Evolution, 59(6), 2005, pp. 1334–1347

STRONG SELECTION AGAINST HYBRIDS AT A HYBRID ZONE IN THE *ENSATINA* RING SPECIES COMPLEX AND ITS EVOLUTIONARY IMPLICATIONS

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Abstract.—The analysis of interactions between lineages at varying levels of genetic divergence can provide insights into the process of speciation through the accumulation of incompatible mutations. Ring species, and especially the *Ensatina eschscholtzii* system exemplify this approach. The plethodontid salamanders *E. eschscholtzii xanthoptica* and *E. eschscholtzii platensis* hybridize in the central Sierran foothills of California. We compared the genetic structure across two transects (southern and northern Calaveras Co.), one of which was resampled over 20 years, and examined diagnostic molecular markers (eight allozyme loci and mitochondrial DNA) and a diagnostic quantitative trait (color pattern). Key results across all studies were: (1) cline centers for all markers were coincident and the zones were narrow, with width estimates of 730 m to 2000 m; (2) cline centers at the northern Calaveras transect were coincident between 1981 and 2001, demonstrating repeatability over five generations; (3) there were very few if any putative F_1s, but a relatively high number of backcrossed individuals in the central portion of transects; and (4) we found substantial linkage disequilibrium in all three studies and strong heterozygote deficit both in northern Calaveras, in 2001, and southern Calaveras. Both linkage disequilibrium and heterozygote deficit showed maximum values near the center of the zones. Using estimates of cline width and dispersal, we infer strong selection against hybrids. This is sufficient to promote accumulation of differences at loci that are neutral or under divergent selection, but would still allow for introgression of adaptive alleles. The evidence for strong but incomplete isolation across this centrally located contact is consistent with theory suggesting a gradual increase in postzygotic incompatibility between allopatric populations subject to divergent selection and reinforces the value of *Ensatina* as a system for the study of divergence and speciation at multiple stages.

Key words.—Concordant clines, *Ensatina eschscholtzii*, hybrid zones, linkage disequilibrium, maximum likelihood, ring species, selection against hybrids, speciation.

Received March 10, 2004. Accepted March 20, 2005.

Recent theoretical studies have confirmed the plausibility of speciation via accumulation of incompatible mutations (the Dobzhansky-Muller effect), but emphasize that the gradual nature of the process is enhanced by divergent selection (Gavrilets 2003). To provide empirical support to this theory, it is necessary to examine genetic interactions among lineages with varying levels and forms of divergence (Turelli et al. 2001). As has long been recognized (Mayr 1942, 1970), ring species provide a natural experiment for this purpose. Here, we take advantage of an unusual opportunity to quantify selection against hybrids in a natural contact zone in the center of a geographic ring involving the plethodontid salamander *Ensatina eschscholtzii* (Stebbins 1949; Dobzhansky 1958; Wake 1997). These fully terrestrial, direct-developing lungless salamanders inhabit coniferous forests and oak woodland along the Pacific Coastal region of North America, from southern British Columbia to northern Baja California, extending inland to the western slopes of the Cascades, the Sierra Nevada, and the Peninsular Ranges (Stebbins 1949; Wake and Yanev 1986). The current taxonomy (Stebbins 1949; Wake and Schneider 1998; but see Frost and Hillis 1991; Graybeal 1995; Highton 1998) recognizes a single species, *E. eschscholtzii*, and seven subspecies strongly differentiated in color and pattern with two main pattern classes: (1) the blotched forms (*platensis*, *croceater*, and *klauberi*), which occur in the Sierra Nevada and various mountain ranges in Southern California; and (2) the unblotched forms (*eschscholtzii*, *oregonensis*, *picta*, and *xanthoptica*), which occur throughout the rest of the range (Fig. 1). The historical biogeographic hypothesis proposed for *Ensatina* by Stebbins (1949), broadly supported by allozyme and mitochondrial DNA data (Larson et al. 1984; Wake and Yanev 1986; Moritz et al. 1992; Wake 1997), proposes that the species originated in present-day northwestern California and southwestern Oregon and spread southward. Along the coast the species evolved a uniform reddish-brown dorsal coloration and a light pink to orange ventral coloration. In the inland mountains the species evolved a cryptic, spotted, or blotched color pattern. As the two arms of the expanding distribution moved southward, on either side of the large Central Valley, they came into sympatry in the southern Peninsular Ranges. Midway along the length of the Central Valley there has been a ''transvalley leak'' (Stebbins 1949), and *xanthoptica* has ex-

