than 0.25, and a dotted line indicates presence but at a frequency of 0.25 or less. Populations numbers are according to table 1, and frequencies are from tables 2 and 4.

and found to be left-skewed ($g_1 = -0.3$). All rootings between subspecies in all trees show *oregonensis* (including *picta* for this analysis) and *klauberi* as monophyletic groups. No trees support *platensis* as a monophyletic group. When *platensis* is forced to be monophyletic, the tree is 10 steps longer than the most parsimonious trees. For *platensis,* even groups of populations that display weak differentiation (e.g., our northern *platensis*) are not monophyletic. The tree displayed shows *croceater* as a paraphyletic group; *croceater* is monophyletic in some trees. A representative tree is presented as a phylogram (fig. 5A), showing minimum, average, and maximum branch lengths over all possible reconstructions using McClade (Maddison and Maddison 1992).

In the vicinity of Lassen Peak, *oregonensis* and *platensis* approach to within 8 km with no evident intergradation, as judged either by color pattern or by allozymes (fig. 6). The area immediately to the west and northwest of Lassen Peak has low population density, and specimens are

TABLE 5. Genetic distances for study 1. Above diagonal, Nei (1978) genetic distance; below diagonal, Nei (1972) genetic distance.

Population	31	32	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
31	—	0.014	0.032	0.047	0.167	0.101	0.121	0.130	0.378	0.366	0.358	0.336	0.399	0.451	0.467	0.494	0.451
32	0.022	—	0.029	0.028	0.132	0.078	0.095	0.106	0.375	0.369	0.375	0.352	0.412	0.453	0.468	0.493	0.455
34	0.043	0.040	—	0.015	0.082	0.051	0.058	0.064	0.371	0.316	0.360	0.336	0.399	0.414	0.425	0.487	0.418
35	0.060	0.041	0.030	—	0.045	0.049	0.022	0.033	0.381	0.375	0.383	0.364	0.431	0.482	0.478	0.520	0.482
36	0.177	0.141	0.093	0.059	—	0.038	0.015	0.056	0.421	0.391	0.424	0.389	0.391	0.410	0.425	0.467	0.413
37	0.114	0.091	0.066	0.067	0.052	—	0.041	0.091	0.442	0.421	0.450	0.411	0.411	0.430	0.437	0.481	0.438
38	0.128	0.101	0.067	0.033	0.023	0.052	—	0.021	0.367	0.350	0.367	0.339	0.399	0.435	0.428	0.484	0.438
39	0.142	0.118	0.079	0.050	0.070	0.108	0.032	—	0.376	0.357	0.374	0.356	0.425	0.478	0.446	0.528	0.470
40	0.384	0.381	0.380	0.392	0.427	0.453	0.371	0.386	—	0.026	0.052	0.080	0.126	0.142	0.135	0.150	0.139
41	0.379	0.382	0.331	0.393	0.405	0.439	0.362	0.375	0.037	—	0.029	0.049	0.091	0.085	0.082	0.125	0.080
42	0.369	0.385	0.373	0.398	0.435	0.466	0.375	0.389	0.060	0.044	—	0.006	0.073	0.079	0.076	0.092	0.077
43	0.344	0.360	0.347	0.377	0.398	0.424	0.346	0.368	0.087	0.062	0.017	—	0.070	0.072	0.074	0.095	0.066
44	0.406	0.418	0.408	0.442	0.398	0.422	0.403	0.436	0.131	0.103	0.082	0.077	—	0.015	0.024	0.027	0.012
45	0.457	0.459	0.422	0.492	0.416	0.440	0.439	0.488	0.145	0.096	0.087	0.078	0.019	—	0.018	0.017	0.003
46	0.476	0.476	0.436	0.491	0.434	0.450	0.434	0.459	0.142	0.096	0.087	0.083	0.031	0.024	—	0.014	0.012
47	0.507	0.505	0.501	0.537	0.480	0.498	0.494	0.544	0.160	0.142	0.107	0.108	0.037	0.027	0.027	—	0.022
48	0.461	0.465	0.431	0.497	0.425	0.453	0.446	0.485	0.147	0.095	0.089	0.077	0.020	0.011	0.023	0.036	—

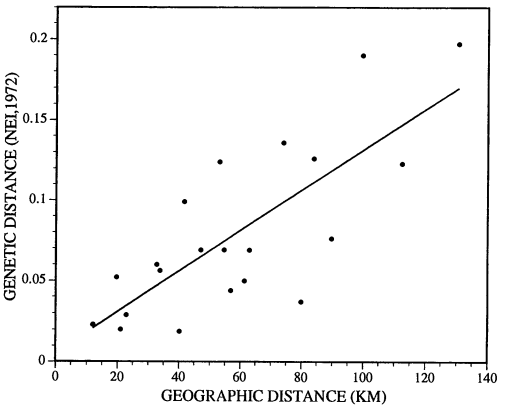

FIG. 3. Pattern of relation of geographic distance to Nei (1972) genetic distance across northern California populations of *oregonensis* (4–10). Although the points are not independent, a regression is shown to illustrate the linear relationship discussed in the text.

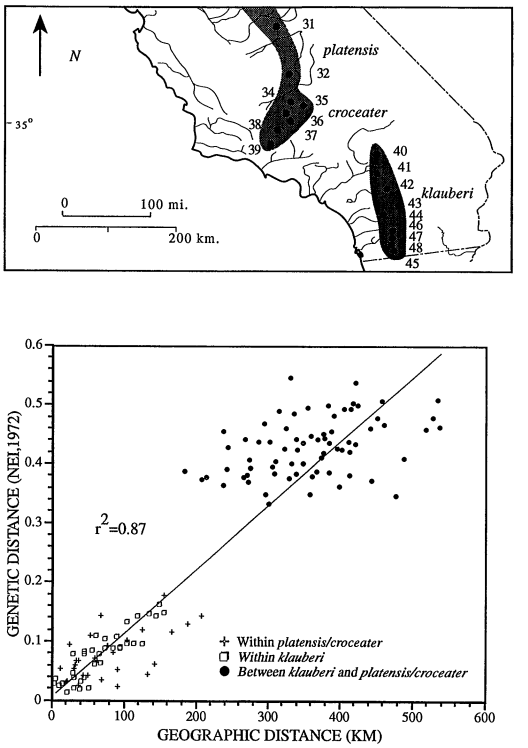

FIG. 4. Pattern of relation of geographic distance to Nei (1972) genetic distance within southern *platensis-croceater,* within *klauberi,* and between the two groups (upper cluster of points). Although the points are not independent, a regression is shown to illustrate the linear relationship discussed in the text.

difficult to find. We have located a few specimens from sites near and between our populations 13 (morphologically and genetically similar to *oregonensis*), 14, and 15 (the latter two are morphologically and genetically similar to northern *platensis*). In all instances, these small samples (secondary localities in fig. 6) are readily identifiable to subspecies on morphological criteria, and these identifications are supported by allozyme data (not presented here). The genetic distances in this area (fig. 6) are inflated locally in the vicinity of populations 13–15 by the presence of unique variants noted earlier for population 14.

