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GEOGRAPHIC VARIATION IN ALLOZYMES IN A "RING SPECIES," THE PLETHODONTID SALAMANDER *ENSATINA ESCHSCHOLTZII* OF WESTERN NORTH AMERICA

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Abstract.—The ring species *Ensatina eschscholtzii* (a plethodontid salamander) of western North America has a circle of subspecies surrounding the Central Valley of California which come into contact and are sympatric in southern California. We examined 26 proteins in 19 populations (maximum of 10 specimens per population) collected throughout the range in order to gain an understanding of the degree of differentiation in the group. Allozymic differentiation is profound, with genetic distances in excess of 0.5 (Rogers or Nei) between populations. Naturally hybridizing populations differ by genetic distances greater than 0.4. Two general classes of color morphs, blotched and unblotched, are segregated geographically, but they do not form discrete genetic units. Both are deeply differentiated, and genetic distances among populations of either class exceed those measured between the classes where they are sympatric in southern California. This study disclosed little evidence of gene exchange around the ring of populations and sampling of many additional populations in regions between populations sampled thus far will be required to determine whether smooth intergradation occurs. Although genetic distances measured exceed those between some co-occurring species of plethodontid salamanders, we find no evidence of borders between cryptic species.

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The nature of speciation, by which we mean the attainment of reproductive closure (sensu Larson, 1984), has again become a central issue in evolutionary biology, and much recent literature emphasizes the central role of speciation in establishing patterns in the history of life (Eldredge and Gould, 1972; Gould and Eldredge, 1977; Gould, 1982; Stanley, 1979; Vrba, 1980, 1985). All workers recognize that speciation can take many forms, but debate focuses on the questions of whether or not one mode of speciation dominates (e.g., Carson and Templeton, 1984; Barton and Charlesworth, 1984; Mayr, 1982), and whether morphological change is concentrated in speciation events (Gould, 1982; Larson, 1984; Stanley, 1979; Wake, 1981; Wake et al., 1983). Speciation often is envisaged as a process that occurs fairly rapidly, is frequently initiated by founder effects, and occurs in small populations at the margin of the ranges. It is as though much of the extensive literature on geographic variation in species and its relevance to speciation (see reviews in Cain, 1954; Mayr, 1963) has been forgotten. Even Mayr, who did so much to popularize geographic speciation, has shifted his emphasis from the so-called "dumbbell" model, an extreme in which two large, subequal portions of the species are separated from each other, to the "peripatric" model, where a strong disparity in size exists between the isolated populations (Mayr, 1982). But Futuyma and Mayer (1980) and Paterson (1981, 1982) have defended the classic form of geographic speciation, as developed by Mayr (1942). "Ring species" (species in which terminal parts of chains of subspecies overlap and behave as species) historically have offered one of the strongest lines of evidence for gradual speciation and for the allopatric mode of speciation by subdivision (Cain, 1954; Dobzhansky, 1958). Most examples have not been investigated using starch-gel electrophoresis, to measure genetic differentiation between adjacent and distant populations in the ring. In this paper, we re-examine a classic case of a ring species in the process of gradual allopatric speciation by subdivision—the salamanders of the genus *Ensatina*.

Prior to the work of Stebbins (1949), *Ensatina* was known as a group of species with strongly differentiated color patterns. Coastal populations from British Columbia to southern California (the species *eschscholtzii*) were more or less uniformly col-

ored, but populations in the inland mountains of California were strongly marked (blotched), usually with large spots or bands of red-orange to yellow pigment on a dark brown to black background (Fig. 1). Blotched populations were variously regarded as species or subspecies. Slevin (1930) recognized a single species with three subspecies: a blotched northern subspecies in the Sierra Nevada (*sierrae*), a more boldly blotched subspecies in the southern mountains (*croceater*), and a more uniformly and lightly pigmented coastal form (*eschscholtzii*). Other workers (e.g., Bishop, 1943) recognized these subspecies as full species.

Stebbins (1949) demonstrated that the different populations of *Ensatina* were linked by populations with intermediate phenotypes, which he interpreted as occupying zones of intergradation. He recognized the three taxa of Slevin and two taxa described subsequent to Slevin's paper (*picta*: unblotched, but rather boldly pigmented populations from northwestern California and adjacent Oregon; *klauberi*: a strongly blotched series of populations in inland mountains of extreme southern California) as subspecies, and described another subspecies, the unblotched *xanthoptica* from the inner Coast Range near San Francisco Bay. Stebbins used the older name *platensis* instead of *sierrae*.

On the basis of a detailed analysis of color variation Stebbins (1949) demonstrated that the subspecies intergraded into one another from southern California through the inland mountains and the Sierra Nevada to northern California, around the northern end of the Sacramento Valley, and then back down the Pacific Coast to San Diego County (Fig. 2). He thought that the ranges of *eschscholtzii* and *klauberi* overlapped in southern California, without any merging of characters, but he had no direct evidence of microsympatry. In his view, the subspecies in southern California behaved as if they were discrete species even though they were linked together by a circuitous sequence of intermediate populations. The populations were grouped into subspecies, on the basis of suites of morphological traits held in common, and the subspecies were shown to intergrade. Thus, the species has a special character—it is a *Rassenkreis* (a group of races) with terminal overlap, or a ring species.