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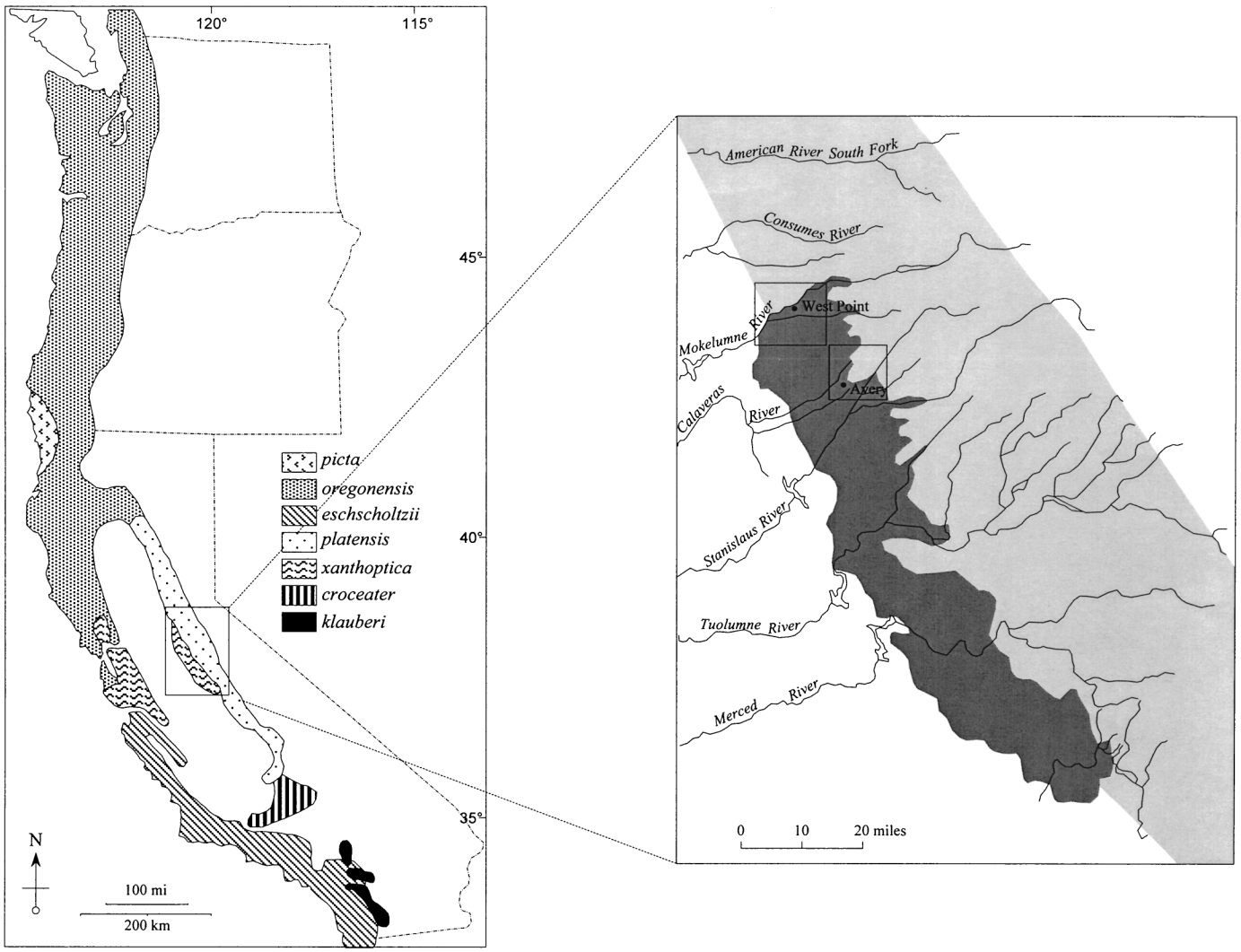

FIG. 1. The *Ensatina* complex, showing the distribution of taxa recognized by Stebbins (1949), but with borders based on molecular markers rather than morphological traits (Wake 1997), and the general location of the hybrid zone between *E. eschscholtzii xanthoptica* and *E. e. platensis*. The more detailed map shows the locations of the two studied transects in the northern part of the hybrid zone (Calaveras Co.).

panded its range into the foothills of the Sierra Nevada where it is parapatric with the blotched *platensis*. However, genetic evidence also indicated a much deeper history than originally envisioned with multiple episodes of expansion, isolation, and secondary contact within and across the ring driven by long-term climate change and geomorphological evolution of California since the Miocene (Jackman and Wake 1994; Wake 1997). Presently, there is a diversity of secondary contacts among lineages with varying levels of divergence for neutral genes, habitat requirements, and predator avoidance strategies (Wake 1997). Sympatry with little hybridization occurs between the southernmost forms, the coastal *E. e. eschscholtzii* and the inland *E. e. klauberi* (Wake et al. 1986). By contrast, two previously isolated nonsister lineages of *E. e. platensis* appear to be merging in Central Sierra Nevada (Wake and Schneider 1998; J. Alexandrino unpubl. data). Understanding genetic interactions in contact zones within and between phenotypic forms is central to using this geographic ring to elucidate speciation processes (Highton 1998; Wake and Schneider 1998).

Species formation can be envisaged as the process by which selection acts to generate barriers to gene flow between two populations. The mechanisms preventing gene flow can be investigated where the barriers are not yet complete, that is, in hybrid zones where genetically distinct populations meet and produce hybrids (Barton and Hewitt 1985; Arnold 1997). Differentiated multilocus genotypes may then be broken up by recombination with natural selection acting on the newly generated combinations of alleles (Harrison 1990, 1993). The simplest result of this kind of interaction is the formation of clines at different loci or of phenotypic traits, the position and width of which are determined by a balance between dispersal into the zone and selection against hybrids. Until a selection/migration equilibrium is reached, cline dynamics (e.g., shape, movement) will depend on the forces that maintain the cline. The dynamics of clines maintained

by endogenous selection will be independent from the environment (Bazykin 1969; Barton 1979; Mallet and Barton 1989; Johnson et al. 1990; Gavrilets 1997), whereas those clines maintained by exogenous selection across an ecotone will settle on the boundary between environments (Endler 1977; Moore and Price 1993; Kruuk et al. 1999). The coincidence of the cline centers and the concordance of cline widths is thought to originate from secondary contact between populations and/or strong gametic disequilibria across the entire genome that causes the center of the hybrid zone to act as a barrier (Barton and Hewitt 1985). However, multiple characters may introgress to different extents because of different selection pressures, causing nonconcordant clines, while epistatic interactions may force cline centers apart causing noncoincident clines. A narrow hybrid zone with coincident and concordant clines and strong selection against hybrids is more likely to effectively maintain isolation between diverged forms and further promote speciation than is a hybrid zone with wider clines associated with neutral mixing.

Selection gradient (Slatkin 1973, 1975; May et al. 1975; Endler 1977) and tension zone (Barton and Hewitt 1985, 1989; Barton and Gale 1993) hybrid zone models differ by assuming that selection operating against hybrids is exogenous or endogenous, respectively, but are remarkably similar mathematically in providing estimates of selection from cline width and dispersal (Barton and Hewitt 1985, 1989; Barton and Gale 1993; Kruuk et al. 1999). We follow a population genetic analysis under the tension zone model framework because it makes use of several hybrid zone properties (cline width, cline concordance/coincidence, linkage disequilibrium) to inform us about not only the genetic differences between the populations involved, but also the rate of individual dispersal and the barriers to allele exchange (i.e., strength of selection) between differentiated gene pools (Szymura and Barton 1986; Barton and Gale 1993).

