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and Its Consequences

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CHAPTER 6

SYMPATRY AND HYBRIDIZATION IN A "RING SPECIES": the Plethodontid Salamander *Ensatina eschscholtzii*

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A central question in evolutionary biology relates to mode of speciation. Do species arise gradually, by the divergence of geographically disjunct or distant populations, or do species arise suddenly, on the margins of ranges, in newly occupied habitats? Although few would question that species can arise by other means as well, much controversy over modes of speciation relates to this question. Furthermore, most workers would accept that the answer to the question asked is neither one nor the other, but rather a mixture of these and other modes. But there have been many recent discussions of so-called "founder-effect" or "peripatric" speciation, and this mode seems to have been on the ascendency in recent years.

Speciation by geographic subdivision remains in the lexicon of speciation modes (Endler 1977; Templeton 1981; Mayr 1982; Woodruff 1981), but somewhat forgotten are the cases of isolation, and resulting speciation, by distance, best illustrated by "ring species." Such species played a role in the formulation of the geographic model of speciation (Mayr 1942, 1963), but their numbers have been eroded as various problems have arisen with different specific cases (Mayr 1970). In this chapter, we present a progress report of our continuing study of one of the best known "ring species," the lungless salamander *Ensatina eschscholtzii* of California.

The salamander *E. eschscholtzii* is a strictly terrestrial member of the lungless family Plethodontidae. It has seven subspecies, most of which are wrapped in a ring-like fashion around the Central Valley of California (Stebbins 1949; Wake and Yanev 1986). Occupancy of California has been from the North, via southward migration through the Coast Ranges and the Sierra Nevada and other interior mountain ranges; no *Ensatina* occupy the Central Valley currently. Stebbins (1949) believed that the subspecies intergrade at the north end of the valley. Midway along the length of the valley there has been a "transvalley leak," and a population from the coastal region has established itself in the foothills of the Sierra Nevada. Two subspecies with strikingly distinct color patterns meet along a front more than 100 km in length and everywhere they meet they hybridize (Brown 1974). In southern California two subspecies with even more strikingly distinct color patterns meet. Sympatry has been found in four mountain ranges (Wake et al. 1986). In three of these hybridization occurs, but in the fourth (Cuyamaca Mountains of San Diego County) no hybridization is found and the two morphs appear to be distinct biological species.

We are studying genetic differentiation throughout the range of *Ensatina* throughout western North America, with a concentration on zones of intergradation and hybridization. In this chapter we focus principally on a zone between about 900 and 1200 m elevation in the foothills of the central Sierra Nevada in southern Calaveras County, California. This region was studied by Brown (1974), who reported hybridization between *Ensatina eschscholtzii platensis* (a form with a spotted or blotched color pattern, consisting of red-orange spots or blotches on a dark brown background), a widespread Sierran subspecies, and *E. e. xanthoptica* (a form with a uniform, lively orange color pattern), a coastal subspecies with an outpost in the Sierran foothills. Although the area in which parental types cooccurred was restricted, Brown thought that the zone of hybrid influence might be relatively wide, on the order of tens of kilometers. We used electrophoretic analysis of variable electromorphic loci to investigate interactions in the hybrid zone. Our results are compared with our findings (Wake et al. 1986) from the contact zones in southern California.

MATERIALS AND METHODS

We established a study zone in southern Calaveras County, California, lying between two relatively large water barriers, San Antonio Creek (the major tributary of the Calaveras River system) and the Stanislaus River (Figure 1). Most salamanders were captured through the use of pitfall traps. From 20 to 50 such traps were placed in restricted areas of a favorable habitat in the vicinity of the village of Avery, at elevations between 975 and 1200 m. Two additional sites were located at higher elevations to the northeast, near the town of Arnold and the village of Camp Connell.

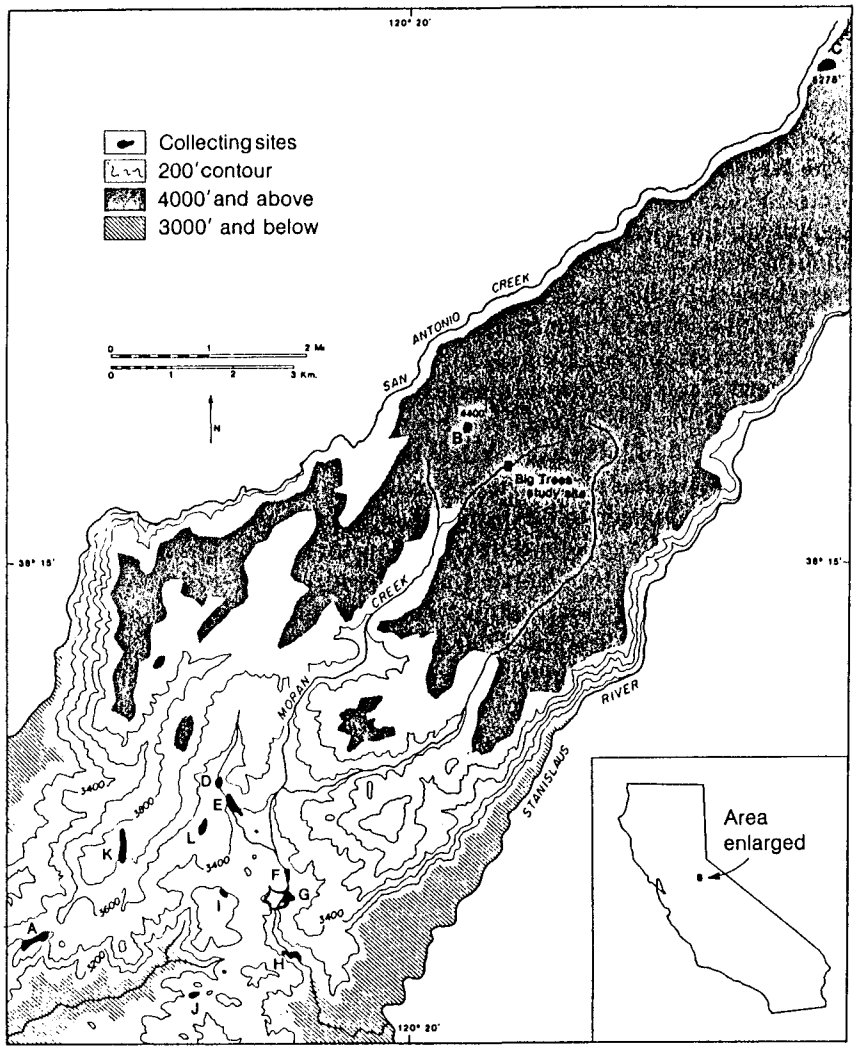

FIGURE 1. Map of portion of southern Calaveras County, California, indicating locations of populations (Table 1) used in this study. The only contour maps available are in English units (1 foot = 0.3048 m).

Details will be published elsewhere. For the purposes of the present study all sites at which 10 or more specimens were obtained were used (two sites thus were excluded from detailed analysis), and some sites that lay in very close proximity to each other were combined. The sites were no more than about 200 m in diameter, and we believe that we are justified in treating

them as local populations on the basis of population studies of the species (Stebbins 1954). We have 12 sites in this region (Table 1).