The present study was undertaken primarily to gain an understanding of the amount of genetic differentiation within *Ensatina* relative to that in other plethodontid salamanders, estimated from an analysis of protein variation (see detailed review of the extensive data existing for plethodontids in Larson, 1984). Dobzhansky (1958) considered *Ensatina* to be a case of incomplete speciation, on the grounds that the species was united by an unbroken series of intermediate populations, joining the co-existing blotched and unblotched populations in the south. He argued that these two groups "*can* exchange genes, not directly but by a long circuitous route, through the other races." A secondary goal of our paper is to gain some preliminary assessment of the degree to which protein variants are shared among the widespread populations of the species. Because of the scale of the present study, we recognized that it might be difficult to answer this second question, and additional studies directed to this issue are in progress. A companion paper (Wake et al., 1986) presents data relative to the overlap portion of the ring in southern California, and documents the co-existence of blotched and unblotched populations without hybridization at a single locality in southern California, the southernmost spot at which intraspecific sympatry with or without hybridization occurs. Work completed and in progress addresses in detail, at the levels of proteins, osteology, external morphometrics, and color pattern, various subsections of the ring, especially those areas of secondary contact where intraspecific hybridization occurs (Brown, 1974).

Materials and Methods

Samples from 19 populations collected throughout the range (Table 1, Fig. 2) of the species were examined using starch-gel electrophoresis. For this study, we largely ignored subspecific taxonomy and concentrated on obtaining samples from all major portions of the range. Sample size was 10, with the exception of three samples of 7, 5, and 3 (Table 3). Freshly sacrificed specimens were dissected and the viscera stored

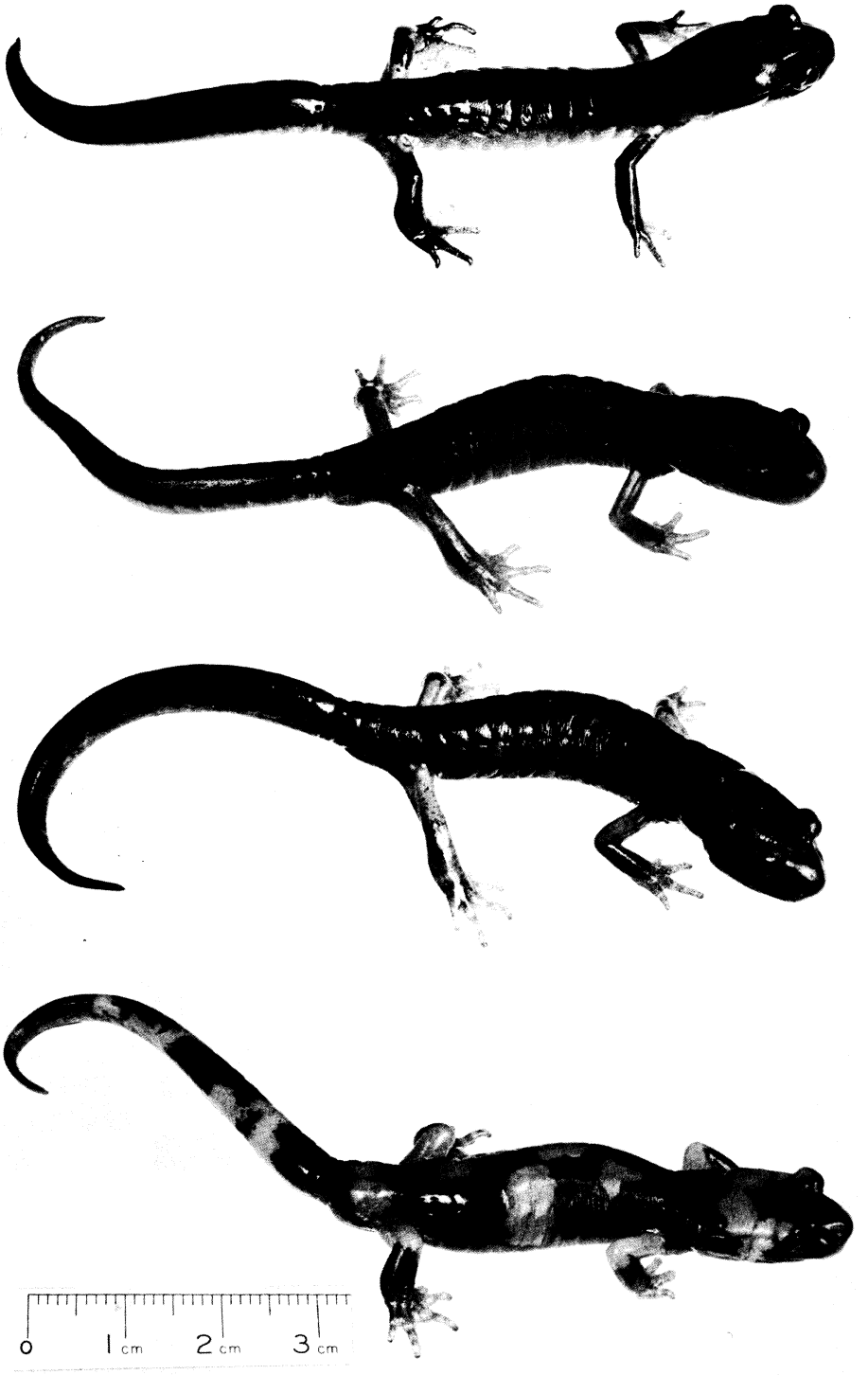

FIG. 1. Color-pattern variation in the polytypic species *Ensatina eschscholtzii*. From top: *E. e. oregonesis* from Humboldt Co., CA; *E. e. xanthoptica* from Calaveras Co., CA; *E. e. platensis* from Calaveras Co., CA; *E. e. klauberi* from San Diego Co., CA. The top two animals have uniform dorsal color patterns of a red- to

at −76°C until used; carcasses were preserved and deposited in the Museum of Vertebrate Zoology. Aqueous mixed homogenates of liver, stomach, and intestine 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). Electromorphs are designated alphabetically with *a* being the fastest migrant. Polymorphism is based on all observed variants (thus the minimum criterion was 0.05 for $N = 10$), and heterozygotes were recorded from direct counts. Two standard estimates of genetic distance between populations, the distance measures of Nei (1972, 1978; D_N) and Rogers (1972; D_R) were computed from observed allozyme frequencies.