Ordination of Genetic Distances.—Multidimensional scaling of genetic distances is a useful technique for exploratory analysis of the geography of genetic variation. The technique simplifies representation of the genetic distance data without imposing a hierarchical structure (Felsenstein 1982; Lessa 1990). Multidimensional scaling can therefore be heuristic in detecting clinal or reticular associations that would be missed by phenetic clustering of populations, as in UPGMA. If genetic distances reflect isolation by distance, then coordination of the genetic distances should roughly correspond to a geographic map of the populations sampled (Felsenstein 1982). The first two axes, when appropriately rotated, are expected to display the populations arrayed in the same order as they are geographically; deviance from the map indicates either higher or lower amounts of gene flow than characteristic of the group of populations as a whole. Whereas Felsenstein (1982, p. 10) cautions that historical branching events could lead to the false impression of gene flow, we have avoided this problem by independently testing for gene flow (see below). The results of multidimensional scaling are the same whether Nei (nonmetric) or Rogers (metric) genetic distances are used (see also Lessa 1990). We have used the former to be consistent with other genetic distances used in this paper.

Within *oregonensis* and *platensis,* multidimensional scaling of the matrix of genetic distances produces an array that roughly corresponds to a geographic map of the populations, with eastern (e.g., 8–13) and western (e.g., 3) populations of *oregonensis* (as well as 2, *picta*), and northern (e.g., 14–26) and southern (e.g., 28–33) populations of *platensis,* lying at opposite poles (fig. 7). Populations of the two subspecies lie along different planes. A gap exists between the two taxa and where the northern (e.g., 14–26) pop-

TABLE 6. Genetic distances for study 2. Above diagonal, Nei 1978; below diagonal, Nei 1972.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	—	.185	.097	.083	.043	.054	.037	.078	.099	.098	.131	.150	.114	.293	.209	.200
2	.199	—	.159	.102	.213	.270	.262	.288	.291	.318	.306	.396	.345	.400	.326	.356
3	.109	.173	—	.114	.136	.168	.141	.201	.222	.238	.263	.294	.250	.345	.248	.269
4	.092	.113	.123	—	.043	.117	.088	.129	.184	.185	.195	.237	.204	.339	.309	.299
5	.055	.227	.148	.052	—	.047	.016	.060	.118	.109	.151	.172	.134	.340	.282	.265
6	.064	.281	.178	.124	.056	—	.012	.013	.038	.025	.055	.075	.040	.331	.234	.217
7	.051	.277	.155	.099	.029	.023	—	.049	.059	.060	.104	.118	.073	.377	.271	.256
8	.088	.299	.211	.136	.069	.020	.060	—	.062	.038	.049	.073	.050	.314	.241	.196
9	.108	.301	.230	.190	.126	.044	.069	.069	—	.008	.068	.070	.019	.319	.209	.199
10	.113	.335	.253	.197	.123	.037	.076	.050	.019	—	.036	.045	.008	.334	.236	.230
11	.142	.319	.274	.203	.162	.063	.116	.057	.075	.049	—	.026	.022	.369	.269	.269
12	.158	.405	.301	.241	.179	.080	.126	.078	.074	.055	.031	—	.025	.359	.283	.276
13	.124	.356	.260	.211	.143	.047	.084	.057	.025	.020	.030	.030	—	.338	.238	.229
14	.304	.412	.356	.347	.350	.339	.388	.322	.326	.346	.378	.364	.346	—	.102	.123
15	.226	.344	.265	.323	.298	.248	.289	.256	.222	.255	.284	.295	.252	.116	—	.060
16	.214	.376	.288	.315	.283	.232	.275	.212	.214	.250	.285	.289	.244	.139	.082	—
17	.204	.347	.245	.293	.271	.230	.278	.215	.241	.255	.275	.287	.255	.106	.050	.060
18	.167	.318	.208	.245	.222	.194	.241	.187	.219	.231	.271	.281	.244	.154	.058	.061
19	.194	.331	.229	.280	.262	.217	.253	.222	.190	.228	.269	.252	.220	.150	.040	.059
20	.158	.316	.204	.232	.203	.170	.217	.166	.199	.206	.246	.255	.219	.174	.059	.075
21	.170	.312	.209	.248	.231	.193	.240	.191	.208	.226	.264	.274	.234	.167	.048	.069
22	.174	.306	.213	.252	.235	.209	.250	.210	.239	.256	.286	.305	.267	.179	.057	.079
23	.174	.318	.214	.251	.231	.206	.248	.205	.228	.239	.289	.299	.258	.170	.057	.070
24	.185	.315	.218	.257	.237	.231	.255	.235	.263	.277	.323	.339	.297	.194	.075	.091
25	.187	.303	.225	.263	.245	.232	.265	.230	.259	.273	.304	.332	.290	.182	.063	.084
26	.209	.343	.241	.283	.259	.255	.278	.255	.294	.313	.350	.363	.324	.216	.094	.097
27	.209	.351	.240	.275	.259	.266	.273	.276	.307	.328	.380	.390	.346	.277	.188	.178
28	.299	.456	.349	.366	.341	.348	.356	.366	.390	.406	.482	.496	.441	.404	.283	.282
29	.352	.519	.407	.429	.399	.402	.414	.421	.444	.463	.546	.558	.499	.445	.311	.313
30	.361	.499	.413	.419	.397	.419	.419	.439	.461	.483	.571	.586	.524	.464	.343	.326
31	.430	.593	.496	.502	.454	.465	.462	.489	.509	.522	.621	.635	.563	.562	.403	.395
32	.358	.491	.412	.432	.409	.417	.418	.440	.455	.472	.556	.577	.515	.486	.406	.371
33	.415	.565	.459	.495	.477	.494	.493	.521	.536	.541	.642	.670	.601	.567	.484	.467

ulations of *platensis* and the eastern (e.g., 8–13) populations of *oregonensis* meet near Lassen Peak, the two planes diverge. Some populations of the two taxa are closer in multidimensional space to each other than they are to geographically remote members of their own group. The multidimensional scaling (fig. 7) shows that a cluster of populations (15–26) in the northern Sierra Nevada is nearly undifferentiated. At both the northern (population 14) and southern (population 27) ends of this region of relative uniformity are instances of much genetic change across short geographic distances.