These salamanders use underground retreats by day and during the extended dry season, and they are relatively difficult to find on the surface. Pitfall trapping was the only effective means of collection. Specimens encountered by turning cover objects in the study sites were included, but these were rare (less than 5% of the total). The period of collection extended over 5 years, and we have combined years. These animals are known to have very localized movements and are known to live for many years, and females are thought to reproduce every other year following sexual maturity at about age 4 years (Stebbins 1954), so we do not believe that combining years compromised our study.

Specimens were returned living to the laboratory and were scored for color pattern using a modification of the hybrid index devised by Brown (1974), who scored five separate color traits ranging from 1 (theoretical "pure" *platensis*) to 10 (theoretical "pure" *xanthoptica*). He presented an average score between 1 and 10 for each animal. Our study of coloration is incomplete at this time, and we present here information obtained from three traits, scored according to the index established by Brown. These traits are degree of iris iridophore development, degree of erythrophore development on ventral surfaces, and degree of melanophore development on ventral surfaces. We note here that Brown's Figure 3 has

TABLE 1. Populations used in study and sample size.

Designation	Locality (Calaveras Co., California)	Sample size
A	4 km WSW Avery	27
B	1 km NE Arnold	11
C	1 km NE Camp Connell	22
D	1.7 km N Avery	32
E	1.3 km NNE Avery	25
F	1.3 km E Avery	11
G	1.4 km ESE Avery	41
H	2.3 km SE Avery	26
I	0.5 km S Avery	12
J	2.6 km SSW Avery	26
K	2 km WNW Avery	53
L	0.9 km NNW Avery	21

transposed standard 1 and 4 for ventral melanophores, and we corrected this error prior to using the index. Furthermore, instead of averaging the scores for the three traits we simply summed them; our index ranges from 3 through 30.

Following scoring for color the specimens often were photographed (mainly suspected hybrids) and prepared for electrophoretic analysis (see Wake and Yanev 1986, for details). An initial survey of two populations (plus some additional material from nearby areas) as a part of our study of geographic variation in the species included 26 scorable proteins. We found fixed or nearly fixed differences between reference populations of *platensis* and *xanthoptica* in eight proteins (Nei $D = 0.419$), and for purposes of analysis in this chapter we treat these as genetic loci; the electromorphs were treated as alleles. We established a genetic hybrid index on the basis of these eight loci ranging from 0 (theoretical "pure" *platensis*) to 16 (theoretical "pure" *xanthoptica*). These alleles are indicated in Table 2. There are a few low-frequency alleles, and for purposes of initial analysis each of these was assigned either to *plantensis* or *xanthoptica*. This assignment was based on several factors. We used geographically remote samples of *xanthoptica* and *platensis* for comparative purposes. Our outside sample of *xanthoptica* is a combination of several sites in western Calaveras County below 750 m elevation (total of 25 specimens). The outside sample of *platensis* is a combination of a sample from Blodgett, El Dorado County, California (total of 10 specimens), and of samples B and C (Figure 1,

TABLE 2. Allele assignments for the eight loci used in this study.

Locus	Symbol	EC Number	*platensis* alleles	*xanthoptica* alleles
Phosphogluconate dehydrogenase	Pgd-A	1.1.1.44	*f*	*g*
Malate dehydrogenase (mitochondrial)	M-Mdh-A	1.1.1.37	*c*	*b*
Isocitrate dehydrogenase (cytosolic)	S-Icdh-A	1.1.1.42	*g*	*d*
Proline dipeptidase	Pep-D	3.4.13.9	*d*	*f*
Aspartate aminotransferase (cytosolic)	S-Aat-A	2.6.1.1	*b*	*a*
Tripeptide aminopeptidase	Pep-B	3.4.11.4	*h*	*g*
Glycerol-3-phosphate dehydrogenase	Gpd	1.1.1.8	*a*	*c*
L-Iditol dehydrogenase	Iddh-A	1.1.1.14	*d*	*b*

together totalling 32 specimens). For the outside samples of *xanthoptica* the alleles listed in Table 2 had a frequency of 1. Because each sample was relatively small, we probably missed rare alleles, such as an allele found in a heterozygous state in single individuals in both populations A and J. Because these populations were like *xanthoptica* in almost every other respect, and because these rare alleles otherwise were not found, they were counted as if they were *xanthoptica* allele *f*. When low-frequency alleles were encountered only in the hybrid zone and they did not appear in either set of outside samples they were assigned to one form or the other on the basis of cooccurrence with other alleles, and in every case assignment was unambiguous. At one locus (*PAP*) we had difficulty separating two fast-migrating bands in *platensis*, but since the *xanthoptica* allele was very slow in its migration, the separation of the two, and the identification of heterozygotes, was unambiguous, so we scored the two fast-migrating alleles as if they are one allele (designated *g* here; cf. Wake and Yanev 1986).

Coordination of allele designations throughout all of our studies on this species will be difficult because of the extraordinary number of alleles involved, but as far as possible we have used the allele designations of Wake and Yanev (1986). Alleles thought to be newly discovered in this study are given arbitrary designations that are simply alphabetical extensions of our earlier study, and they do not relate to mobility. Details can be obtained from D. B. Wake.

We restricted our study to the loci that had fixed or nearly fixed differences and that were readily scorable. We found 24 alleles for these 8 loci, but the 16 listed in Table 2 strongly dominated and the remaining 8 are absent in most populations and generally have a frequency of less than 0.05 when present. For only one locus does such an allele reach a moderate frequency. LGG allele *k* (assigned to *xanthoptica* on the basis of its presence in four heterozygotes in population J and its absence in all *platensis* populations) reaches a frequency of 0.19 in population H and 0.16 in population G. Several of these eight alleles are "rare," and three are found only in single heterozygotes. The greatest number of low-frequency alleles are found in populations G and K, both in the heart of the hybrid zone, but both also the largest samples.

Although scores of 0 and 16 in our allozyme hybrid index unambiguously identify parental *platensis* and *xanthoptica* individuals, respectively, the presence of certain alleles of one form in the other complicates the situation somewhat. We used population A as a reference for *xanthoptica* and populations B and C as reference populations for *platensis*. The profiles of these populations relative to outside comparative populations (Figures 2 and 3) show that such a procedure is justifiable. We assume that there is residual background variation in the form of low-frequency alleles