RESULTS

Electrophoretic Variation

We consistently scored 26 proteins in our samples (Table 3). Variation is great within this species, and only one protein (GLUD) is monomorphic. The frequency of polymorphic proteins in the 16 samples of 10 specimens ranges from 12% (population 12) to 77% (population 10) (mean = 39% ± 16.7%). We identified 126 allozymes for the 26 proteins studied. The mean number of allozymes per locus per population is 1.45. Mean individual heterozygosity is variable, and in the 16 samples of 10 individuals it ranges from 0.019 (population 12) to 0.250 (population 11) (mean = 0.112 ± 0.066) (Table 3). The two smallest samples show low variability, as would be expected, but variability in the sample with $N = 7$ (population 5) was just a bit below the mean values (polymorphism = 35%, heterozygosity = 0.082).

Much regional differentiation is evident. A single electromorphic variant predominates in all populations for only three proteins (ICD-1, LAP, PGM), and this is nearly the case for four additional proteins (ADA-1, ADH-2, GOT-2, LDH-1). No single pattern of the sharing of variants among populations is repeated for the other 18 polymorphic proteins. In general, neighboring populations around the ring possess similar patterns of protein variation, but

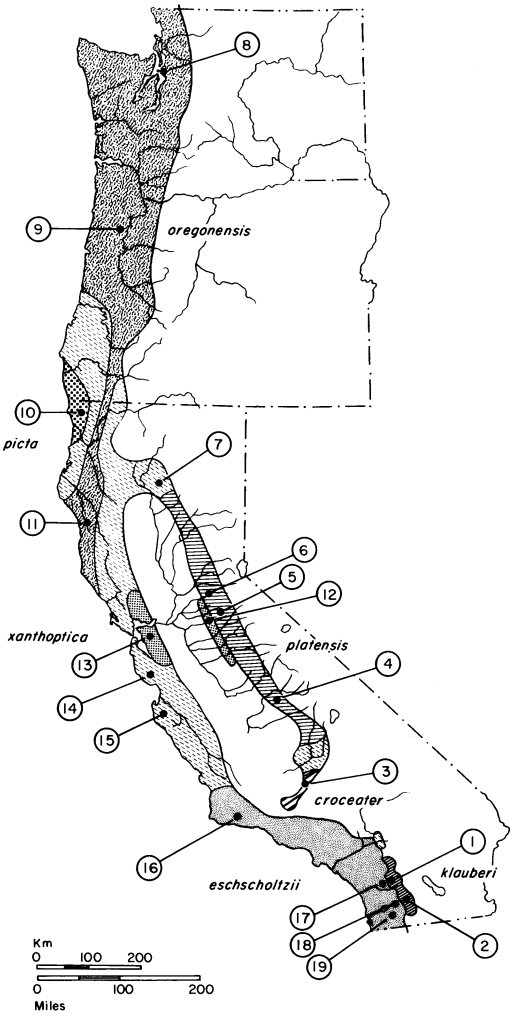

FIG. 2. Ranges of the subspecies of *Ensatina eschscholtzii* according to Stebbins (1949), and populations sampled in this study. Limits of subspecies are indicated by sharp lines, but they are arbitrarily drawn in the region when intergradation zones are thought to start. Intergradation zones are indicated by the broken diagonal lines. Locality numbers correspond to Table 1.

←

orange-brown. The bottom two animals have much darker dorsal ground color, varying from rich dark brown in *E. e. platensis* to black in *E. e. klauberi*. The small dorsal spots of *E. e. platensis* are dark red-orange, but the large blotches of *E. e. klauberi* are light tan to orange-tan. See Stebbins (1949) for color illustrations.

TABLE 1. Collecting localities for *Ensatina eschscholtzii* used in electrophoretic analysis. Numbers refer to populations in Figure 1. Subspecies designated according to Stebbins (1949).