Isolation by Distance.—To test the hypothesis that isolation by distance is taking place in *Ensatina,* the data for 23 populations (from populations 1–33) were analyzed using a program developed by Slatkin (1993) to determine if they fit his model of isolation by distance. Pairwise comparisons of $\hat{M}$, a measure of gene flow (Nm), were calculated. Values of M can range from 0 to infinity, but values greater than 1 indicate high levels of gene flow, more than one migrant per generation. In the northern part of the range (*picta* and *oregonensis*), all values between nearest neighbors exceed 1, with the exception of a single comparison, Buckhorn Summit (population 8) to Hazel Creek (population 9), which is a little less than 1. The geographic distance between these populations is the greatest nearest-neighbor distance among the populations sampled for this analysis. In the northern Sierra Nevada, all values between Yankee Jim (population 19) and Tuolumne (population 25) are greater than 1, indicating that this group of populations has experienced recent gene flow. From Yankee Jim to Kern River (population 33) all neighboring populations have values of M exceeding 1 except for an area in the middle of the range on either side of Wagner Ridge (population 27), where nearest-neighbor values are 0.62 and 0.65. From Wagner Ridge to the south, the only values of M that

TABLE 6. Extended.

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
.193	.160	.185	.147	.161	.165	.166	.174	.175	.198	.195	.288	.323	.346	.414	.340	.405
.334	.309	.320	.303	.302	.296	.309	.303	.290	.331	.335	.443	.488	.482	.575	.472	.554
.235	.201	.220	.193	.200	.204	.206	.207	.213	.230	.226	.338	.377	.398	.480	.394	.449
.285	.240	.274	.224	.242	.245	.246	.249	.254	.275	.263	.358	.402	.407	.488	.418	.488
.261	.215	.253	.192	.223	.227	.223	.227	.234	.249	.246	.330	.370	.382	.438	.392	.468
.222	.190	.211	.162	.187	.203	.201	.223	.223	.247	.254	.339	.375	.407	.451	.402	.487
.266	.232	.243	.205	.230	.240	.239	.243	.252	.266	.258	.344	.383	.403	.444	.399	.482
.207	.182	.216	.157	.184	.204	.199	.227	.221	.247	.264	.357	.394	.426	.475	.425	.513
.234	.215	.185	.192	.203	.234	.224	.256	.251	.287	.297	.383	.418	.450	.496	.442	.530
.242	.222	.216	.193	.215	.244	.228	.263	.260	.300	.311	.393	.431	.466	.503	.452	.529
.266	.266	.262	.236	.256	.279	.283	.314	.295	.341	.367	.472	.519	.558	.606	.540	.634
.282	.279	.248	.249	.270	.301	.296	.333	.325	.357	.381	.490	.534	.576	.623	.565	.665
.247	.239	.213	.210	.228	.261	.253	.288	.282	.316	.334	.433	.473	.511	.550	.500	.594
.097	.149	.143	.165	.161	.172	.164	.185	.173	.207	.265	.395	.418	.451	.548	.471	.559
.035	.046	.027	.044	.035	.044	.044	.060	.047	.079	.170	.268	.277	.324	.382	.384	.470
.044	.048	.044	.058	.054	.064	.057	.074	.067	.081	.158	.265	.278	.305	.373	.347	.451
—	.047	.059	.046	.043	.050	.047	.061	.054	.086	.135	.247	.259	.295	.374	.347	.442
.052	—	.021	.002	.007	.009	.003	.022	.014	.038	.106	.191	.199	.239	.312	.300	.373
.067	.025	—	.023	.020	.031	.025	.044	.034	.062	.132	.212	.221	.263	.331	.329	.403
.055	.008	.031	—	.001	.008	.004	.022	.016	.039	.110	.179	.183	.229	.288	.297	.373
.050	.011	.026	.009	—	.006	.005	.024	.018	.043	.118	.201	.208	.252	.315	.319	.387
.057	.012	.037	.016	.011	—	.002	.007	.002	.026	.097	.183	.177	.226	.292	.288	.358
.054	.006	.030	.011	.009	.007	—	.009	.007	.028	.095	.174	.175	.214	.281	.281	.351
.070	.028	.052	.032	.032	.015	.015	—	.000	.007	.066	.155	.156	.183	.244	.239	.320
.064	.020	.042	.026	.025	.009	.013	.010	—	.019	.088	.174	.174	.209	.274	.267	.340
.095	.043	.070	.048	.050	.033	.034	.016	.029	—	.092	.188	.193	.212	.231	.262	.355
.147	.115	.143	.123	.129	.108	.105	.079	.101	.105	—	.092	.091	.101	.175	.140	.230
.257	.197	.220	.189	.209	.191	.181	.165	.184	.197	.106	—	.007	.000	.042	.062	.116
.286	.223	.247	.211	.234	.203	.201	.184	.203	.221	.122	.035	—	.006	.058	.061	.155
.308	.249	.275	.242	.264	.238	.225	.196	.223	.225	.117	.013	.038	—	.027	.056	.118
.389	.323	.344	.303	.328	.305	.293	.259	.289	.246	.193	.057	.091	.046	—	.092	.148
.363	.312	.343	.313	.333	.302	.294	.255	.284	.278	.159	.078	.095	.076	.114	—	.082
.450	.377	.410	.382	.393	.365	.356	.328	.348	.363	.242	.125	.182	.130	.162	.097	—

exceed 1 are among nearest neighbors, with two exceptions (in both instances, second nearest neighbors). At the southern end of the Sierra Nevada two nearest-neighbor values are a little less than 1 (0.88 and 0.92). These results support our interpretation of isolation by distance within each of the subspecies.

The plot of geographic versus genetic distances (fig. 2) for our northern samples (1–13) shows a pattern of increasing genetic distance as geographic distance increases. The outlying populations above the diagonal involve comparisons with the westernmost populations, and those below the diagonal involve comparisons with the easternmost populations. This is the pattern predicted if dispersal has taken place from the west to the east (D. Good in prep.; see below). The plot of geographic versus genetic distances (fig. 4) for southern samples of *platensis,* for *croceater,* and for *klauberi* shows a pattern of increasing genetic distance as a function of geographic distance for the combined *platensis-croceater* sample, and for *klauberi.* For the comparison of *klauberi* with *platensis-croceater,* there is a relatively wide scatter. However, a regression through all of the points extends through the origin as do regressions through the within-group comparisons. The regression for the between-group comparisons alone is much flatter with an intercept high on the ordinate.