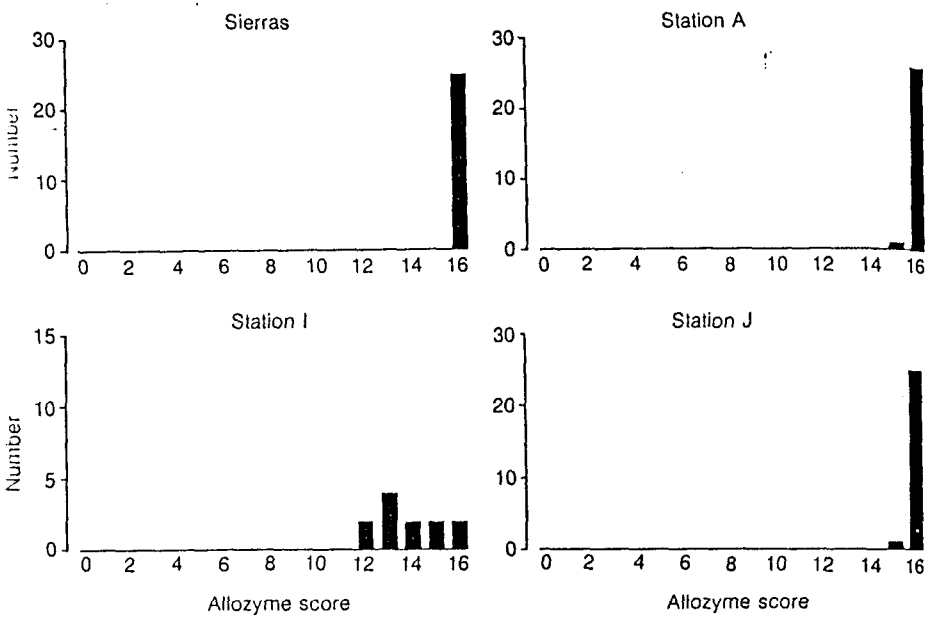

FIGURE 2. Frequency histograms for individuals in four samples classified according to allozyme hybrid index. The four samples include salamanders that have the general coloration of *xanthoptica*. The Sierras sample is used as an allozyme outgroup for this study. Samples from populations A, I, and J lie along the western edge of the hybrid zone. A score of 16 is considered to be "pure" *xanthoptica*, whereas a score of 15 is considered to be ambiguous (based on the presence of the same low-frequency allele in populations A and J, suggesting that this might be a low-frequency "background" allele in *xanthoptica* rather than a *platensis* allele). All scores lower than 15, and some scores of 15, represent incorporation of *platensis* alleles.

in both parental stocks (e.g., our Blodgett sample contains a few alleles in low frequency that we assigned to *xanthoptica*). A score of 15, although slightly ambiguous, is treated as a parental *xanthoptica* pattern. Similarly, the somewhat more variable *platensis* has hybrid index values of 1 and 2 assigned to it. Only by making this compromise can we deal effectively with all eight loci simultaneously.

RESULTS

Based on analysis of genotypes and allozyme hybrid index, as well as the color hybrid index, we found both parental types and hybrids in three pop-

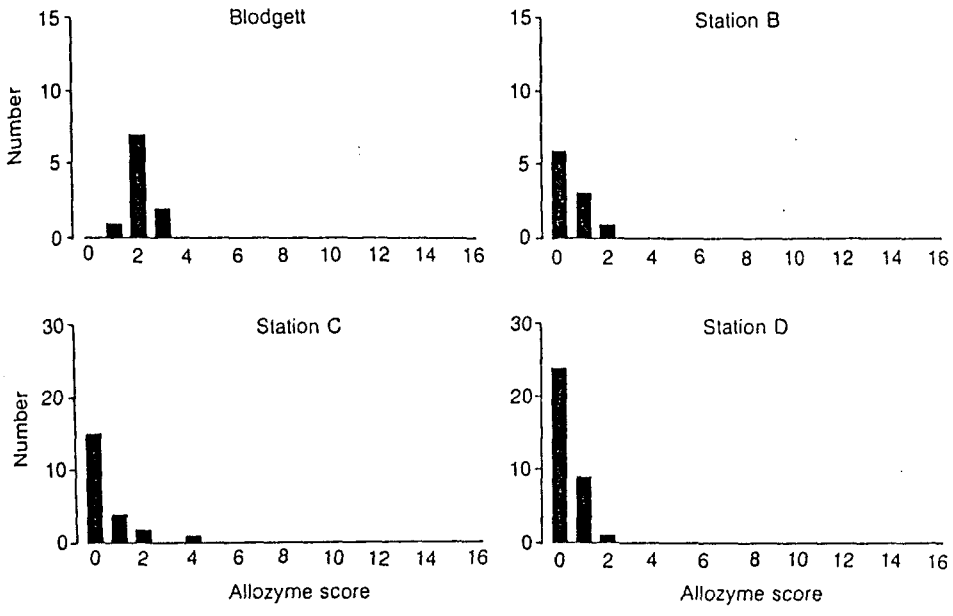

FIGURE 3. Frequency histograms for individuals in four samples classified according to allozyme hybrid index. The four samples include salamanders that have the general coloration of *platensis*. Sample D lies on the eastern and northern edge of the hybrid zone, whereas the other three samples are outgroups for the present study. A score of 0 is an unambiguous "pure" *platensis*. There is more background variation in *platensis* than in *xanthoptica*, and we have found no case of a population having an overall score of 0.

ulations: G, K, and L (Figure 4). Population K (our largest sample) contains one unambiguous F_1 hybrid, but the only other unambiguous F_1 we have found is in population F, which otherwise has only *platensis* genotypes. Individuals with scores of 8 elsewhere in the sample cannot be F_1.

The hybrid zone is very narrow. Endler (1977) has recommended estimating the width of hybrid zones as that distance between frequencies of 0.8 and 0.2 for a given allele. The distance between localities along Mill Creek and a tributary (Figures 1, 5, and 6) that have average gene frequencies (based on *platensis*) dropping from 0.88 to 0.2 (Table 3) is approximately 1.4 km.

The hybrid zone also can be crossed indirectly by a transect extending along a mainly south-facing slope between sites for samples A and B (Figure 1). Here the samples are less regular in relation to each other than those lying along Mill Creek. Both samples K and L lie on the hybrid zone,

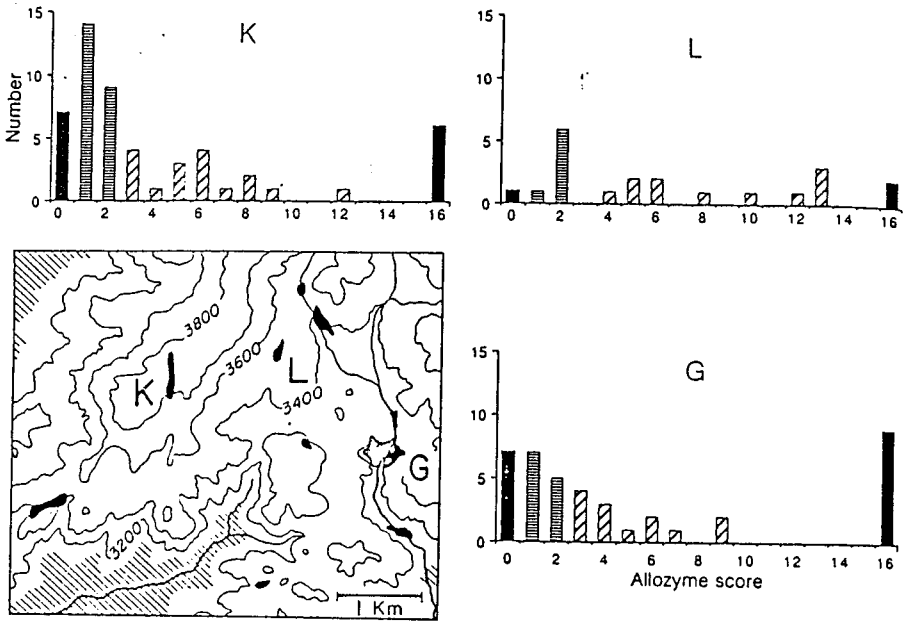

FIGURE 4. Location of the three sample sites near Avery, Calaveras County, California, at which "pure" parental *platensis* and *xanthoptica* are present. These samples lie along the middle of the narrow hybrid zone. Frequency histograms are based on the allozyme hybrid index. A single F_1 (sample K) was found. Scores of 1 and 2 are differentiated from scores of 0 or of 3 and higher to indicate their somewhat ambiguous nature (see text). The only contour maps available are in English units (1 foot = 0.3048 m).

which accordingly is even narrower than is apparent from Figure 7. Furthermore, we have a small sample (three specimens) from a site 500 m west of the western edge of K that has allozyme scores of 16, 16, and 15 (this last score is based on the same allele for 6-phosphogluconic dehyrogenase (6-PGD) that gives single scores of 15 in samples A and J), suggesting that the hybrid zone is entered abruptly.