Locality number	Subspecies	Collecting locality
1	*klauberi*	Mendenhall Valley, 3 km NW Palomar Junction, San Diego Co., CA.
2	*klauberi*	Heise Co. Park W of Julian, San Diego Co., CA.
3	*croceater*	Cummings Valley, 4 km SW Hwy 202, Kern Co., CA.
4	*platensis*	Hartland, Tulare Co., CA.
5	*platensis*	3 km NE Arnold, Calaveras Co,. CA.
6	*platensis*	1 km W Blodgett, El Dorado Co., CA.
7	intergrade *oregonensis-platensis*	10 km NE Ingot, Shasta Co., CA.
8	*oregonensis*	5 km NE Granite Falls, Snohomish Co., WA.
9	*oregonensis*	Corvallis watershed, Benton Co., OR.
10	*picta*	Along S Fork Smith River, 10 km SW Hwy 199, Del Norte Co., CA.
11	*oregonensis*	Leggett, Mendocino Co., CA.
12	*xanthoptica*	3.5 km WSW Avery, Calaveras Co., CA.
13	*xanthoptica*	San Pablo Cn. 5 km N Orinda, Contra Costa Co., CA.
14	intergrade *xanthoptica-oregonensis*	Smith Grade, 4 km (air) SW Felton, Santa Cruz Co., CA.
15	intergrade *xanthoptica-eschscholtzii*	S Fork Little Sur River at Coast Road, Monterey Co., CA.
16	*eschscholtzii*	Zaca Creek 3 km W Zaca Lake, Santa Barbara Co., CA.
17	*eschscholtzii*	NW edge of Will Valley, 7 km (air) SE Palomar Junction, San Diego Co., CA.
18	*eschscholtzii*	6 km E Alpine, San Diego Co., CA.
19	*eschscholtzii*	3 km (air) NNE Dulzura, San Diego, Co., CA.

disjunctions are common and many differences accumulate over the sampled range. We attempt no more detailed analysis of protein variants at this time, for our results indicate that many additional populations must be sampled in order to meaningfully analyze patterns in protein variant distribution. There are a number of instances of fixed differences between adjacent samples in the present study, and accordingly we have begun more intensive sampling programs to add populations in regions of critical importance.

An indication of the high level of subdivision is the standardized variance in gene frequency (Wright, 1965), $F_{ST} = 0.705$ for the 19 samples, indicating that the majority of variation sampled is apportioned among rather than within these populations (cf. with data for other salamanders in Larson et al., 1984; Larson, 1984).

Genic Differentiation

Levels of protein differentiation among samples are high (Table 4; cf. Larson, 1984). The minimum genetic distance between samples is $D_N = 0.021$; the maximum $D_N = 0.765$. There are 38 entries in the matrix of D_N that exceed 0.5, and most of these involve the blotched populations 1 and 2, and the unblotched population 8; these three are the most peripheral populations in the sample (Fig. 2).

Figure 3 displays D_N between geographically adjacent samples both around the ring and across it. Most of the genetic distances are large, even for salamanders, a group that is highly differentiated relative to other vertebrates (Avise and Aquadro, 1982; Larson, 1984). Distances across (i.e., comparisons between blotched and unblotched samples) the ring increase slightly from north to south, but these differences are not statistically significant because of the high standard errors associated with samples of this size and genetic distances of such magnitude (Nei, 1972).

The pattern of genic differentiation around the ring is not smooth. For example, the northernmost blotched population (6) is little differentiated from its nearest neighbor to the south (5), but both populations are

TABLE 2. Buffer system assay combinations for starch-gel electrophoresis.

Buffer	Source	Assay	Symbol	Notes
Tris citrate pH 8.0	1	Aconitase (2 loci)	ACON	5
		Isocitrate dehydrogenase (2 loci)	ICD	
		Malate dehydrogenase (2 loci)	MDH	
		Mannose phosphate isomerase	MPI	
		Phosphoglucomutase	PGM	
		Phosphogluconate dehydrogenase	PGD	6
Tris citrate pH 7.0	2	Glucose phosphate isomerase	GPI	5
		Lactate dehydrogenase (2 loci)	LDH	
		Leucine aminopeptidase	LAP	7
Lithium hydroxide	1	Glutamate dehydrogenase	GLUD	5
		Glutamate oxalate transaminase (2 loci)	GOT	
Poulik	1	Peptidase 1-leucyl-1-alanine	LA	5
		Peptidase 1-leucylglycylglycine	LGG	5
		Peptidase 1-phenylalanyl-1-proline	PAP	5
Histidine	3	Adenosine deaminase (2 loci)	ADA	
		Alcohol dehydrogenase (2 loci)	ADH	8
Phosphate citrate	1	Superoxide dismutase	SOD	
Tris citrate lithium borate	4	Alpha glycerophosphate dehydrogenase	GPD	

[1] Selander et al. (1971).
[2] Ayala et al. (1972).
[3] Harris and Hopkinson (1976).
[4] Hashimoto et al. (1978).
[5] Stained using 1% agar overlay.
[6] 2 ml 1% NADP in gel buffer and in cathode tray buffer.
[7] 15% glycerine in gel.
[8] Substrate trans-2-Heven-2-01.

relatively well differentiated from the populations neighboring them to the north and south (4 and 7; Fig. 3, Table 4). Population 7 is well differentiated from everything else, but it supposedly is an intergrade between the blotched subspecies *platensis* and the unblotched subspecies *oregonensis*. There are several fixed or nearly fixed differences between population 7 and its nearest neighbors. From the Monterey Bay region (sample 15) to the southern part of the range (population 19) along the coast there is relatively little differentiation among the unblotched group despite the great geographic distance (maximum $D_N = 0.12$ over a distance of about 550 km).

DISCUSSION

At the level of resolution of our study, there is little evidence of smooth, continuous intergradation of populations within *Ensatina*. There is no apparent relation between genetic distance and geographic distance around the ring, although the lowest genetic distance recorded for a population does tend to be to the nearest geographic neighbor. This tendency is greater if we make the assumption that the color morphs are distinct, and thus count as nearest neighbors those populations which share the same color morph (in such a comparison, population 13 would be the nearest neighbor of population 12, not population 5). A surprisingly large amount of intraspecific genic differentiation is found. This differentiation is equivalent to that which occurs between sympatric congeneric species of related genera (Highton and Larson, 1979). However, there are also cases, particularly among plethodontid salamanders in California, in which intraspecific differentiation is of approximately the magnitude encountered in *Ensatina* (e.g., *Batrachoseps*; Yanev, 1978, 1980).