In the present study, we focus on the genetic characteristics of a ''mid-ring'' secondary contact in the plethodontid salamander ring species *Ensatina* located in the foothills of the Central Sierra Nevada (between about 900 and 1200 m elevation; Fig. 1). The hybrid zone between *E. e. platensis* (a form with a spotted and 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 uniformly bright orange color pattern), a coastal subspecies with an outpost in the Sierran foothills, is most likely the result of secondary contact between lineages that evolved in allopatry following expansion from the eastern San Francisco Bay Area (Stebbins 1949; Moritz et al. 1992; Wake 1997). Habitat between the two disjunct ranges of *xanthoptica* is presently inhospitable for these salamanders, but large lakes existed in the Central Valley during the late Quaternary (Sarna-Wojcicki et al. 1985), and bioclimate modeling (J. Alexandrino, unpubl. data) indicates that the intervening areas could have been suitable with just small shifts in climate as occurred during the Holocene (Davis 1999). These paleoecological inferences, combined with low molecular divergence between Sierran and east Bay *xanthoptica* (Wake and Yanev 1986; Moritz et al. 1992; Wake 1997) indicate that the hybrid zone with *platensis* was established in the Holocene. Based on observations of phenotypes, Brown (1974) suggested that the zone of hybrid influence might be relatively wide, on the order of tens of kilometers. By contrast, the first genetic (allozyme) analysis of one transect across the parapatric boundary suggested a steep cline (1.5 km, using Endler's [1977] criteria), but did not further characterize the genetic architecture of the zone (Wake et al. 1989). Here we apply maximum-likelihood tension-zone models (Szymura and Barton 1986, 1991; Barton and Gale 1993; Phillips et al. 2004) to quantify genotypic disequilibria and cline width and replicate the analysis of contact zones in space and time to test for consistency of major features. If selection is operating against hybrids, we expect to observe cline widths that are narrow relative to dispersal, strong genetic disequilibrium in the center of the zones, and patterns that should be repeatable across space and time.

Materials and Methods

Sampling

Hybridization between *E. e. xanthoptica* and *E. e. platensis* occurs along a narrow, approximately 150-km long zone in the foothills of the Sierra Nevada, bounded by the Mokelumne River in the north and the Kings/San Joaquin River in the south (Fig. 1). The hybrid zone generally coincides with a forest transition between typical lower elevation (600–900 m) Sierran foothill oak woodland and higher elevation (>900 m) dense Sierran montane vegetation (for details see Brown 1974). Previous studies of this hybrid zone have concentrated in southern Calaveras Co. (Brown 1974; Wake et al. 1989) but the expansion of the nearby town of Avery rendered the area mostly unsuitable for long-term studies. We here reanalyze data from Wake et al. (1989) collected along the Avery main transect (hereafter SC89; Figs. 1, 2B and see Supplementary Table 1 available online only at http://dx.doi.org/10.1554/04-156.1.s1). Another transect was established in the early 1980s in northern Calaveras County near the junction of the Mokelumne River forks and the town of West Point, and salamanders were captured in 1980 and 1981 (hereafter NC81; Figs. 1, 2A and see Supplementary Table 1 available online). Twenty years later, in 2001 and 2002, we sampled along a similar transect to undertake a comparative study of the zone (hereafter NC01). Most salamanders were captured in pitfall traps throughout two separate fall-spring collecting seasons spanning 20 years, the first in 1980–1981 and the second in 2001–2002. At each sampling site 10 traps were installed, spanning a maximum of 100 m between traps, which is approximately the maximum per generation individual dispersal distance in *E. e. platensis* (Staub et al. 1995). We assumed that each site represented a local population. Throughout the three studies (SC89, NC81, and NC01) we chose to represent each sampling site by its distance to the fitted cline center (see below), attributing negative values to sites with a predominance of *E. e. xanthoptica* alleles and positive values to sites with higher proportion of those alleles typical of *E. e. platensis* (Fig. 2).

Markers

Morphological and allozyme markers known to be diagnostic between *xanthoptica* and *platensis* were used to di-