Effects of Extinction on Patterns of Population Relationships.—We conducted an "extinction experiment" to test the effects of the disappearance of a group of contiguous populations on our phylogenetic analysis. The intent of this experiment is to determine the impact of a recent extinction, such as may have occurred in "Bob's Gap." If isolation by distance is occurring, elimination of some populations (the number depends on the scale of isolation by distance and the distribution of the populations) should produce diagnosable units of the sort that would be

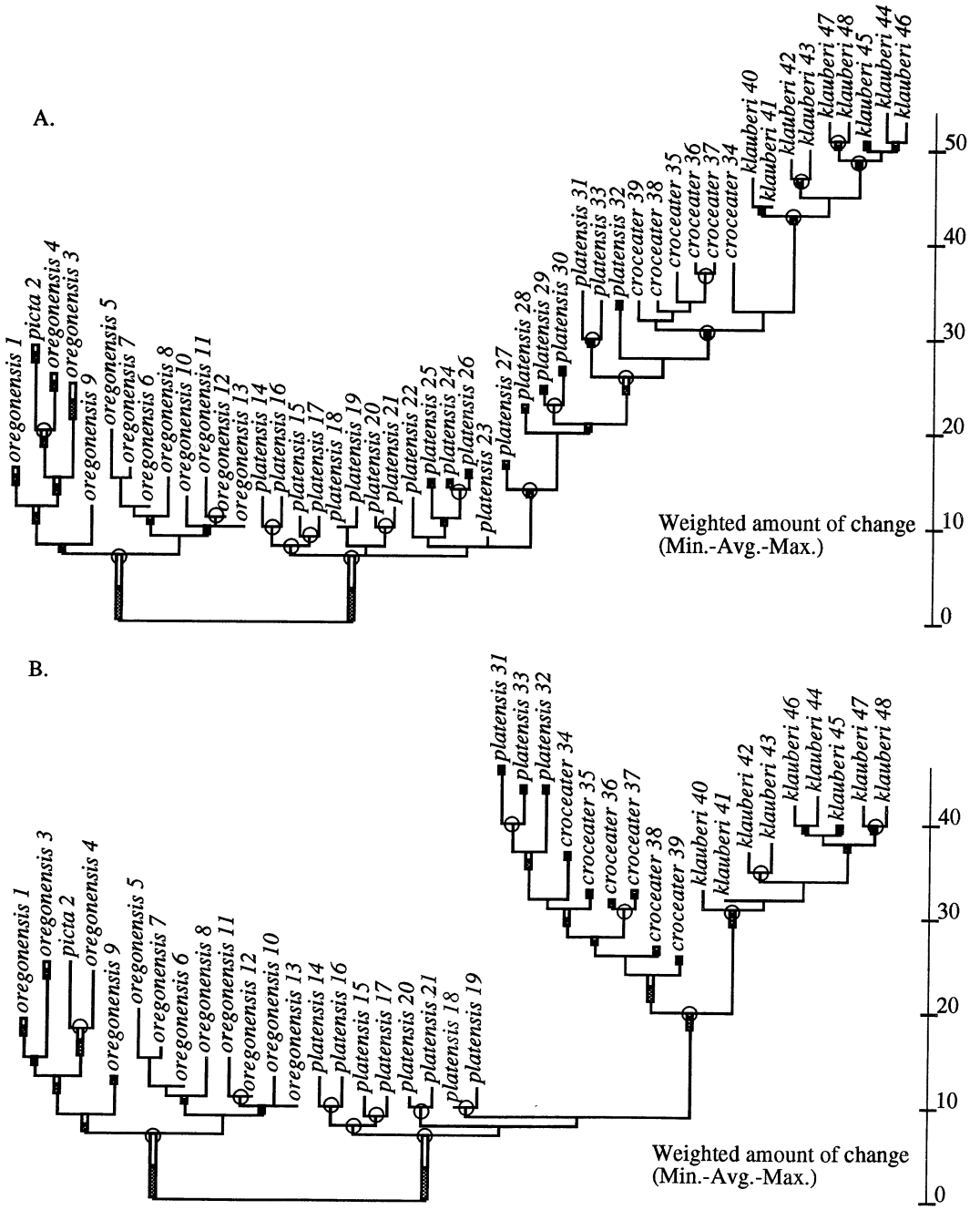

FIG. 5. Phylogenetic analysis of protein variants. (A) One of 1892 equally parsimonious trees having similar topologies, presented as a phylogram. The branch lengths show the minimum, average, and maximum (set at maximum) number of steps using the weighted changes described in the text. Scale at right is the number of steps. Circled nodes are present in all trees. (B) Extinction experiment. A phylogenetic analysis as in (A), but with populations 22–30 removed. This is one of 92 equally parsimonious trees having similar structure. Artificial removal of nine populations produces cladistic structure for southern *platensis* + *croceater* + *klauberi*, with the same number of steps as found in (A) for *klauberi* alone (see text for details).

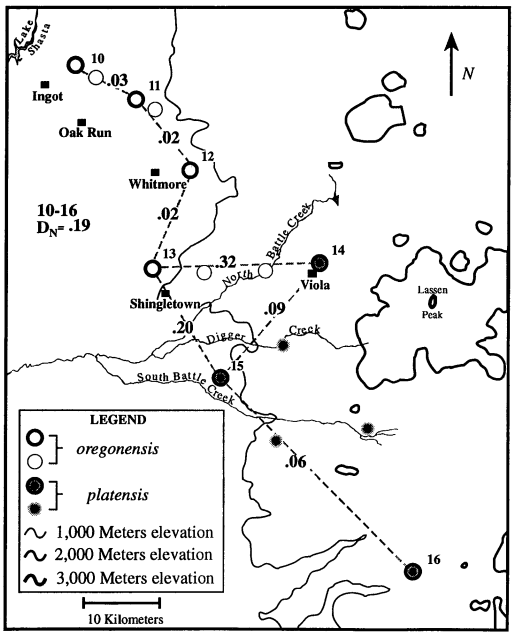

FIG. 6. Lassen Peak region, showing the genetic distances across the contact zone between *oregonensis* and northern *platensis*. Numbered spots refer to sample sites (table 1). Secondary localities with one or two individuals are indicated without numbers but identified taxonomically by morphology and allozyme profiles; these small samples were not used in calculations of genetic distances.