At the level of discrimination of the present study, no genetic distances between any two nearby samples are sufficiently large to suggest than an unrecognized species border exists. For comparison, Larson and Highton (1978) found an abrupt shift in proteins

TABLE 3. Allozyme variation among populations of *Ensatina eschscholtzii*. Population numbers correspond to Table 1.

Enzyme	1	2	3	4	5	6	7	8	9
ACON-1	a (0.95) c (0.05)	a	a	a	d	d	c	a	a
ACON-2	b	b (0.95) c (0.05)	b	b	c	c	b	c	b (0.30) c (0.70)
ADA-1	b (0.40) c (0.60)	b (0.70) c (0.30)	a (0.25) b (0.75)	b (0.75) c (0.20) d (0.05)	b	b	b	b	b
ADA-2	b	b	b	c	c	c	d	c (0.60) e (0.40)	c (0.65) e (0.35)
ADH-1	b	a (0.15) b (0.85)	b	b (0.85) f (0.10) i (0.05)	f	b (0.05) f (0.95)	e	b	b (0.50) g (0.30) h (0.20)
ADH-2	b	b	b	b (0.95) c (0.05)	b	b	b	b	b
GLUD	a	a	a	a	a	a	a	a	a
GOT-1	a (0.95) c (0.05)	a	b	a (0.30) b (0.70)	a (0.07) b (0.93)	a (0.20) b (0.80)	b	b	a (0.05) b (0.95)
GOT-2	a (0.55) b (0.45)	a (0.10) b (0.90)	b	b	b	b	b	b	b
GPD	a (0.95) d (0.05)	a (0.95) d (0.05)	a (0.90) b (0.10)	a (0.70) c (0.30)	a	a	a	a (0.90) c (0.10)	a (0.90) c (0.10)
GPI	a (0.05) b (0.95)	b	c	c	b (0.07) c (0.93)	b (0.05) c (0.95)	c	b (0.95) c (0.05)	c
ICD-1	d	d	c	b (0.20) c (0.70) d (0.10)	c	b (0.05) c (0.90) d (0.05)	b	c	b (0.10) c (0.90)
ICD-2	c	c (0.85) d (0.15)	c	c (0.95) e (0.05)	c	c	c	c	c
LAP	c	c	c	c	c	b (0.20) c (0.65) d (0.15)	a (0.05) c (0.95)	c	c (0.90) d (0.10)
LDH-1	a (0.70) c (0.30)	a (0.45) c (0.55)	a	a (0.70) b (0.30)	a (0.93) c (0.07)	a	a	a	a
LDH-2	d	d	e	e	c (0.07) d (0.64) e (0.29)	d	d	d (0.05) g (0.95)	g
MDH-1	b	b	a (0.05) b (0.95)	a (0.10) b (0.90)	b (0.86) c (0.14)	b	b	a	a (0.15) b (0.85)
MDH-2	a (0.05) b (0.95)	b	b	b	b (0.07) c (0.93)	c	c	c	b (0.50) c (0.50)
MPI	b	b	b	b	a (0.07) b (0.93)	b (0.80) c (0.20)	a (0.10) b (0.90)	b	b
PEP-LA	c (0.55) e (0.45)	c (0.05) e (0.95)	c (0.50) e (0.50)	e	e	c (0.05) e (0.95)	e (0.05) f (0.95)	b	b
PEP-LGG	f (0.20) h (0.80)	d (0.05) f (0.45) h (0.50)	f (0.75) h (0.25)	f (0.05) h (0.95)	f (0.15) h (0.85)	f (0.05) h (0.85) j (0.10)	f (0.95) h (0.05)	b	b

TABLE 3. Extended.