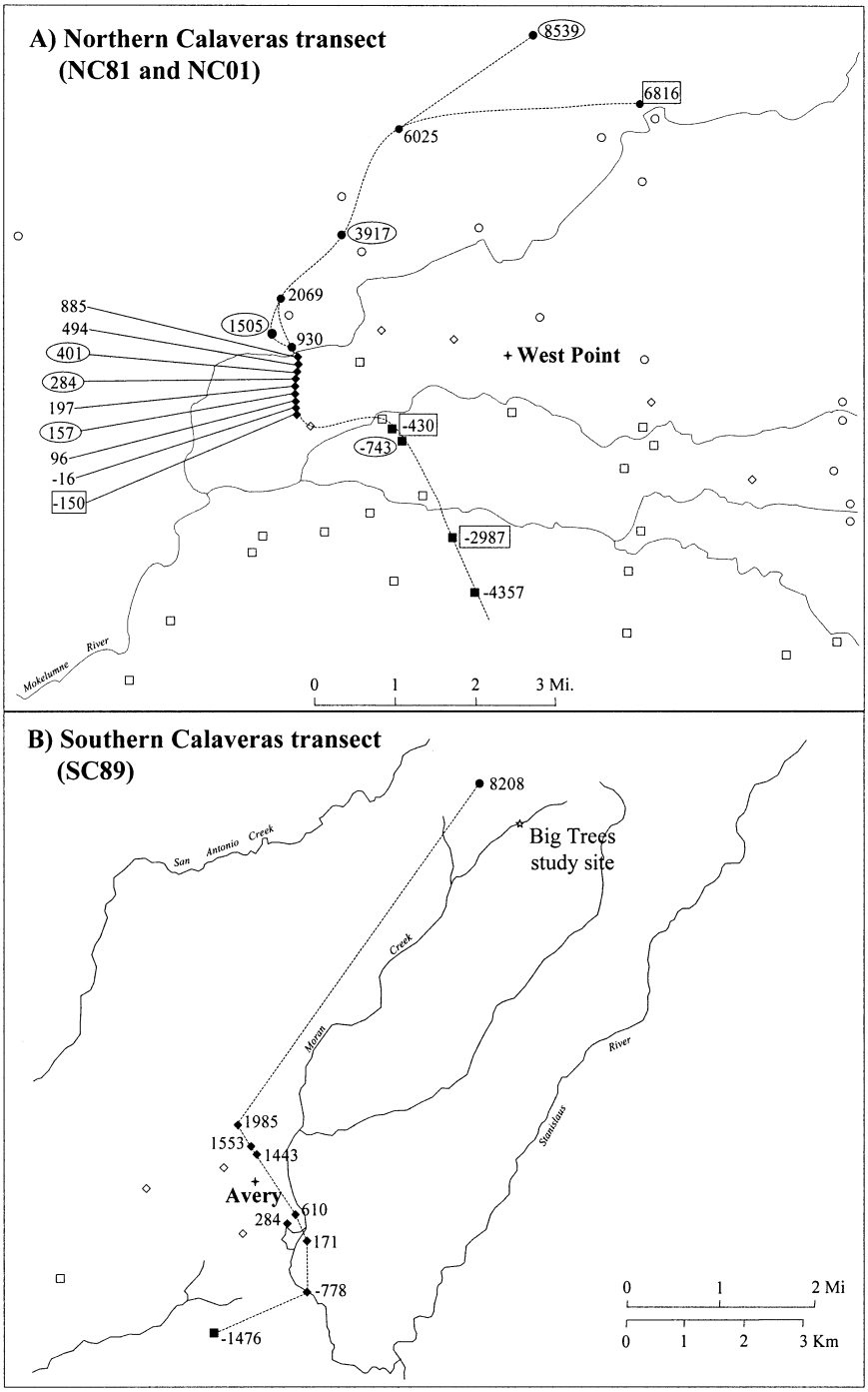

FIG. 2. Details of sampling site location across the northern Calaveras transect (NC81 and NC01) and the southern Calaveras transect (SC89). Sites where *E. e. xanthoptica*, *E. e. platensis*, and presumed hybrids have been collected are represented by squares, circles and diamonds, respectively, with solid black symbols for those in the main transect for the NC81, NC01, and SC89 studies. Sampling sites are designated by their distance in meters from the fitted transect centers (see text), with negative distances in the *xanthoptica* side and positive distances in the *platensis* side. Sites included only in the NC81 and NC01 studies appear outlined by ellipses and rectangles, respectively.

agnose salamanders from SC89 (Wake et al. 1989) and those from NC81 and NC01. Salamanders collected in the NC01 study were additionally typed for a fragment of mitochondrial cytochrome *b* gene.

Color pattern scores.—All specimens were returned living to the laboratory and were scored for color pattern using a modification of Brown's hybrid index that was based on scoring the following traits between 1 and 10 for each animal (Brown 1974): degree of limb melanophore development, degree of iris iridophore development, degree of melanophore development on ventral surfaces, and degree of erythrophore development on ventral surfaces. We used only the last three

traits because the first one was not completely diagnostic between *E. e. platensis* and *E. e. xanthoptica*. Color pattern was summarized by adding the values for all three indexes in each individual. All the resulting scores were then rescaled to generate a color pattern index (CPI) ranging from 0 for ''pure'' *xanthoptica* to 1 for ''pure'' *platensis*.

Allozyme electrophoresis.—Liver, stomach, and intestine were taken from specimens and used for allozyme electrophoresis. Following previous allozyme polymorphism screening (Wake et al. 1989) we used the eight allozyme loci observed to be diagnostic between *E. e. platensis* and *E. e. xanthoptica* (see Supplementary Table 2 available online only at http://dx.doi.org/10.1554/04-156.1.s2): phosphogluconate dehydrogenase (*Pgdh*), malate dehydrogenase (*Mdh*), isocitrate dehydrogenase (*Idh*), aspartate aminotransferase (*Aat*), peptidase B (*Pep-B*), peptidase D (*Pep-D*), glycerol-3-phosphate dehydrogenase (*Gpdh*), and sorbitol dehydrogenase (*Sordh*). Electrophoretic essays were carried out following methods in Wake and Yanev (1986) and Jackman and Wake (1994) and general protocols described in Harris and Hopkinson (1976).

We found fixed or nearly fixed differences between *xanthoptica* and *platensis* populations located at the extremes of the transect. As pointed out by Wake et al. (1989), the coordination of allele designations throughout all electrophoretic studies of *Ensatina* is difficult. In this study, because most loci are essentially diallelic we assumed that the most common alleles observed at each locus in the NC01 study are homologous to those observed in SC89 (Wake et al. 1989) and NC81 (J. R. Macey, unpubl. data).

Mitochondrial DNA typing.—Total cellular DNA was extracted with the QiaGen (Valencia, CA) DNA extraction kit. A diagnostic restriction endonuclease (RE) assay to distinguish between *xanthoptica* and *platensis* haplotypes was designed using available mitochondrial DNA (mtDNA) sequence data (Moritz et al. 1992; Parks 2000). The REs used (*Hinc*II and *Sal*I) cut the *xanthoptica* haplotypes but not *platensis* haplotypes. Polymerase chain reaction amplification of the relevant cytochrome *b* fragment was undertaken as described in Moritz et al. (1992), and RE fragment patterns were scored following digestion and agarose gel electrophoresis.