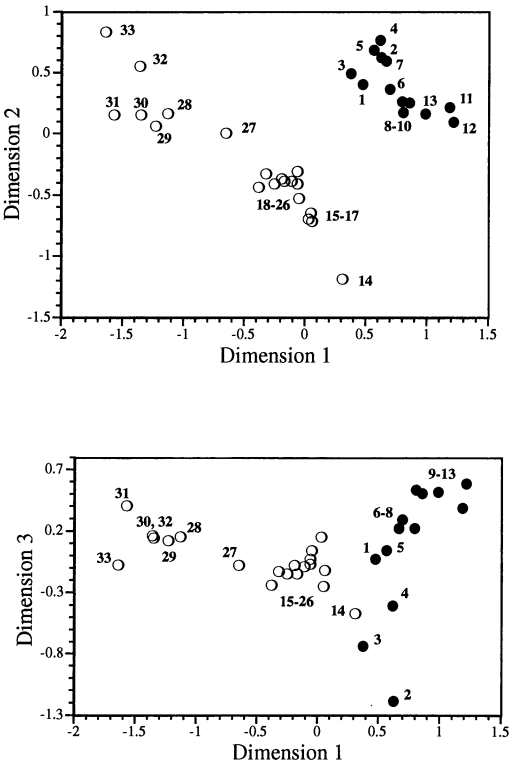

FIG. 7. Multidimensional scaling of genetic distances. Filled circles are *picta* (population 2) and *oregonensis* (populations 1 and 3–13); open circles are *platensis* (populations 14–33). The distribution of *platensis* in the first and second dimensions corresponds roughly to the geography of the populations, aligned with a northwest-southeast axis from 14 to 33 (cf. fig. 1). The distribution of *picta-oregonensis* in the first and third dimensions corresponds roughly to the geography of the populations, except for population 1, aligned with a west-east axis from 2 to 13 (cf. fig. 1).

worthy of taxonomic recognition. We measured the straight-line geographic distance between populations 39 and 40 (on both sides of "Bob's Gap") and then centered an equivalent distance on central Sierran population 27 (see above). The experiment consisted of eliminating populations 22 through 30 and repeating the phylogenetic analysis. The "extinction" creates two distinct groups separated by many steps (fig. 5B). From 11–13 steps (depending on the tree) exist between the remaining northern *platensis* and a cluster including the remaining southern *platensis* + *croceater* + *klauberi*; this approximates the number of steps (10–12) separating *klauberi* from the other populations in both the original and the experimental treatments (fig. 5A, B).

DISCUSSION

Genetic distances in the *Ensatina* complex can be surprisingly large between geographically distant populations within a subspecies. This is the consequence of a general pattern in which genetic distances build gradually as a function of geographic distance, without any evident large break between groups of populations (figs. 3, 4). We postulate a pattern of variation that reflects two phenomena: (1) a general pattern of directional dispersal from west to east in northern California and from north to south along the cordilleran axis, and (2) isolation by distance within recognized taxa. As a result, gene flow throughout the populations studied is slight, and thus genetic distances over geographic distances of the magnitude typical of this study are relatively large. Geographically remote populations within a subspecies are linked by gene flow on a much longer time scale than are contiguous populations; over geological time, gene flow is sporadic, occurring during moister periods when favorable habitats are more continuous. The dichotomy between "ongoing" and "historical" gene flow is artificial;

gene flow occurs on a continuum of scales from recent to ancient, and different scales are detectable in *Ensatina.* On the one hand, we see distant historical events as responsible for the large divergence of the southern *platensis, croceater,* and *klauberi* relative to *oregonensis,* but on the other we see evidence of ongoing or at least recent gene flow in the northern *platensis.*

The pattern of isolation by distance shown by populations of *oregonensis* in figure 2 is consistent with a model of gradual range expansion proposed by Good (in prep.) and tested using a simulation approach developed by Slatkin (1993). According to this argument, stepwise migration in the northern California populations of *oregonensis* that we studied appears to have been from west to east, in accord with the biogeographic scenario of Stebbins (1949).

A relatively large, geographically localized genetic break is found between *oregonensis* and *platensis* in the Lassen Peak area, but genetic distances are lower and more alleles are shared between northern *platensis* and *oregonensis* than between northern and southern *platensis.* We suggest that gene flow took place more recently between northern *platensis* and *oregonensis* than within the range of either subspecies as a whole. Whereas the two subspecies are distinctly different in color pattern and allozymes where they come into contact, the genetic distinction breaks down as one moves away from the immediate zone of contact. Furthermore, in populations north of Lassen Peak that we assign to *oregonensis* (e.g., 10, 11 and 12) individuals are found with color patterns that would qualify as *oregonensis-platensis* intergrades using the criteria of Stebbins (1949).

The area west of Lassen Peak has probably witnessed much local extinction and recolonization. At least three factors contribute to this phenomenon. The southern Cascade range has experienced extensive recent volcanism (Lassen Peak has been active in this century). Many large lava flows exist, and much of the region is unsuitable habitat for *Ensatina.* This is an upland area that was subject to glaciation during Pleistocene times when ice extended as low as about 1500 m and the regional snow line (roughly the level of an average temperature of 0 in the warmest month) was about 2000 m lower than at present (about 4200 m) (Kane 1982). Much of the usable habitat for *Ensatina* would have been eliminated during these periods. We postulate that repeated incidents of extinction and subsequent recolonization (from the northwest and the south) associated with these events may have led to sorting of genetic variants (e.g., by founder effects) and consequent large local genetic distances (fig. 6).

Populations of *platensis* in the northern part of the range (populations 15–26) are relatively undifferentiated genetically (average $D_N = 0.044$). These populations contain a mixture of alleles characteristic of *oregonensis,* on the one hand, and of more southern *platensis,* on the other (table 3). In contrast to the weak differentiation of the northern populations, southern *platensis,* populations 27–33, not only are much more differentiated but also show isolation by distance, with genetic distance accumulating over geographic distance (discussed below).

We examined patterns of relationships among the populations studied by conducting phylogenetic analyses, which impose a hierarchy on what we believe is a network of interactions that is only partially hierarchical (fig. 5). No reason exists to believe that hierarchical representations are appropriate for patterns of within-group variation for northern *platensis, oregonensis* plus *picta,* southern *platensis* plus *croceater,* or *klauberi.* However, the possibility exists that vicariant events may have contributed to the patterns discerned: a secondary contact zone gives identity to *oregonensis* and *platensis* north and west of Lassen Peak, there is a region of reduced gene flow on both sides of the Wagner Ridge population (27) in the central Sierra Nevada that might be interpreted as another region of secondary contact, and there is an apparent geographic gap (Bob's Gap) between *croceater* and *klauberi.* If admixture or reticulation has been associated with vicariant events, as we will argue, evidence should be found in phylogenetic trees. So long as the trees are not rooted within either *oregonensis* or *klauberi,* the northern *platensis* populations that we hypothesize to be admixed should appear near the base of the trees, as is the case (fig. 5); hybrid populations typically appear in basal positions in cladistic analyses (e.g., McDade 1992). Further support for the hypothesis of vicariance and subsequent recontact with admixture comes from a neighbor-joining analysis of genetic distance data (not shown) in which northern *platensis* populations are not only basal but have very short branch lengths, an expected characteristic of populations arising from admixture (Bowcock et al. 1991; Cavalli-Sforza and Piazza 1975).