We consider scores on the allozyme hybrid index from 3 through 14 to be unambiguous hybrids. Such scores are absent from samples immediately adjacent to the hybrid zone: A, D, and J. Percentage of hybrid individuals in samples in and on the margins of the hybrid zone are indicated in Table

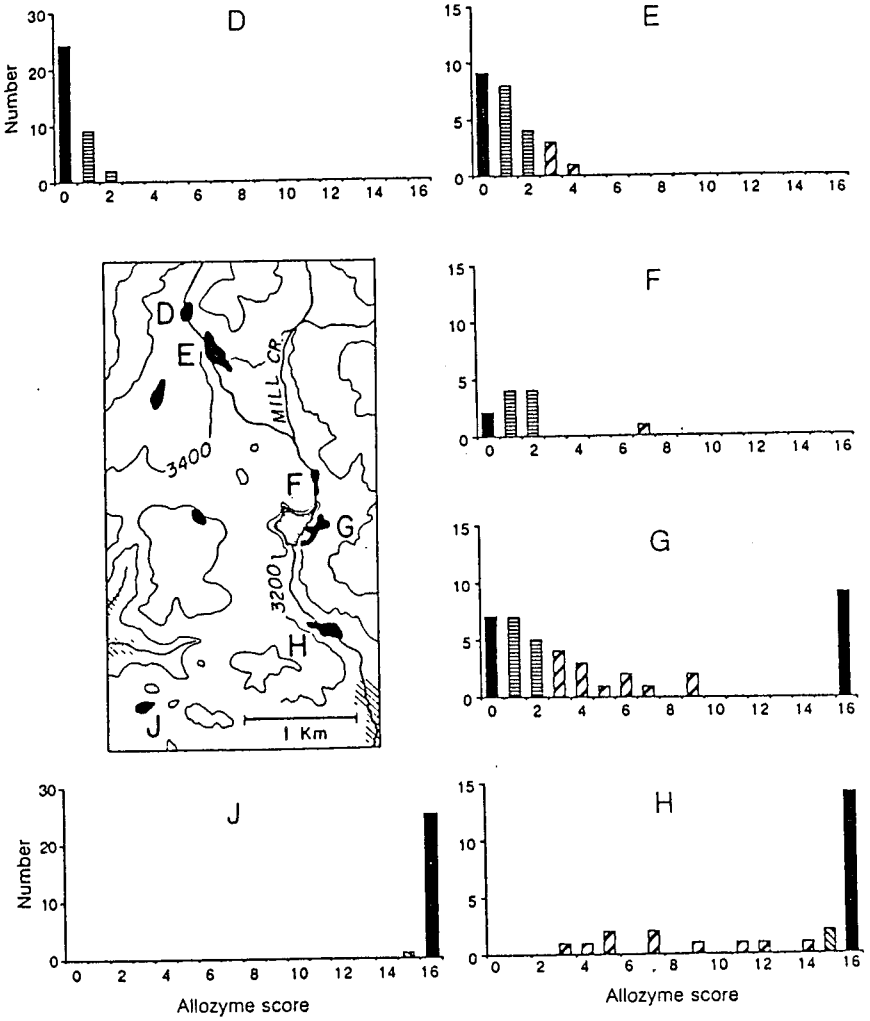

FIGURE 5. Section of the study zone indicating location of samples lying along and near Mill Creek and a tributary near Avery, Calaveras County, California. Frequency histograms based on the allozyme hybrid index are presented for samples extending through the hybrid zone. There is only a single F_1 (sample F), and only a single sample (G) at which "pure" parental *platensis* and *xanthoptica* are present. Scores of 1 and 2 are differentiated from scores of 0 or of 3 and higher to indicate their somewhat ambiguous nature (see text). The only contour maps available are in English units (1 foot = 0.3048 m).

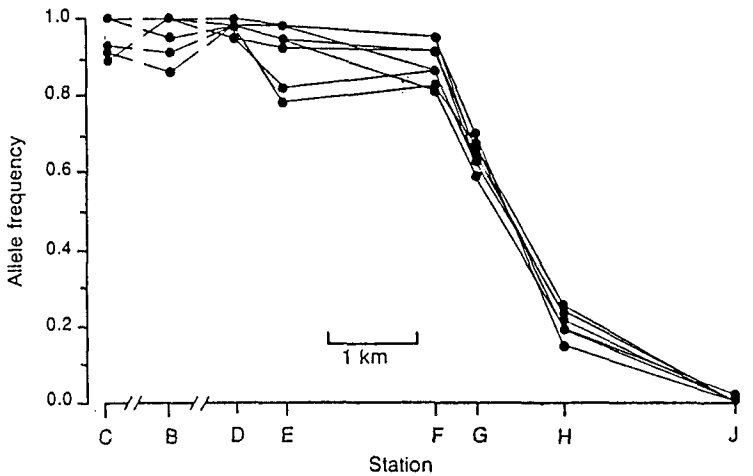

FIGURE 6. Gene frequency cline (based on *platensis* alleles) for eight loci in the samples from Figure 5, plus two relatively remote samples of *platensis* that show the background variation in that form (see also Table 3).

TABLE 3. Average frequency (and standard deviation) of *platensis* alleles for eight loci.

Population	Frequency	Number
A	0.003 (±0.01)	27
B	0.97 (±0.05)	11
C	0.97 (±0.05)	22
D	0.98 (±0.02)	32
E	0.92 (±0.08)	25
F	0.88 (±0.05)	11
G	0.64 (±0.04)	41
H	0.20 (±0.03)	26
I	0.13 (±0.24)	12
J	0.003 (±0.01)	26
K	0.74 (±0.03)	53
L	0.59 (±0.10)	21
	Total	307

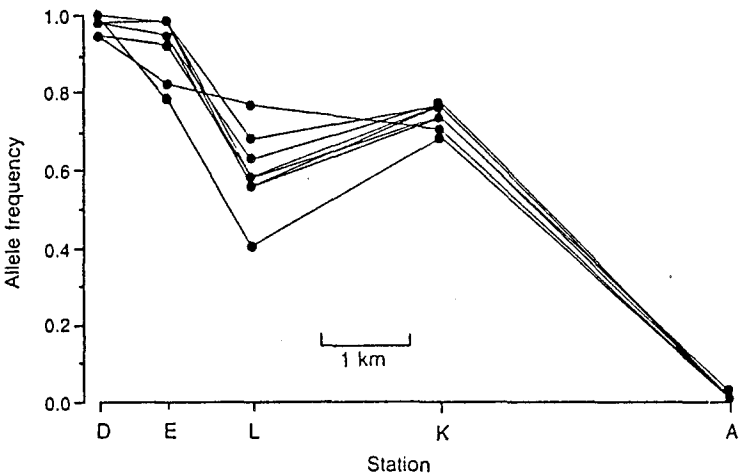

FIGURE 7. Gene frequency cline (based on *platensis* alleles) for eight loci in samples lying along a mainly south-facing slope between samples A and B (cf. Figure 1 and Table 3).