Analyses

All markers were collapsed to two allele systems (i.e., *xanthoptica* alleles and *platensis* alleles; see Supplementary Table 2 available online; Wake et al. 1989). A genetic hybrid index was calculated from allozyme data ranging from 0 (theoretical ''pure'' *xanthoptica*) to 16 (theoretical ''pure'' *platensis*). Maximum-likelihood clines were fitted independently at each locus to population allele frequency data across the transects using the compound hyperbolic tangent function (TANH) and exponential model of Szymura and Barton (1986) implemented in Analyse (Barton and Baird 1995). We fitted sigmoid clines in Analyse using four variables: *pmax*, *pmin* (the maximum and minimum gene frequency values at the tail ends of a cline), cline width, and cline center. We did not explore the more complex stepped-cline hypothesis because its use only seems justified when there is extensive sampling in the tails of clines (Barton and Gale 1993). Given the nature of the available data, we collapsed the sampled sites onto a one-dimensional (1D) transect for the purposes of analysis. Cline fitting was undertaken along a best-fit axis through sampled populations, equating to a compass bearing of 120° for NC81 and NC01 and of 90° for SC89. Error in the orientation of the 1D collapse can only overestimate the width. Locality samples were weighted by effective sample size, a measure of the number of independent sampling events given observed genetic disequilibria: deviation of the population from Hardy-Weinberg equilibrium means the states of the two alleles sampled at a locus are not statistically independent. Effective sample sizes taking into account maximum-likelihood estimates (MLEs) of F_{IS} were calculated for each sample at each locus as (adapted from Philips et al. 2004):

$$N_e = \frac{2N}{1 + F_{IS}}, \tag{1}$$

where N is the number of diploid individuals sampled, and N_e is the effective number of alleles sampled scaled such that complete heterozygote deficit results in effective allele sample size N. The average across sites of the percentage reduction from allele copies sampled to effective independent samplings ranged from 0–22%, 25–37%, and 21–32% across loci, respectively, for NC81, NC01 and SC89.

Cline coincidence and cline concordance between distinct loci and between different studies were evaluated combining maximum-likelihood cline-fitting procedures (Barton and Baird 1995) with the approach recently described by Phillips et al. (2004); for each model and each locus the likelihood surface was explored stepwise along axes for both center position c and width w with the other parameters free to vary at each point. In this way the likelihood profiles (Hilborn and Mangel 1997) for both c and w can be constructed. For example, coincident versus staggered cline center hypotheses can be compared as follows: summing log-likelihood c profiles over a set L of loci results in the log-likelihood profile for the ML shared center of the L loci. The center coincidence ML can be compared with the sum of noncoincident c profile MLs using a likelihood-ratio test, under the assumption that twice the difference in $\log_e$ likelihood ($G = -2\Delta LL$) between two models asymptotically follows a chi-squared distribution with the degrees of freedom being the difference in the number of parameters.

Within the contact zone, Hardy-Weinberg equilibrium (HWE) was assessed for each marker using ML estimates of F_{IS} in Analyse. Hardy-Weinberg equilibrium was also assessed at each site for each marker within the contact zone. Scaled average pairwise linkage disequilibrium ($R = D/(pquv)^{1/2}$) through the cline was assessed for diagnostic markers by partitioning the variance in hybrid index summed across diagnostic loci (Barton and Gale 1993).

To compare disequilibria among allozymes and mitochondrial haplotypes with disequilibria at loci underlying quantitative traits (CPI), we used the disequilibrium measure of D^* (Nürnberger et al. 1996), as follows:

$$D^* = \frac{2\ \mathrm{cov}(z, z')}{(1 + F_{IS})\Delta z \Delta z'}, \tag{2}$$

where Δz and $\Delta z'$ are the differences between means of the quantitative characters z and z' across the hybrid zone.

RESULTS

Allelic Distributions across the Hybrid Zone

Most allozyme loci were essentially diallelic although some low frequency alleles were found in all studies (see Supplementary Table 2 available online). The allele *Pep-D*f+* was found in NC81 only at the most extreme *xanthoptica* locality (frequency of 0.04 at site −4357); *Pep-D*d-* was found in NC01 in three samples near the center of the transect with frequencies ranging from 0.07 to 0.13. At the *Pep-B* locus three low frequency alleles (*Pep-B*h-*, *Pep-B*h--*, and *Pep-B*h+*) were found in NC81 and NC01 with frequencies from 0.03 to 0.17. These alleles were either observed in extreme *platensis* samples or nearer the center of the transect but in samples where *platensis* alleles predominated. Only one low frequency allele was found at the *Gpdh* locus (*Gpdh*a+*, with frequencies of 0.08 to 0.13) in populations near the center of the transect. Overall, fewer rare alleles were found in the NC studies than in SC89 where a total of eight rare alleles were observed (see Supplementary Table 2 available online; Wake et al. 1989). This may be explained by the larger average sample size for SC89 (19) compared with NC81 and NC01 (6 and 10, respectively) as it spanned several fall-spring seasons (see sample sizes in Tables 1 and 2). To reduce the observed variation to two allele systems, all rare and low-frequency alleles were allocated either to *xanthoptica* or to *platensis* depending on their location in the transect (see Supplementary Table 2 available online; Wake et al. 1989). Theoretically, this could be a source of error in allele frequency estimation and subsequent cline analysis, especially for alleles occurring only at sites near the transect center. But for the purposes of our analysis, we verified that the error introduced by erroneous allocation of rare alleles would be within the allele frequency variation between loci at each site (NC81, NC01, and SC89). For example, if rare alleles at the loci *Pep-D*, *Gpdh*, and *Pep-B* had been erroneously allocated in NC01, the change in allele frequencies at these loci across the center of the transect would approach that of *Idh*, *Mdh*, and *Aat*, respectively (not shown). We conclude that wrong rare allele allocation would not affect the main results and inferences derived from cline analysis.