We interpret the weak geographic differentia-

tion of northern *platensis* to be the result of admixture of populations of *oregonensis* and southern *platensis* ancestry. We postulate that populations similar in coloration to present-day *platensis* expanded rapidly northward, possibly after having evolved in the south and being isolated from more northern populations by factors associated with Pleistocene glaciation in the central Sierra Nevada (see below). These northward dispersing populations mixed with resident populations that may have been more like *oregonensis* in coloration [possibly resembling the populations that Stebbins (1949) identified as *oregonensis-platensis* intergrades, but which we find to be genetically identifiable as *oregonensis*]. This argument assumes that the color pattern of *platensis,* apparently cryptic (Stebbins 1949; Brown 1974), is adaptively superior to that of *oregonensis* in the northern portion of the Sierra Nevada. Our scenario envisions the *platensis* color pattern spreading rapidly to the north, replacing the *oregonensis* pattern; however, the less adaptive, or selectively neutral, protein variants of the merging populations would have mixed in a more haphazard manner. The northward movement of the adaptive phenotype was curtailed by the same climatic and geologic factors that led to restrictions or cessation of gene flow, thereby establishing the current *platensis-oregonensis* border. The mitochondrial genes have moved even more slowly than selectively neutral allozymes. A large break is evident between northern and southern *platensis* in mtDNA sequences (Moritz et al. 1992). The point at which allozyme distances change from uniformity to isolation by distance (between populations 26 and 27) does not correspond to the break in mitochondrial types, which recently has been pinpointed between populations 24 and 23, within the allozymically uniform group of *platensis* (Schneider and Wake in prep.).

We suspect that the history of *Ensatina* has seen extensive admixture following local extinction and recolonization events at various points in the chain. We still see evidence of the past separation in *platensis,* but elsewhere in the chain of populations these contact zones mainly have been obliterated by subsequent gene flow.

From the perspective of our allozyme data, *croceater* is not detectable, either by phenetic or cladistic analysis. Isolation by distance occurs throughout southern *platensis* and continues without interruption into *croceater.* The two subspecies differ in color pattern, and there is a narrow transition zone between the two. The blotches become less numerous, larger, and more clearly defined as one moves into the southern Sierra Nevada, and in the lower Kern River Canyon the color of the blotches changes from red orange to lemon yellow across the river. Individuals with red-orange spots are found occasionally on the south side of the river, but from that point south, including the northernmost of the populations that are diagnosed by allozymes as *klauberi* (40), the spots are lemon yellow.

The subspecies *klauberi* is diagnosable by our allozyme data and by mtDNA sequence data (Moritz et al. 1992). However, from figure 4 it is unclear whether the allozymic differences found between *croceater* and *klauberi* reflect lack of information about "Bob's Gap" or an older vicariant event. A regression line through all of the points in figure 4 goes through the origin, as would be expected in the case of isolation by distance with very recent extinction. The populations that Stebbins (1949) identified as *croceater-klauberi* intergrades on the basis of coloration fall out with *klauberi* genetically; isolation by distance is relatively great within *klauberi,* and there is allozymic differentiation from the northern to the southern end of its range. We cannot eliminate the possibility that "Bob's Gap" was occupied until recently by populations that were similar to *croceater* in coloration, as are the northern populations that are diagnosed by allozymes as *klauberi.* These populations may have shown a pattern of isolation by distance like those of the combined southern *platensis-croceater-klauberi* data set. R. Stebbins and D. Wake think it possible that populations of blotched *Ensatina* may remain undiscovered in the rugged San Gabriel Mountains.

Our "extinction experiment" was designed to determine if a sudden geographic gap introduced into a continuous range of populations showing isolation by distance would lead to cladistic resolution, and it did. The number of steps separating populations 31–48 from the remaining populations in the north approximates the number separating *croceater* from *klauberi* over a similar geographic distance (fig. 5). The most important and general message from this experiment is that recent extinction can produce a pattern that is apparently hierarchical, even when the populations involved have been joined by intermediates with gene flow occurring between near neighbors until the moment of the extinction event. Extinction in such cases produces dis-

tinct groups of populations that would be interpreted by those with evolutionary species concepts as species, with no additional biological processes being necessary.

The biogeographic hypothesis of Stebbins (1949) predicts a continuous pattern of isolation by distance from *picta* and *oregonensis* through *platensis* and *croceater* to *klauberi,* with regions of low buildup of genetic distance as a function of geographic distance in the main body of the range of each subspecies and high buildup of genetic distance in the intergrade zones. Only one region, that including the northern populations of *platensis,* shows lower than average genetic differentiation. In contrast, genetic distance builds mainly as a function of geographic distance within southern *platensis-croceater,* within *klauberi,* and within our northern transect that includes one population of *picta,* some *picta-oregonensis* intergrades, and *oregonensis.*

In view of the above analysis, we propose a modified biogeographic hypothesis for the blotched forms of *Ensatina.* We postulate three major historical events, each of which is inferred from the integrated allozyme and the published mtDNA data (fig. 8). First, we hypothesize a vicariant event during which ancestors of a clade consisting of the populations currently grouped in the subspecies *klauberi, croceater,* and southern *platensis* (best seen in cladistic analyses of the cytrochrome B sequence data, Moritz et al. 1992) became separated from an ancestral group that resembled present-day *oregonensis* from northern California. However, *oregonensis* is so heterogeneous that some of its populations are more similar in allozymes to some northern *platensis* populations than they are to other *oregonensis.* The northern *platensis* mtDNA is so different from that of other *platensis* and from all *oregonensis* (which is also heterogeneous in mtDNA) so far discovered that it cannot be placed with confidence in any phylogenetic hypothesis (Moritz et al. 1992; Schneider et al. in prep.).