10	11	12	13	14	15	16	17	18	19
a (0.30) c (0.30) d (0.40)	c (0.60) d (0.40)	c (0.05) d (0.95)	d	d	b (0.05) c (0.90) d (0.05)	c	b	b	b
b (0.20) c (0.75) e (0.05)	a (0.45) b (0.15) c (0.40)	c	c	c	c (0.95) d (0.05)	b (0.50) c (0.50)	b (0.30) c (0.70)	c	c
b (0.90) c (0.10)	b (0.85) c (0.15)	b	a (0.30) b (0.70)	b	b	b	b	b	b
c (0.80) d (0.20)	c (0.70) d (0.30)	c	c	a (0.60) c (0.40)	d	d	d	d	d
c (0.05) g (0.20) j (0.75)	d (0.35) j (0.65)	g	g	b (0.35) g (0.65)	b (0.25) h (0.75)	b (0.10) h (0.90)	b (0.05) h (0.95)	h	h
b	b (0.95) c (0.05)	b	b	b	b	b	b	a (0.60) b (0.40)	b
a	a	a	a	a	a	a	a	a	a
b (0.90) d (0.10)	b	a	a (0.75) c (0.25)	a (0.75) b (0.25)	a	a (0.95) c (0.05)	a	a	a
a (0.35) b (0.65)	a (0.40) b (0.60)	b	b	g	b	b	b	b	b
a (0.45) c (0.55)	a (0.60) c (0.40)	c	c (0.95) d (0.05)	c	a	a	a	a	a
b (0.05) c (0.95)	c	c	c	b (0.05) c (0.95)	c	c	c	c	c
a (0.05) b (0.35) c (0.60)	b (0.05) c (0.25) d (0.65) e (0.05)	d	c (0.25) d (0.75)	b (0.05) c (0.90) d (0.05)	d	d (0.95) f (0.05)	d	d	d
c	c (0.95) d (0.05)	c	a (0.15) c (0.85)	c	b (0.10) c (0.90)	c (0.90) e (0.10)	c	c	c
c	c (0.95) d (0.05)	c	c	c	c	c	c	c	c
a	a	a	a	a	a	a	a	a	a
b (0.75) c (0.05) d (0.20)	a (0.05) b (0.50) d (0.45)	d	d	a (0.05) d (0.90) f (0.05)	b (0.05) d (0.95)	c (0.05) d (0.90) e (0.05)	b (0.05) d (0.80) g (0.15)	d	d
b	b (0.90) c (0.10)	b	b	a (0.05) b (0.95)	b	b	b	b	b
b (0.25) c (0.75)	b (0.15) c (0.85)	b	b	b	b	b	b	b	b
a (0.05) b (0.80) e (0.10) f (0.05)	b	b	b (0.55) g (0.45)	b (0.20) e (0.05) g (0.75)	b	b (0.90) c (0.10)	b	b	b
d (0.25) e (0.75)	a (0.05) b (0.90) e (0.05)	e (0.85) f (0.15)	e (0.80) f (0.20)	e (0.20) f (0.80)	b (0.05) e (0.75) f (0.05) g (0.15)	e (0.85) f (0.15)	e (0.95) f (0.05)	e	e
a (0.05) c (0.25) e (0.55) g (0.10) i (0.05)	b (0.35) e (0.65)	g	g	c (0.25) g (0.75)	a	a	c	c	a (0.67) c (0.33)

TABLE 3. Continued.

Enzyme	1	2	3	4	5	6	7	8	9
PEP-PAP	a (0.45) b (0.55)	a (0.60) b (0.40)	a (0.50) b (0.50)	a (0.50) b (0.50)	a (0.50) b (0.50)	b	c	c	a (0.05) c (0.90) d (0.05)
6-PGD	a (0.20) e (0.80)	e	f	a (0.55) e (0.40) f (0.05)	f	f (0.95) g (0.05)	d (0.15) f (0.65) g (0.20)	f	c (0.10) f (0.90)
PGM	b	b	b	b (0.95) c (0.05)	b	b	b	b	a (0.05) b (0.95)
SOD	a	a	a (0.95) b (0.05)	b	b	b	a (0.05) b (0.95)	b	b
SORDH	d	b (0.05) d (0.95)	d	b (0.05) d (0.95)	d	d	d	d	d
Sample size	10	10	10	10	7	10	10	10	10
Number of alleles	38	38	33	44	36	38	33	30	40
Number of alleles per locus	1.46	1.46	1.27	1.69	1.38	1.46	1.27	1.15	1.54
H	0.138	0.119	0.073	0.123	0.082	0.069	0.038	0.050	0.131

equivalent to a D_N of about 1.5 in *Plethodon,* and this subsequently proved to be a species border when sympatry was discovered (Highton, 1979). This and other cases among plethodontid salamanders are reviewed by Larson (1984). The greatest genetic distances in *Ensatina* are recorded between geographically remote populations, except in the areas of intraspecific sympatry in the central Sierra Nevada (populations 5 and 12) and in the southern parts of the range. In both instances, blotched and unblotched populations are involved in the comparisons. At the southern end of the range, D_N between the blotched and unblotched populations ranges between 0.40 and 0.52, although geographic distance is slight (20 km or less). This result is in accordance with Stebbins's (1949) hypothesis that the blotched and unblotched populations in southern California entered the region independently, rather than diverging along a selection gradient from some single ancestral population that occurred in the region. The blotched populations are thought to have originated from the inland mountains and the unblotched ones from the coastal regions to the north. Blotched populations in southern California are more similar genetically to populations of similarly colored animals to the north than they are to the nearby unblotched populations, and the analogous comparison is even more pronounced for the unblotched populations.

But the surprise in our results is the high level of differentiation achieved in general. For example, among the blotched populations, levels of D_N are as high as 0.596 (between samples 1 and 5); and among the unblotched populations, distances are as high as 0.765 (between populations 8 and 12). While these genetic distances are greater than those measured between the supposedly terminal parts of the ring where the overlap occurs in southern California, both examples cited are between relatively remote parts of the ranges of these two general classes of color patterns. However, the fact that such high levels of intramorph genetic differentiation are recorded suggests that the evolutionary history of the genus may be more complex than previously considered.

Based on analysis of color patterns, Brown (1974) argued that blotched and unblotched groups hybridized in two geographic areas:

TABLE 3. Extended.