Northern Calaveras.—Fixed or nearly fixed differences were observed at all markers between populations located in the extremes of the transect both for NC81 and NC01 (Table 1). A nearly complete transition in allele frequencies across the hybrid zone was more rapidly attained at all allozyme loci in NC01 (1360 m between −430 m and 930 m from the zone center) than in NC81 (4660 m between −743 m and 3917 m from the zone center). The widest zone of change was observed in NC81 at *Pep-B* (6768 m), penetrating deeper into the *platensis* side of the zone (frequency of 0.60 at site 6025). Mitochondrial haplotype screening was only carried out in NC01 and revealed a zone of complete change from *xanthoptica* to *platensis* haplotypes between −430 m and 494 m from the zone center (a total of 924 m). Color pattern changed from *xanthoptica* to *platensis* scores across zones of

TABLE 1. Frequencies for *Ensatina eschscholtzii platensis* alleles and means of color-pattern (CPI) traits across the hybrid zone for the northern Calaveras (NC81 and NC01) studies of the *E. e. xanthoptica–platensis* hybrid zone. *n*, number of individuals scored for the allozyme loci at each site. Dashes indicate no data for that year.

		Allozyme loci									
Locality	*n*	*Pgdh*	*Mdh*	*Idh*	*Pep-D*	*Aat*	*Pep-B*	*Gpdh*	*Sordh*	mtDNA	CPI
8539[1]	3/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	—/—	0.98/—
6816[2]	—/12	—/1.00	—/0.96	—/1.00	—/1.00	—/1.00	—/1.00	—/1.00	—/1.00	—/1.00	—/0.90
6025	5/18	0.90/1.00	1.00/1.00	1.00/1.00	0.90/1.00	1.00/1.00	0.60/1.00	1.00/1.00	1.00/1.00	—/1.00	0.92/0.93
3917[1]	7/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	1.00/—	—/—	0.97/—
2069[3]	6/1	0.67/1.00	0.58/1.00	0.67/1.00	0.67/1.00	0.67/1.00	0.50/1.00	0.75/1.00	0.58/1.00	—/1.00	0.63/0.96
1505[1]	5/—	0.80/—	0.80/—	0.60/—	0.90/—	0.80/—	0.90/—	0.80/—	0.88/—	—/—	0.81/—
930	4/8	0.75/0.94	0.63/0.94	0.50/1.00	0.75/1.00	0.63/1.00	1.00/0.94	0.63/1.00	0.88/1.00	—/1.00	0.63/0.94
885	3/3	1.00/0.83	1.00/0.67	0.83/1.00	1.00/1.00	1.00/1.00	1.00/0.83	1.00/1.00	1.00/0.83	—/1.00	0.86/0.79
494	12/7	1.00/0.79	0.71/0.71	0.83/0.50	0.96/0.71	0.75/0.83	0.79/0.75	0.80/0.83	0.92/0.71	—/1.00	0.94/0.68
401[1]	4/—	0.75/—	0.88/—	0.88/—	0.63/—	0.63/—	0.75/—	0.63/—	0.38/—	—/—	0.76/—
284[1]	3/—	0.83/—	0.50/—	0.33/—	0.67/—	0.67/—	0.83/—	0.83/—	0.67/—	—/—	0.96/—
197	6/15	0.92/0.70	0.58/0.73	0.92/0.73	0.75/0.67	0.75/0.80	1.00/0.87	0.92/0.62	0.83/0.70	—/0.71	0.84/0.70
157[1]	7/—	0.86/—	0.86/—	0.71/—	1.00/—	0.71/—	1.00/—	0.86/—	0.92/—	—/—	0.85/—
96	2/9	1.00/0.72	0.25/0.67	0.75/0.67	1.00/0.78	0.50/0.50	0.75/0.67	0.75/0.61	1.00/0.72	—/0.78	0.94/0.67
−16	8/14	0.44/0.50	0.25/0.43	0.44/0.54	0.50/0.46	0.44/0.46	0.38/0.43	0.44/0.54	0.40/0.43	—/0.55	0.47/0.46
−150[2]	—/3	—/0.33	—/0.33	—/0.33	—/0.33	—/0.17	—/0.33	—/0.33	—/0.33	—/0.33	—/0.43
−430[2]	—/15	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.02
−743[1]	8/—	0.00/—	0.00/—	0.00/—	0.00/—	0.00/—	0.00/—	0.00/—	0.00/—	—/—	0.01/—
−2987[2]	—/7	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.00	—/0.04
−4357	13/5	0.08/0.00	0.00/0.00	0.08/0.00	0.08/0.00	0.00/0.00	0.04/0.00	0.00/0.00	0.07/0.00	—/0.00	0.11/0.03

[1] Sampled only for NC81. [2] Sampled only for NC01. [3] Not used for analysis in NC01.

TABLE 2. Frequencies for *Ensatina eschscholtzii platensis* alleles and means of color-pattern (CPI) traits across the hybrid zone for the southern Calaveras (SC89) study of the *E. e. xanthoptica–platensis* hybrid zone. *n*, number of individuals scored for the allozyme loci at each site.

		Allozyme loci								
Locality	*n*	*Pgdh*	*Mdh*	*Idh*	*Pep-D*	*Aat*	*Pep-B*	*Gpdh*	*Sordh*	CPI
8208	11	1.00	0.86	1.00	1.00	0.91	1.00	0.95	1.00	0.82
1985	32	0.95	0.98	0.98	1.00	0.98	1.00	0.98	0.95	0.89
1553	13	0.88	0.88	0.88	1.00	0.96	0.62	1.00	0.88	0.91
1443	10	0.85	1.00	1.00	0.95	1.00	0.95	0.95	1.00	0.93
610	11	0.86	0.82	0.91	0.95	0.95	0.82	0.86	0.91	0.82
284	33	0.74	0.67	0.71	0.77	0.74	0.74	0.71	0.70	0.69
171	9	0.33	0.28	0.33	0.39	0.39	0.33	0.33	0.28	0.31
−778	26	0.19	0.19	0.23	0.15	0.19	0.19	0.21	0.25	0.32
−1476	26	0.02	0.00	0.00	0.02	0.00	0.04	0.00	0.00	0.07

change of 4660 m, in NC81, and 1360 m, in NC01, in a similar way to that observed for allozyme loci but without complete fixation at any of the extreme sites of the zone.