Second, following extinction of populations in the present-day northern and central Sierra Nevada, we hypothesize that southern *platensis* and *oregonensis* interacted to give rise to present-day northern *platensis.* This admixture is reflected in patterns of allele sharing between northern *platensis* and *oregonensis,* which has involved the flow of southern *platensis* alleles over an *oregonensis*-like (in allozymes and coloration) population that has largely preserved an ancient mtDNA (Moritz et al. 1992; Schneider et al. in prep.). Further support for this hypothesis is gained from the basal placement of northern *platensis* in the phylogenetic analyses. The existence of incipient blotching in populations of *oregonensis* in the extreme southeastern part of its range, just north of the Lassen Peak area, supports the hypothesis (Stebbins 1949; Brown 1974) of the adaptive value of this color pattern, and was the basis for the identification of these populations as intergrades by Stebbins (1949). Allozymes present in southern populations may have "hitchhiked" with the adaptively important alleles associated with the more organized blotching characteristic of southern *platensis* and moved through the resident populations as the color pattern moved northward. Males appear to be the dispersing sex in *Ensatina* (Stebbins 1954; Staub and Wake unpubl. data), and this may account for the lag in the northward spread of mtDNA relative to color pattern and allozymes. If the admixture detected in the allozyme data was mainly the result of unidirectional movement, the border between mtDNA types would also be expected to shift northward, but less than the most rapidly dispersing allozymes, because of the more sedentary nature of females. We believe this to be the case, because the border between the two major types of mtDNA detected in *platensis* (Moritz et al. 1992) occur within the allozymically more uniform group of northern *platensis* (Schneider et al. in prep.).

Third, more recently, following the first admixture-reticulation event, repeated vicariant events associated with volcanism and glaciation near Lassen Peak have locally amplified the differences between *oregonensis* and northern *platensis.* In the south, populations in the San Gabriel Mountains have largely and possibly completely disappeared, creating "Bob's Gap."

Taxonomic Implications

In northeastern California, the blotched and unblotched forms of the *Ensatina* complex approach each other very closely, within about 8 km, without showing morphological or genetic intergradation. We interpret this as a dynamic zone in which there has been a sequence of extinction and recolonization. The most recent colonizations have been from the south by *platensis* and from the north and west by *oregonensis.* Fixed genetic differences exist between the two forms where they contact each other, and the local populations are easily diagnosable. However, the situation is complex because of the possibility of a

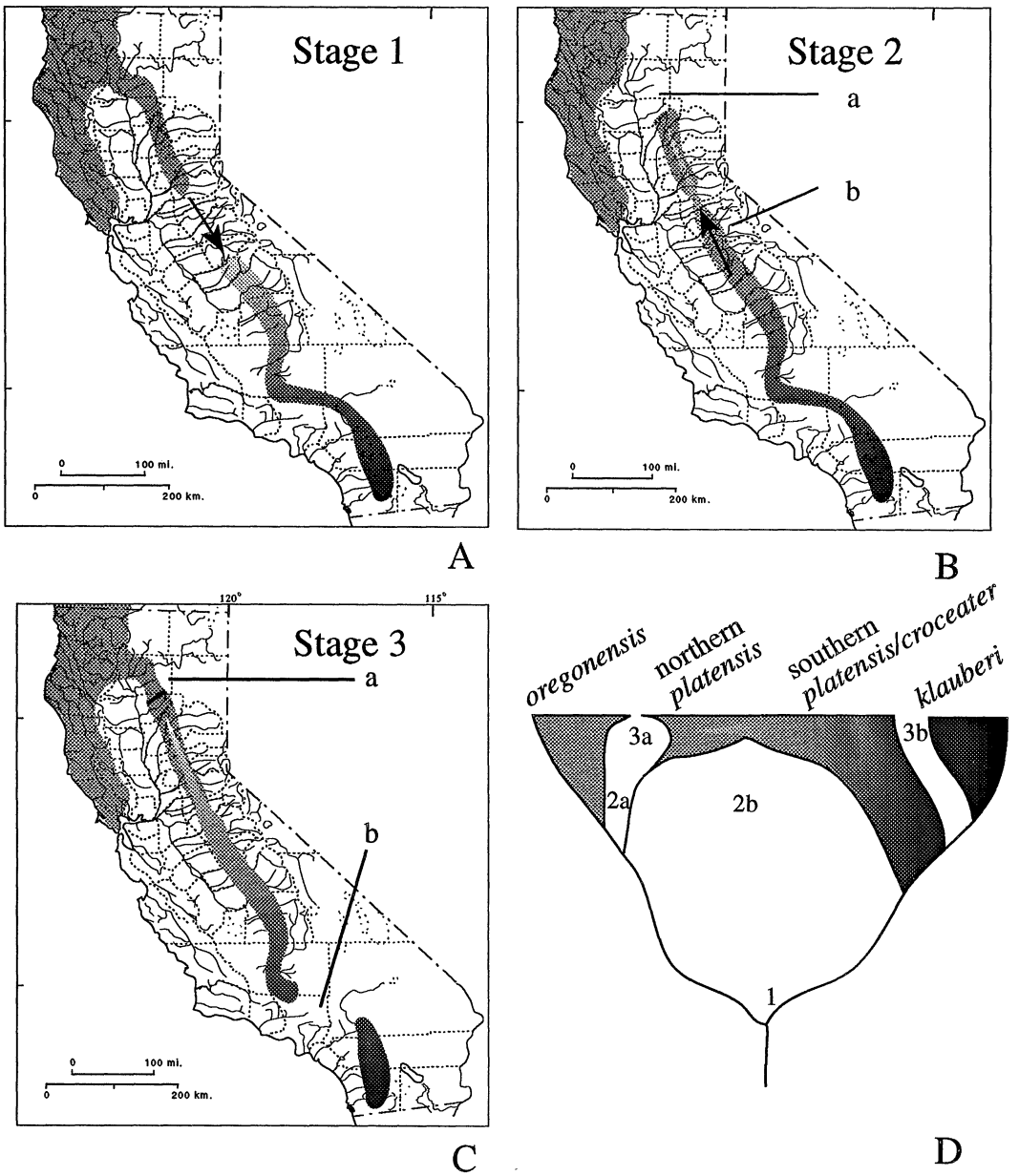

FIG. 8. Biogeographic scenario for the blotched forms of the *Ensatina* complex. (A) Stage 1. Hypothesized origin of the southern *platensis-croceater-klauberi* clade, and its isolation from more northern ancestors (*oregonensis*). (B) Stage 2, A. Separation of northern *platensis* from *oregonensis*. Stage 2, B. Northward movement of southern *platensis* to contact northern platensis. (C) Stage 3, A. Recontact of northern *platensis* and *oregonensis* in vicinity of Lassen Peak. Stage 3, B. Formation of "Bob's Gap" in the San Gabriel Mountains. (D) Tree illustrating the three stages. Note the diphyletic nature of *platensis*. Only geographically relevant populations of the paraphyletic *oregonensis* are indicated.

dual origin of *platensis*, and *platensis* is not diagnosable as a unit on character data from either allozymes or mtDNA (data herein; Moritz et al. 1992; Schneider et al. unpubl. data). Northern populations of *platensis* show greater allozymic resemblance to some populations of *oregonensis* than they do to southern *platensis*, and the sequences of mitochondrial cytochrome B that have

been studied are unique, and currently their phylogenetic placement is ambiguous (but most likely basal or nearly so). Only color pattern, as analyzed by Stebbins (1949), and the multidimensional analysis of genetic distance data presented herein (which, however, is confounded in that *croceater,* which has a different color pattern, is included) offer potentially diagnosable features for a taxon (*platensis*) that may be composite in origin.