TABLE 4. Percentage individuals in different samples counted as hybrids.

Sample	Operational hybrids (%)	Sample	Operational hybrids
A	0	G	32
B	0	H	42
C	0[a]	I	67
D	0	J	0
E	16	K	30
F	9	L	52

[a]One individual in this population has a score of 4, but the alleles contributing to this score have a high probability of being *platensis* alleles; see text.

4. Two samples, I (N = 12) and L (N = 21) have more than 50% hybrids. In sample I there are no individual scores lower than 12 and the population is dominated by *xanthoptica* alleles (average frequency of *platensis* alleles in only 0.13 (SD = 0.24; Table 3). In contrast, in sample L the distribution of genotypes approximates that expected in a hybrid swarm (Figure 4), and only three scores of 0 or 16 are recorded.

The color hybrid index modified from Brown (1974) was established without knowledge of genetic information. The relationship between the two indices is illustrated in Figures 8, 9, and 10. Boxed values in these figures represent coincident occurrences in populations taken as representing parental conditions (A for *xanthoptica*; B and C for *platensis*). The *platensis* color pattern is much more variable than that of *xanthoptica*, and the color index ranges from 3 through 10. That for *xanthoptica* ranges only from 28 through 30. The two F_1 individuals found in this study have color scores of 15 and 16. Accordingly, we believe that the color index is suitable for comparison with the allozyme index.

Those populations in the heart of the hybrid zone (G, K, and L) have both color and hybrid scores skewed in the direction of *platensis* (Figure 8). No allozyme scores from 0 through 8 are missing, and no color scores from 3 through 15 are missing. In contrast, two allozyme scores from 8 through 16 are missing, and five color scores from 15 through 30 are miss-

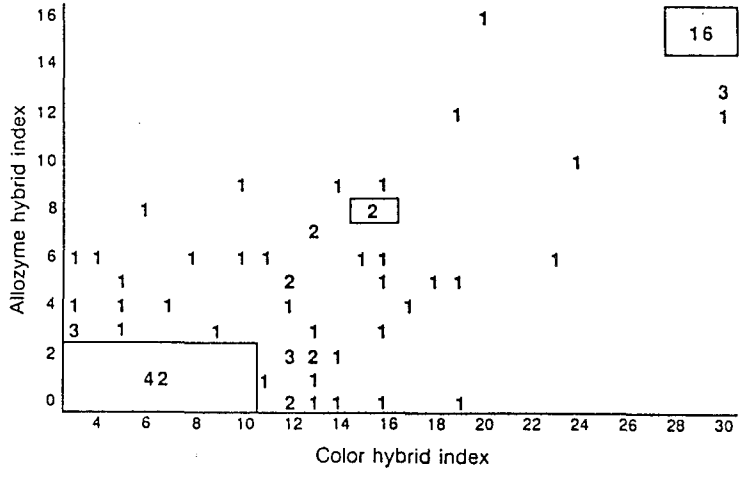

FIGURE 8. Relationship between allozyme hybrid index and color hybrid index in three populations in which both parental genotypes and hybrid genotypes are present. Color scores of 0, 1, and 2 cannot exist using our methods (see Materials and Methods). The box in the lower left outlines the coincidence between allozyme scores of 0 to 2 and color scores in populations B and C, which, for purposes of analysis, are considered to be typical parental *platensis*. The box in the upper right outlines the coincidence between allozyme scores of 15 and 16, and color scores in population A, which, for purposes of analysis, are considered to be typical parental *xanthoptica*. The box in the center surrounds the combined scores of the two F_1 individuals encountered in this study. The numbers identify individuals from samples G, K, and L for which information both on allozymes and color is available.

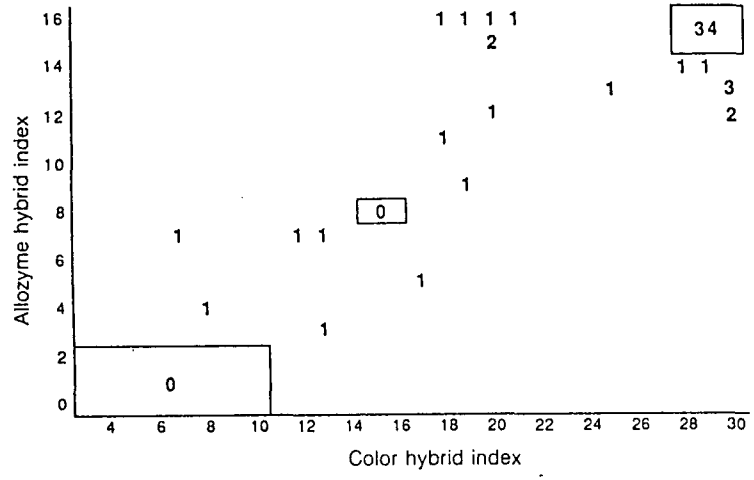

FIGURE 9. Relationship between allozyme hybrid index and color hybrid index for three samples (H, I, and J) on the *xanthoptica* side of the hybrid zone. See Figure 8 for further explanation.

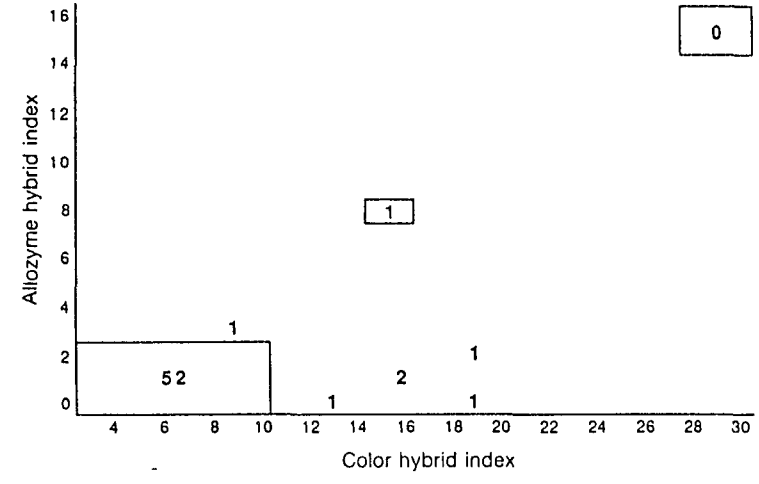

FIGURE 10. Relationship between allozyme hybrid index and color hybrid index for three samples (D, E, and F) on the *platensis* side of the hybrid zone. See Figure 8 for further explanation.

ing. Only nine individuals have scores from 8 through 16 for allozymes and simultaneously from 15 through 30 for color (excluding those in the parental box). In contrast, there are 35 individuals with scores from 0 through 8 for allozymes and simultaneously from 3 through 15 for color (again, excluding those in the parental box). Only two individuals have allozyme scores above 8 and color scores below 15, but nine individuals have allozyme scores below 8 and color scores above 15.