Southern Calaveras.—Allele frequencies were not fixed at all loci at any given site in this hybrid zone transect (Table 2). The transition zone from an allele frequency range of 0.82–0.95 (site 610) to 0.00–0.04 (site −1476) spanned a total distance of 2096 m. Only three sites (284, 171, and −778) showed intermediate frequencies between these extreme allele frequency values. The transition zone for color pattern was located within precisely the same sites as the allozyme transition zone.

Hardy-Weinberg Equilibrium and Genotypic Disequilibrium across the Hybrid Zone

Northern Calaveras.—For NC81, analysis of genotype frequencies produced estimates of F_{IS} that were not significantly different from zero across all allozyme loci (Table 3). Significant deficiency or excess of heterozygotes was also absent when consensus F_{IS} estimates were plotted for all eight allozyme loci against distance along the transect (Fig. 3A, NC81). However, significant deficiency of heterozygotes was observed at sites 401 (*Pep-B*), 157 (*Aat*), −16 (*Idh* and *Pep-B*), and −4357 (*Pgdh*, *Idh*, *Pep-D*, and *Sordh*). At this last site, located in the far *xanthoptica* side of the transect, we observed significant values of consensus F_{IS} (0.86; 2 unit bounds 0.56–1.00) due to the presence of one individual homozygous for *platensis* alleles at the *Pgdh*, *Idh*, *Pep-D*, and *Sordh* loci in a sample of 13 otherwise *xanthoptica*-like individuals. In NC01, by contrast, F_{IS} at all loci indicated heterozygote deficiency with the exception of *Pgdh* (Table 3). The variation of consensus F_{IS} estimates across the transect showed that the deficiency of heterozygotes occurred at sites 197, 157, 96, and −16 with maximum values at the last two sites near the center of the cline (Fig. 3A, NC01).

TABLE 3. Maximum-likelihood estimates of F_{IS} with the respective two log-likelihood unit confidence limits for the northern Calaveras (NC81 and NC01) and southern Calaveras studies of the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone.

	F_{IS}		
	Northern Calaveras		Southern Calaveras
Locus	NC81	NC01	SC89
Pgdh	0.00 (0.00–0.30)	0.30 (0.00–0.57)	0.23 (0.00–0.37)
Mdh	−0.10 (−0.10–0.13)	0.41 (0.14–0.64)	0.31 (0.16–0.45)
Idh	0.19 (0.00–0.43)	0.56 (0.26–0.77)	0.45 (0.30–0.60)
Pep-D	0.16 (0.00–0.43)	0.38 (0.09–0.63)	0.39 (0.23–0.54)
Aat	0.15 (−0.10–0.41)	0.51 (0.22–0.74)	0.23 (0.00–0.39)
Pep-B	0.00 (0.00–0.29)	0.44 (0.16–0.69)	0.45 (0.30–0.58)
Gpdh	0.00 (0.00–0.26)	0.48 (0.19–0.72)	0.30 (0.14–0.46)
Sordh	0.00 (0.00–0.21)	0.30 (0.02–0.56)	0.41 (0.25–0.55)

Across the eight diagnostic loci, significant scaled average pairwise linkage disequilibrium (*R*) was observed in both the NC81 and NC01 studies (Fig. 3B, NC81 and NC01). In NC81, all sites around the center of the zone (seven sites from −16 to 494) showed significant values of *R* with the exception of sites 401, 284, and 96, all with less than five samples per site. Neither ''pure'' *xanthoptica* nor putative F_1 hybrids were observed here (but see below) and 86% of the individuals showed an intermediate allozyme hybrid index (Fig. 4, NC81). Away from the center of the zone, significant *R*-values were detected at sites −4357 (see above) and 2069. In NC01, most samples collected farther than 500 m from the center of the cline showed fixed allele frequencies, and gametic associations could not be estimated. At sites located between −150 m and 494 m from the center of the zone (five sites), values of *R* ranged from 0.44 at site 494 (two unit bounds 0.38–0.44) to 0.70 at site −16 (two unit bounds 0.64–0.75). Average *R*-values for cytonuclear disequilibria could be estimated at the four sites with *xanthoptica* and *platensis* haplotypes (sites −150 to 197) and ranged from 0.62 at site 197 (two unit bounds 0.07–0.86) to 0.85 at site −16 (two unit bounds 0.49–0.87). Both ''pure'' parental genotype combinations but no putative F_1 hybrids were found at these central NC01 populations, with 58% of individuals being genetically intermediate (Fig. 4, NC01).

In both the NC81 and NC01 studies, color pattern (CPI) was closely associated with molecular markers with overall *D** values of 0.21 and 0.24, respectively (Fig. 5B, NC81 and NC01). *D** values across the hybrid zone were always higher and statistically significant at sites near the center of the hybrid zone (*D** of 0.04–0.22 and 0.12–0.22 for NC81 and NC01, respectively; Fig. 5A).

Southern Calaveras.—At this transect, F_{IS} estimates in the SC89 study show heterozygote deficiency at six loci, *Mdh*, *Idh*, *Pep-D*, *Pep-B*, *Gpdh*, and *Sordh* but not at the *Pgdh* and *Aat* loci (Table 4). Significant deficiency of heterozygotes as