10	11	12	13	14	15	16	17	18	19
a (0.50) c (0.50)	a (0.15) c (0.85)	d (0.05) f (0.95)	d (0.15) e (0.15) f (0.70)	d (0.15) e (0.85)	c (0.25) d (0.20) e (0.55)	c (0.30) d (0.70)	d (0.55) e (0.45)	d	d
c (0.10) f (0.75) g (0.10) i (0.05)	a (0.05) b (0.30) f (0.45) g (0.20)	g	g (0.95) h (0.05)	c (0.10) f (0.50) g (0.40)	c (0.05) f (0.95)	c (0.25) f (0.75)	f	f	c (0.33) f (0.67)
a (0.15) b (0.85)	b	b	a (0.10) b (0.90)	b	b	b	b	b	b
b (0.95) c (0.05)	b (0.75) c (0.25)	b	b	b	b	b	b	b	b
b (0.05) d (0.90) e (0.05)	a (0.05) d (0.95)	b	b (0.80) d (0.20)	b (0.05) d (0.95)	c (0.15) d (0.85)	d	d	d	d
10	10	10	10	10	10	10	10	5	3
59	53	29	38	43	38	37	32	27	28
2.27	2.04	1.12	1.46	1.65	1.46	1.42	1.23	1.04	1.08
0.246	0.250	0.109	0.154	0.135	0.073	0.092	0.077	0.015	0.025

midway in the ring in the central Sierra Nevada, and at the southern end of the ring. The argument of Stebbins (1949), amplified and extended by Brown (1974), is that intergradation occurs between races, most critically between the blotched subspecies *platensis* and the unblotched subspecies *oregonensis* at the northern end of the ring, north of the Sacramento Valley, and that the two regions of hybridization are the result of secondary contact.

We examined one population from the region of hypothesized intergradation of the two races (population 7). This population is well differentiated from all other populations examined, although it is more similar to populations 10 and 11 than to any others. Populations 5 and 6, both blotched, are less differentiated from unblotched populations 10 and 11 than they are from blotched populations at the southern end of the range (see Fig. 3; Table 4). We currently are conducting an intensive sampling of the mountainous region around the north end of the Sacramento Valley, but populations are sparse and widely scattered. A difficulty in analyzing this situation is that gene flow may be so low that local differentiation in the isolated and semi-isolated intermediate populations may obscure any pattern of intergradation (cf. Larson et al., 1984).

Our results support the argument that hybridization in the foothills of the Sierra Nevada is the result of secondary contact. Stebbins (1949) postulated that the unblotched populations in the Sierra Nevada foothills had invaded the region recently, from the region east of San Francisco Bay. Our populations 12 and 13 are very little differentiated ($D_N = 0.021$) despite the fact that they occur on either side of the inhospitable Great Central Valley. Furthermore, population 12, representing the immigrants, is the least variable population (considering only those with a sample size of 10) in our sample, and it has relatively very few protein variants (29) and low heterozygosity (0.019); again this is in accordance with expectations for relatively recently founded populations.

The overlap of the blotched and unblotched populations in southern California also was postulated to be a secondary contact by Stebbins (1949), and our data do not reject this hypothesis (see also Wake et al., 1986).

Stebbins (1949) argued that the subspe-

TABLE 4. Genetic distance measures of Nei (1972) (above diagonal) and Rogers (1972) (below diagonal) by electrophoretic comparisons of populations of *Ensatina eschscholtzii*. Population numbers correspond to Table 1.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	—	0.036	0.284	0.327	0.596	0.582	0.623	0.659	0.599	0.613	0.595	0.625	0.655	0.641	0.450	0.425	0.451	0.520	0.470
2	0.105	—	0.281	0.314	0.555	0.562	0.599	0.646	0.584	0.586	0.615	0.563	0.596	0.611	0.410	0.386	0.404	0.466	0.419
3	0.276	0.282	—	0.177	0.345	0.403	0.425	0.445	0.293	0.354	0.451	0.708	0.682	0.502	0.463	0.440	0.448	0.529	0.502
4	0.332	0.313	0.220	—	0.255	0.302	0.462	0.451	0.282	0.275	0.392	0.470	0.458	0.419	0.417	0.378	0.398	0.474	0.429
5	0.471	0.455	0.324	0.276	—	0.023	0.370	0.344	0.265	0.156	0.254	0.419	0.392	0.318	0.345	0.375	0.356	0.382	0.359
6	0.481	0.468	0.377	0.317	0.080	—	0.373	0.381	0.295	0.193	0.272	0.409	0.381	0.317	0.341	0.372	0.356	0.379	0.354
7	0.488	0.478	0.371	0.411	0.339	0.334	—	0.483	0.350	0.287	0.253	0.645	0.644	0.495	0.337	0.298	0.396	0.461	0.429
8	0.499	0.497	0.372	0.405	0.316	0.348	0.397	—	0.095	0.328	0.305	0.765	0.752	0.545	0.542	0.593	0.582	0.637	0.609
9	0.488	0.478	0.303	0.306	0.282	0.302	0.334	0.139	—	0.209	0.194	0.541	0.522	0.383	0.372	0.387	0.387	0.446	0.416
10	0.493	0.485	0.372	0.323	0.250	0.273	0.333	0.360	0.272	—	0.107	0.369	0.348	0.286	0.318	0.330	0.332	0.377	0.353
11	0.479	0.490	0.410	0.376	0.315	0.339	0.308	0.341	0.265	0.195	—	0.429	0.432	0.394	0.316	0.325	0.378	0.426	0.390
12	0.485	0.451	0.514	0.411	0.366	0.358	0.486	0.542	0.449	0.382	0.420	—	0.021	0.208	0.339	0.371	0.368	0.389	0.347
13	0.511	0.480	0.506	0.410	0.373	0.359	0.498	0.554	0.457	0.373	0.423	0.081	—	0.144	0.354	0.384	0.380	0.403	0.358
14	0.503	0.488	0.426	0.390	0.321	0.312	0.410	0.450	0.364	0.334	0.399	0.248	0.224	—	0.315	0.365	0.331	0.380	0.353
15	0.402	0.368	0.396	0.385	0.328	0.324	0.316	0.440	0.352	0.346	0.356	0.302	0.340	0.315	—	0.028	0.094	0.123	0.072
16	0.391	0.361	0.391	0.367	0.348	0.346	0.290	0.472	0.358	0.351	0.358	0.328	0.357	0.346	0.081	—	0.101	0.122	0.064
17	0.402	0.367	0.385	0.373	0.325	0.331	0.350	0.454	0.347	0.350	0.382	0.321	0.364	0.331	0.129	0.132	—	0.028	0.036
18	0.439	0.402	0.431	0.414	0.344	0.342	0.386	0.480	0.396	0.391	0.420	0.334	0.378	0.361	0.158	0.165	0.063	—	0.037
19	0.405	0.369	0.416	0.384	0.329	0.324	0.358	0.465	0.374	0.365	0.391	0.302	0.346	0.333	0.118	0.110	0.078	0.062	—