Those populations on the *xanthoptica* side of the hybrid zone (H, I, and J) have a strong predominance of coincident color and allozyme scores that are those identified as parental *xanthoptica* (Figure 9). There are 19 individuals with allozyme scores between 8 and 16 and color scores between 15 and 30 (excluding those in the parental box), but there are only 5 with allozyme scores from 0 through 8 and simultaneously with color scores from 3 through 15 (and there are no individuals in the parental box).

Those populations on the *platensis* side of the hybrid zone (D, E, and F) have a strong predominance of coincident color and allozyme scores that are those identified as parental *platensis* (Figure 10). No individuals have allozyme scores above 8, and only four individuals have color scores above 15 (19 is the highest).

DISCUSSION

Our analysis of allozyme variation in the study area has shown that hybridization between *platensis* and *xanthoptica* occurs in a geographically narrow zone in the foothills of the Sierra Nevada in Calaveras County, California. This area was chosen for study because it was known that a contact zone between *platensis* and *xanthoptica* existed there, and hybridization between the two had been demonstrated based on analysis of color pattern variation (Brown 1974). We located the exact sites used by Brown and added some additional ones. His series were as follows (our population designation follows in parentheses): (1) Dorrington (C); (2) Arnold (B); (3) Mill Creek (D); (4) Avery and Hunter Reservoir (G); (5) Indian Creek (A, our site is located a few hundred meters SE of his).

Brown (1974) estimated that the distance of overlap between the two parental types was about 0.3 miles (approximately 480 m), but stated that more intensive work would probably show the zone to be wider than he had observed. He thought that he detected genetic influence of *xanthoptica* on the coloration of *platensis* some distance from the zone of overlap (e.g., in the Arnold series). Introgression was believed to be taking place in both directions, and its effect was increased variability in color pattern in the parental forms (*platensis* was thought to be more affected than *xanthoptica*).

Using criteria from his color analysis, Brown (1974) constructed a hybrid index that gave an estimate of 8% hybrids in this entire region. In his Avery and Hunter Reservoir site (our population G), 22% (from his Figure 8) were hybrids. In the present study percentage hybrid individuals in population G by the operational definition using our allozyme and color indices is 32 and 38, respectively.

We have extended Brown's study in two ways: by adding more sites in the immediate vicinity of the hybrid zone near Avery, and by surveying variation in eight polymorphic proteins. Our results confirm Brown's identification of hybridization in a narrow zone. Along Mill Creek and its tributary the hybrid zone is only a little over 1 km in width, and both parental forms occur only in one site over a linear distance of about 300 m or less. The two other areas of overlap are equally narrow. We find no evidence of pervasive introgression, and only ambiguous evidence of gene flow beyond the immediate limits of the hybrid zone.

Prior work (Wake and Yanev 1986) showed that *platensis* has considerably more polymorphic proteins than does *xanthoptica* (1.38 to 1.69 alleles per locus, versus 1.12 for *xanthoptica*; heterozygosities for *plantensis* ranged from 6.9 to 12.3%, versus 1.9% for *xanthoptica*). Thus, although our hybrid index based on eight proteins is relatively certain for *xanthoptica*, the potential for overlooking rare *xanthoptica* alleles in the low-frequency background variation of *platensis* is relatively greater. For example, examination of allozyme hybrid index scores above zero in population D discloses that 5 of the 10 individual variants contributing such scores involve alleles of three proteins that also are present in reference populations B and C. Furthermore, these 10 variants occur in only 6 of the 8 surveyed proteins, whereas in the adjacent population E (which is a few hundred meters closer to the zone of overlap) the 32 variants contributing to scores above 0 are found in all eight proteins. Thus, little gene flow (possibly involving as few as 5 out of a total of 512 alleles) can be measured across the hybrid zone from *xanthoptica* through the zone of overlap to population D. Even less gene flow is occurring in the opposite direction. In population A only 1 of 430 alleles and in population J only 1 of 414 alleles (a single protein in one individual each in these two populations could not be scored) could be considered a *platensis* allele.

We do not believe that gene flow from *xanthoptica* is responsible for allozyme hybrid index scores greater than 0 in populations B and C. First, the Blodgett sample (Figure 3) is from an area approximately 75 km from B, far north of the Sierran range of *xanthoptica*, so scores greater than zero in that sample cannot be attributed to gene flow. Second, the one score in the hybrid range (a score of 4 in an individual in population C) is based on four variants that are present in Blodgett.

Population D has even fewer potentially *xanthoptica* variants than do more remote populations of *platensis*, in spite of the fact that it is a larger

sample (N = 32) than the others and lies very near the zone of overlap (Figure 3). This suggests not only that there is little gene flow, but also that some of the low-frequency background alleles might have been lost. This population is the last of the "pure" *platensis* populations, and is at the extreme margin of the range of the subspecies, both in terms of elevation and in habitat. At lower elevations, and only a few meters away from the stream, as well as farther to the west generally, the forest thins and becomes interspersed with areas of brush and grasslands. Typically *platensis* lives in closed canopy, relative dense forest at higher elevation, whereas *xanthoptica* lives in lower, more open forest and brush mosaic habitats. Thus, it is possible that combinations of inbreeding and drift might increase homozygosity and reduce variability in the finger-like projections of the population of *platensis*, causing it to break up into relatively small and semi-isolated demes at the population front.

We found only 2 F_1 hybrids; all others are backcrosses. Our samples are too small to permit a detailed analysis of the pattern of backcrossing, but it appears to be restricted to the hybrid zone and to involve mainly other hybrid individuals. If there were more backcrossing into parental stocks we would find more evidence of gene flow. Thus, we conclude that hybridization between sympatric parental genotypes is rare.

Inspection of the allozyme hybrid index profile in population G (Figure 4) suggests that the *xanthoptica* individuals present in the population have not been outbreeding, for all classes from 9 through 16 are absent. There is only one individual in this range in station K, but there are several in Station L (Figure 4). Furthermore, in both station H (Figure 5) and I (Figure 2) there are numbers of individuals with scores between those of F_1 and "pure" *xanthoptica*. Thus, it seems unlikely that the absence of a series of potential genotypes from population G is the result of inviability or poor performance. However, on both sides of the contact zone, and even in the zone itself, the pattern of skew encountered suggests an absence of free interbreeding.

We believe that our hybrid zone arose from secondary contact resulting from the "transvalley leak" of *xanthoptica* from the west, probably during Late Pleistocene times (see Stebbins 1949, for a detailed consideration of this issue). The close genetic similarity of Sierran *xanthoptica* to populations in the hills just east of San Francisco Bay (Nei genetic distance is 0.021) argues in favor of this hypothesis, and the very low variability of Sierran *xanthoptica* compared with that of other populations of *Ensatina* is consistent with establishment of these populations by recent founder events (data in Wake and Yanev 1986). Further evidence in favor of the hypothesis of secondary contact is the fact that 8 of 26 proteins sampled show drops in frequency from greater than 0.95 to less than 0.05 in a distance just over 5 km, and they change in a highly concordant manner. The hybrid zone has been stable for at least 20 years.