Stebbins (1949) used color pattern to diagnose *croceater* in relation to both *platensis* and *klauberi.* Our protein data indicate that *croceater* and southern *platensis* form a continuous and intergrading group of populations showing isolation by distance. No allele data diagnose *croceater,* a *platensis* that excludes *croceater,* or a *croceater* (the older name) that includes *platensis.*

At present, the blotched forms of *Ensatina* are recognized as three subspecies of the *Ensatina eschscholtzii* complex. Because *klauberi* is sympatric with *eschscholtzii* in southern California, with only limited or no hybridization, and because it is physically separated from *croceater* by a substantial geographic gap, Frost and Hillis (1990) considered its status as an independent species to be obvious, and suggested that *klauberi* be recognized as a species taxon separate from the remaining members of the complex. Whereas *klauberi* is monophyletic and diagnosable, it is simply the end of a nearly continuous chain of populations; the advantage or desirability of raising it to species rank is unclear. Populations exist that are morphological intergrades between *oregonensis* and *platensis, platensis* and *croceater,* and *klauberi* and *croceater* (Stebbins 1949). We have shown that *klauberi* and *croceater* are separated by a genetic distance that approximates what would be predicted for the geographic distance, corrected for recent land movements, based on patterns elsewhere in the complex, and our "extinction experiment" shows that we can generate cladistic support for groups of populations we know to be united by gene flow simply by sudden elimination of the linking populations. The geographic gap between *klauberi* and *croceater,* if real, is likely to be recent in origin.

Our data fail to reject the general zoogeographic hypothesis of Stebbins (1949), although the general picture in northeastern California is more complicated than he believed was the case. To recognize *klauberi* as a separate species would leave behind a heterogeneous ancestral species that still contains rings within it (*xanthoptica* and *platensis* behave as separate species in the central Sierra Nevada, Wake et al. 1989; and *xanthoptica* and *oregonensis* meet in a secondary contact in Sonoma County, north of San Francisco Bay, Wake et al. unpubl. data); thus, the concept of a ring species is not at risk in whatever taxonomic decision is made concerning *klauberi.*

The *Ensatina* complex appears to be breaking up into units that are not yet fully distinct and which have complicated relationships to one another. Local and regional extinctions have occurred frequently, and if such extinctions occur in appropriate places and are not recolonized, the breakup itself will produce cladistically distinct units. Such extinctions in space, when they create cladistically distinct units, are logically equivalent to the kinds of extinction that are implicated in speciation (Nixon and Wheeler 1992). Already, some clusters of populations of *Ensatina* are "candidate species," and depending on one's taxonomic philosophy several options exist. A cohesive-species concept or a biological-species concept might continue to recognize a single species, because of their focus on process; an evolutionary or phylogenetic-species concept would minimally recognize *klauberi* as a distinct species, but at present might not go beyond that point. In the interests of taxonomic stability and because we cannot reject the Stebbins' scenario, we choose to recognize a single species and refer to the assemblage as the *Ensatina eschscholtzii* complex. To start taking apart the complex taxonomically before it is fully understood will serve no useful purpose.

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LITERATURE CITED

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourão, and S. Pérez-Salas. 1972. Enzyme variation in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics 70:113–139.

Bowcock, A. M., J. R. Kidd, J. L. Mountain, J. M. Herbert, L. Carotenuto, K. K. Kidd, and L. L. Cavalli-Sforza. 1991. Drift, admixture, and selection in human evolution. A study with DNA polymorphism. Proceedings of the National Academy of Sciences, USA 88:839–843.

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

Cavalli-Sforza, L. L., and A. Piazza. 1975. Analysis of evolution: evolutionary rates, independence, and treeness. Theoretical Population Biology 8:127–145.

Dobzhansky, T. 1958. Species after Darwin. Pp. 19–55 *in* S. A. Barnett, ed. A century of Darwin. Heinemann, London.

Felsenstein, J. 1982. How can we infer geography and history from gene frequencies? Journal of Theoretical Biology 96:9–20.

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

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam.

Kane, P. 1982. Pleistocene glaciation, Lassen Volcanic National Park. California Geology 35:95–105.

Lessa, E. 1990. Multidimensional analysis of geographic genetic structure. Systematic Zoology 39: 242–252.

Maddison, W. P., and D. R. Maddison. 1992. MacClade, Version 3.01; analysis of phylogeny and character evolution. Sinauer, Sunderland, Mass.

McDade, L. 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46:1329–1346.

Moritz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Zoology 41:273–291.

Nei, M. 1972. Genetic distance estimates between populations. American Naturalist 106:283–292.

———. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nixon, K. C., and Q. D. Wheeler. 1992. Extinction and the origin of species. Pp. 119–143 *in* M. J. Novacek and Q. D. Wheeler, eds. Extinction and phylogeny. Columbia University Press, New York.

Rohlf, F. J. 1989. NTSYS Version 1.5. Numerical taxonomy and multivariate analysis system. Stony Brook, N.Y.

Schoenherr, A. 1976. The herpetofauna of the San Gabriel Mountains, Los Angeles County, California. Special Publication of the Southwestern Herpetological Society 1–95.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics University of Texas Publications 6:49–90.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47: 264–279.

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

———. 1954. Natural history of the salamanders of the plethodontid genus *Ensatina*. University of California Publications in Zoology 54:47–124.

Swofford, D. C. 1991. PAUP: Phyolgenetic analyses using parsimony, version 3.0 Computer program distribution. Illinois Natural History Survey Champaign, Il.

Swofford, D., and R. B. Selander. 1981. A computer program for the analysis of allelic variation in genetics. Journal of Heredity 72:281–283.

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

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

Wake, D. B., K. P. Yanev, and M. M. Frelow. 1989. Sympatry and hybridization in a "ring species": the plethodontid salamander *Ensatina eschscholtzii*. Pp. 134–157 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, Mass.

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