cies *picta* has evolved conservatively and that it preserves what might have been the patterns of color variation characteristic of an hypothesized ancestral population. Our data offer an interesting perspective on that argument. The genetic variability of the populations from the range, or near the range, of *picta* is very great. Populations 10 and 11 have heterozygosities of about 25% and they have 59 and 53 proteins, respectively, at only 26 presumptive loci (Table 3). These are extraordinarily high levels of variability (cf. Nevo, 1978), and the number of alleles present will only rise with increasing sample size. Perhaps these populations are old, as Stebbins hypothesized, and we also believe them to be very large (based on frequency of encounter in the field, compared with other parts of the range).

Our data conflict with the conclusion of Dobzhansky (1958) that the far-flung populations of *Ensatina* are united by the exchange of genes, which inhibit the attainment of reproductive closure. Stebbins (1949) argued that the species is at a stage in its evolution at which it is breaking into discontinuous populations. Our data indicate that *Ensatina* is well differentiated genetically, with many fixed differences being found in comparisons of populations throughout the range. Populations of *Ensatina* appear to be large and gene flow nonhomogeneous. If one assumes that most of the variants we have sampled have only minor or no adaptive significance, our data suggest that the species is relatively old and that genetic differentiation has been taking place over a long period of time. In contrast, the fact that differentiation is as great within as between the blotched and unblotched forms suggests that these two general color morphs, which have distinct geographic ranges, might represent separate adaptive responses to provincial selection pressures rather than either discrete historical entities or incipient species.

Ensatina poses a number of challenges to those interested in speciation phenomena. The blotched and unblotched populations in the south behave as if they are discrete species (see Brown, 1974; Wake et al., 1986), but there is no evidence of species borders to the north of this region. At intermediate geographic levels (the central Sierra Nevada) in the ring of taxa the two color morphs hybridize, but the stable and narrow hybrid zone in the central Sierra Nevada (Brown, 1974; Wake and Yanev, unpubl.) suggests that here, too, the morphs behave as species, albeit less discrete ones than to the south. *Ensatina* offers an instance of gradual divergence in allopatry, with morphological differentiation being only very roughly associated with genetic divergence. Investi-

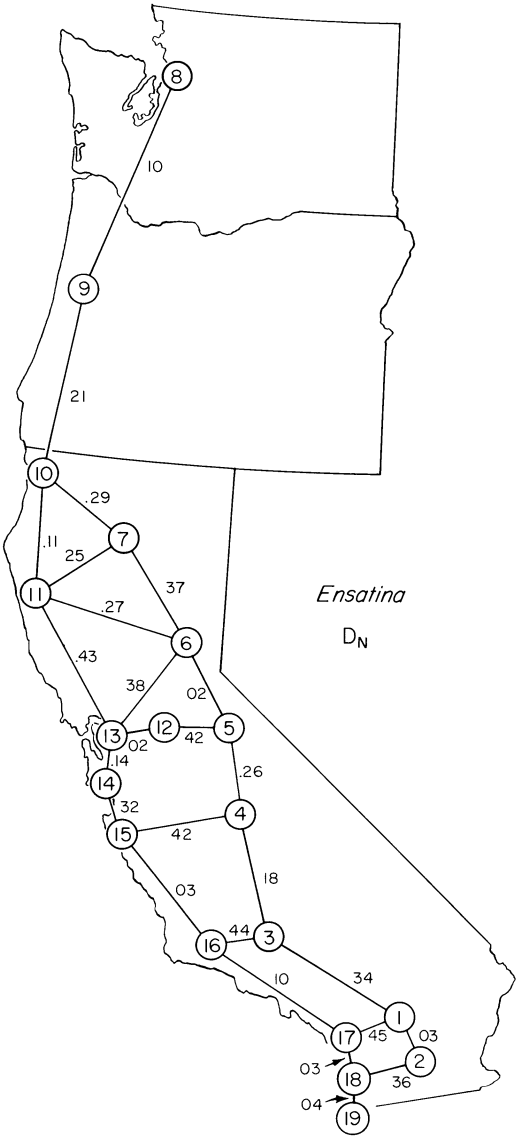

FIG. 3. Nei's genetic distance between adjacent samples both around and across the ring of subspecies. Locality numbers as in Table 1 and Figure 2.

gations in progress will examine intervening sections of the ring, and zones of hybridization, in greater detail.

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