A factor contributing to the narrowness of the hybrid zone is the tendency of terrestrial plethodontid salamanders to have small home ranges and to have no special pattern of dispersal. These salamanders lay direct-developing eggs on land and do not migrate to water. Stebbins (1954) studied *xanthoptica* in the East Bay region over approximately 4 years, and based on a mark-recapture study he reported on density (about 600-700 per acre), home range, and movements. Home ranges were small, with "greatest width" averaging 10.1 m for females and 19.6 m for males. The widest home range recorded was 41.4 m, and this also was the greatest movement. Sexual maturation does not occur until the fourth year, and individuals may live to be 10 to 15 years of age, so movements of single individuals over their lifetime will be difficult to follow. Dispersal distances are expected to be greater than home range sizes, but as yet little information of dispersal in *Ensatina*, or other terrestrial salamanders, exists. A mark-recapture study on a 100 by 300 m plot in Calaveras Big Trees State Park (Figure 1) is designed to find potential dispersing individuals, and preliminary results suggest that Sierran *platensis* move greater distances (at least one individual has moved more than 100 m) than do the *xanthoptica* Stebbins (1954) studied. Possibly his plot size was too small to record dispersers. Although the species is a relatively sedentary one, narrowness and persistence of the hybrid zone cannot be attributed to neutral diffusion based on lack of movement by the animals.

Much attention has been given to hybrid zones recently (e.g., Barton and Hewitt 1981, 1983, 1985; Woodruff 1981), and there have been some important theoretical advances. For example, Barton and Bengtsson (1986) concluded that strong selection against hybrids at numerous genetic loci is required to create a genetic barrier at linked neutral loci. We believe that hybrid individuals are at a disadvantage in the hybrid zone, and that the allozyme patterns we report are a consequence of that selection, even though the allozymes themselves may be selectively neutral. There are three main candidates for selective agents: habitat choice, predation, and mating behavior.

In general *xanthoptica* occurs at lower elevations than *platensis*, in warmer and drier environments, and in open pine to pine-oak forest, and mosaics of forest and grassland-brush habitats. Where *xanthoptica* approaches the range of *platensis* it reaches its highest elevational limit on south-facing slopes with relatively open forest, whereas *platensis* reaches its lowest elevational limit on north-facing slopes with more closed forest or in stream bottoms. For example, along Mill Creek and its tributaries *platensis* has been found as low as 975 m (station G), whereas just a short distance away *xanthoptica* has been taken as high as 1190 m (station K). Station K can be subdivided at the ridge-top into north and south halves, the latter south facing and covered by a relatively more open forest. Using our operational values with the allozyme hybrid index, the ratio of *xanthop-*

tica to *platensis* is 5:11 on the south half, but 1:16 on the north half, and the single F_1 from the site is from the south half. We do not know what sets the limits of either form, but both to the north and to the south of the range of *xanthoptica* in the Sierra Nevada, *platensis* reaches lower elevations than in the Avery area. Perhaps the combination of deteriorating habitat and competition with *xanthoptica* sets the lower elevational limits of *platensis* in this area. There is no apparent reason why *xanthoptica* could not live at slightly higher elevations. The winter snow zone commences between 1000 and 1200 m, but the environment is relatively mild. However, densities of *platensis* are relatively high immediately at the edge of the hybrid zone, and it seems likely that competition by preemptive occupancy of space could limit incursion of *xanthoptica*. The zone of overlap is surprisingly narrow given the known ability of single individuals to undertake movements equal to more than one-third the width of the known zone of overlap.

Several vertebrates are known or suspected to prey on *Ensatina* (Stebbins 1954). Raccoons regularly disturb pitfall traps and eat salamanders, and jays and other birds also feed on *Ensatina*. The cryptic (in natural habitat) color pattern of *platensis* contrasts with the vivid, conspicuous coloration of *xanthoptica* (Stebbins 1949; Brown 1974). Both Stebbins (1949) and Brown (1974) have argued that *xanthoptica* is a mimic of the highly poisonous salamanders of the genus *Taricha*, which have only one known predator (a garter snake). The robust tail of *Ensatina* is richly supplied with poison glands and it is an effective deterrent to at least some snake predators (Hubbard 1903). Furthermore, the tail can be autotomized. On several occasions of raccoon depredation of can traps, only the detached tail of the *Ensatina* has been left behind. Sierran populations of *xanthoptica* are an especially vivid yellow-orange, much brighter than the East Bay populations, and the Sierran populations of *Taricha torosa* are also the most brightly colored in that species (Riemer 1958). Hybrids between *platensis* and *xanthoptica* are neither mimics nor are they cryptic. They stand out, but do not look like anything else. Increased predation on them (for which we have no evidence) could contribute greatly to selection against hybrids and backcrosses. Presumably the mimics could ascend to higher elevations and the cryptic forms to lower elevations, so the position of the hybrid zone is unlikely to be set by increased predation on hybrids. Cryptic forms are less cryptic at lower elevations, because of the more open nature of the low elevation forests, and mimics move out of the range of *Taricha* at higher elevations, reducing the effectiveness of their mimicry. Selection related to habitat choice along the elevational gradient, in combination with increased predation on hybrids, could help define the elevational limits of the zone.

Mate recognition systems also may be important in the vicinity of the hybrid zone. Plethodontid salamanders have an involved courtship behavior, and in *Ensatina* it is a very lengthy process with many components (Stebbins 1954). We have no evidence as yet concerning the possibility of isolating mechanisms, or differences in mate recognition signals, but the existence of a courtship that is dependent on chemical and tactile cures contributes to the likelihood that such may be the case. F_1 individuals are rare, and all genotypic classes recorded could be the result of relatively limited mating among the hybrids themselves. We suspect on the basis of the genic patterns recorded that there is little mating of parental types with F_1s or other hybrids.

Parapatric distributions, in which two genetically distinct forms have closely abutting geographic ranges, are common in salamanders (see review by Larson 1984; Good et al. 1987), and frequently there is no hybridization between the taxa. Elevational replacement is also a well-known phenomenon in salamanders (Hairston 1951; Wake and Lynch 1976), and competition between closely related species of plethodontids has been demonstrated (see review by Hairston 1988). No direct evidence of competition in *Ensatina* exists, but arguing by analogy from studies on other species of salamanders, competition between *xanthoptica* and *platensis* may contribute to the location of the contact zone. We know that the zone varies in elevation from place to place in the Sierra Nevada, as Hairston (1951) demonstrated for *Plethodon jordani* and *P. glutinosis* in North Carolina, and this suggests that different combinations of factors are acting in concert to set the location of the zone in different areas. If we are correct in our argument that *xanthoptica* has invaded the Sierra Nevada, it probably became established first in regions of low elevation and open habitat, unoccupied by *platensis*. As it spread eastward and into higher elevations, it came into contact with *platensis*, and the amount of geographic overlap between the two has remained remarkably slight. We postulate that a combination of preemptive occupancy of space by *platensis*, competition, and different patterns of environmental adaptation has created a standoff between the two forms.

Comparisons with other contact zones in *Ensatina* show that although gene flow is low and very limited in geographic extent in Calaveras County, there is even less genic exchange in southern California. There are 10 nearly fixed genetic loci (out of 26 loci sampled) differentiating the blotched *klauberi* (the morphological analog of *platensis*) and unblotched *eschscholtzii*, and with this increased genetic differentiation there also is an apparent decrease in the number of hybrids found and the extent of backcrossing (Wake et al. 1986). A hybrid index based on fixed differences in 10 proteins can be used to display variation in the four main contact zones in southern California (Figure 11). Our samples for southern California reported here are regional ones, including sites in which both parental

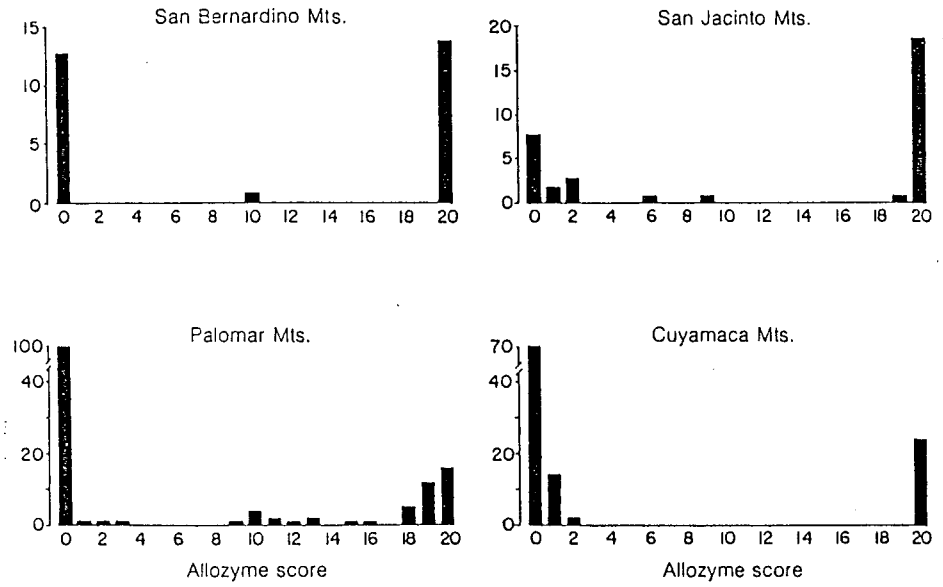

FIGURE 11. Frequency histograms for four samples of *Ensatina* in southern California. These samples represent amalgamated local samples in four discrete mountain regions. A score of 0 is unambiguous "pure" *klauberi*, whereas 20 is unambiguous "pure" *eschscholtzii*. Scores of 1 and 2 are also considered to be *klauberi*, and scores of 19 are accepted as *eschscholtzii*. All other scores are considered to be hybrids. Sympatry with no hybridization occurs in the Cuyamaca Mountains.

forms occur as well as areas in which only one occurs. In this figure *klauberi* has scores of 0 to 2, and *eschscholtzii* has scores of 19 and 20. We found only two hybrids in the San Bernardino Mountain region, both F_1 taken in close proximity to both parental types. In the San Jacinto Mountains we found only two hybrids, neither an F_1. In the Palomar Mountains there are more hybrids, including a potential F_1, and there is an apparent skew in the direction of *eschscholtzii*. Finally, in the Cuyamaca Mountains, our southernmost sample, there is complete sympatry with no evidence of hybridization; however, *eschscholtzii* is relatively uncommon (only 8 have been found, compared to more than 50 *klauberi*). Thus, in the zone of contact in southern California there is substantially less hybridization than in the Sierran zone of contact, and there even is local sympatry with no hybridization.

In both the southern California and Sierra Nevada hybrid zones the interacting units appear to be at the species level of differentiation. However, because of the complexities associated with the possibility of indirect connections between the units, we recommend no taxonomic changes as yet. Studies of genic variation, morphological variation, and ecology are in progress, and we are giving special attention to areas that previous workers have identified as zones of intergradation. The general pattern that is emerging is not one of gradual differentiation, but rather one in which there is much local and regional differentiation with occasional sharp zones of change. Speciation seems to have progressed in part by allopatric adaptive divergence and in part by essentially stochastic isolation by distance. We tentatively accept the general scenario of Stebbins (1949) that *Ensatina* moved southward and wrapped around the Central Valley. However, this appears to have been a much more ancient event than envisioned by him. There have been many opportunities for range expansion and contraction, and for adaptive morphological and ecological differentiation. We find genetic differentiation manifest at many levels. Thus, within what Stebbins identified as subspecies, there is substantial geographic differentiation. The amount of genetic differentiation within the blotched and unblotched groups of subspecies is as great as that between these two groups. Intergradation between coastal and inland (montane) groups north of the Sacramento Valley was postulated on the basis of an analysis of coloration (Stebbins 1949). This region must be studied carefully to determine if there is a substantial difference from the kinds of interactions that we have recorded in the Sierran foothills. The apparent natural experiment provided by the transvalley leak, which resulted in the secondary contact with hybridization of *xanthoptica* and *platensis*, may be just a more dramatic form of the secondary meeting of groups that has led to the abrupt change that we find elsewhere in this complex group. The cooccurrence of two biological species in the Cuyamaca Mountains is readily apparent because of the great differences in coloration, and the absence of hybrids. Our discovery of regions of abrupt changes in gene frequencies suggests that it is still too early to determine if *Ensatina* presents several stages in the process of speciation, or if the genus already has speciated extensively and some borders are more readily apparent than are others. Work in progress will contribute to the solution of this problem.

SUMMARY

A complex of morphologically differentiated populations of salamanders of the plethodontid genus *Ensatina* surround the Central Valley of California in ring-like fashion. At the bottom of the ring, in southern California, there is overlap, and sympatry occurs with limited or no hybridization in different locations. At about the midpoint in the ring there has been a "transvalley leak" of coastal populations into the foothills of the Central Sierra Nevada, where coastal unblotched and inland blotched populations

meet along a long, narrow hybrid zone. There are either fixed or nearly fixed allozymic differences between the two interacting population groups, and these were used to study interactions in the hybrid zone. Local sympatry has been found in three places, with both parental types as well as a variety of hybrids being present. F_1s are rare, but there are backcrosses and perhaps multigenerational hybrids present in the sympatric sites and on their borders. The hybrid zone is very narrow; the average gene frequencies (eight loci) drop from 0.88 to 0.2 in about 1400 m, and the width of the zone of overlap between parental types is about 300 m. Gene flow is low, but apparently symmetrical. The two interacting groups appear to have achieved the status of biological species, with strong genetic and morphological, and moderate ecological differentiation. The groups are differently adapted, and there appears to be selection against hybrids. Nevertheless, the groups are linked by a series of intermediate populations around the north end of the Central Valley. This ring species displays several stages of speciation in what appears to be a continuous process of gradual allopatric, adaptive divergence.

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