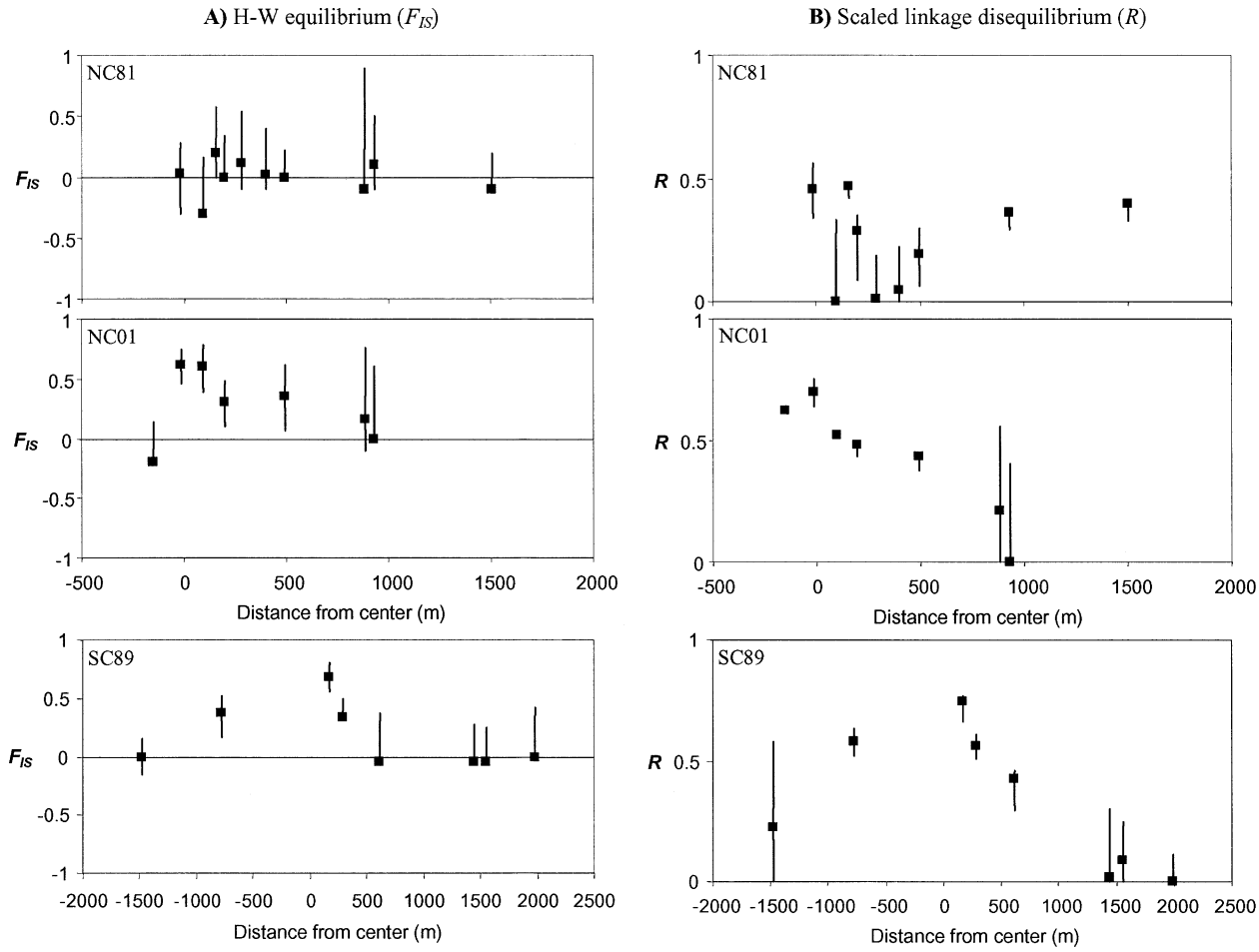

FIG. 3. Hardy-Weinberg equilibrium and genotypic disequilibrium across sites in the northern Calaveras (NC81 and NC01) and southern Calaveras (SC89) studies of the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone. (A) Consensus maximum-likelihood estimates (MLEs) of F_{IS}; (B) MLEs of average scaled linkage disequilibria (R).

measured by consensus F_{IS} values was observed only at sites 284, 171, and −778 located around the center of the zone (Fig. 3A, SC89). Significant values for gametic correlations (R) were found at four sites located between −778 m and 610 m of the hybrid zone center with a maximum at site 171 ($R = 0.75$; two unit bounds 0.66–0.77; Fig. 3B, SC89). At these central sites, one putative F_1 hybrid was found at site 610, but 57% of the individuals had other intermediate genotype combinations (Fig. 4, SC89).

The association between the eight allozyme loci and the average color pattern index was high, with an overall D^* value of 0.24 (Fig. 5B, SC89). Estimates of D^* across the hybrid zone showed higher and statistically significant values at sites near the center of the hybrid zone (D^* of 0.09–0.24; Fig. 5A, SC89).

Cline Width and Coincidence

Northern Calaveras.—Visual inspection of likelihood profiles (not shown) suggested coincidence of cline centers for all markers in both NC81 and NC01 (see also Table 4 and Fig. 6). Constraining all markers to a common center does not result in a significant decrease in likelihood (NC81: $G_{C\text{same}-C\text{diff.}} = 2.054$, 8 df, $P > 0.05$; NC01: $G_{C\text{same}-C\text{diff.}} = 0.923$, 9 df, $P > 0.05$). Additionally, the decrease in likelihood was nonsignificant when the cline centers for NC81 and NC01 were constrained to a common center ($G_{C\text{same}-C\text{diff.}} = 1.281$, 1 df, $P > 0.05$). Thus, based on our data there is no reason to suggest anything other than the most parsimonious hypothesis: that clines in this zone coincide across loci and over temporally separated samples.

Cline widths were variable in NC81 (MLEs 390–4130 m; Table 4) with wider clines at *Mdh, Idh, Pep-D, Aat, Gpdh,* and *Sordh* (MLEs 2230–4130 m) than at *Pgdh, Pep-B,* and color pattern (MLEs 390–580). These differences may be real or an artifact associated with the following: (1) *pmin* > 0 and *pmax* < 1 were used to fit clines for *Pgdh, Pep-B,* and color pattern given that allele frequencies were not completely fixed near the extreme localities of the transect; and (2) allele frequency increases sharply from site −16 (0.25–0.50) to site 157 (0.71–1.00) at all markers, but remains high at *Pgdh, Pep-B,* and color pattern in sites near the transect center (above 0.75 between sites 96–1505; see Table 1), which may have contributed to the lower width estimates for these three markers. Fitting stepped symmetric clines to the NC81 data significantly improved likelihood and produced narrower width estimates that were similar across markers

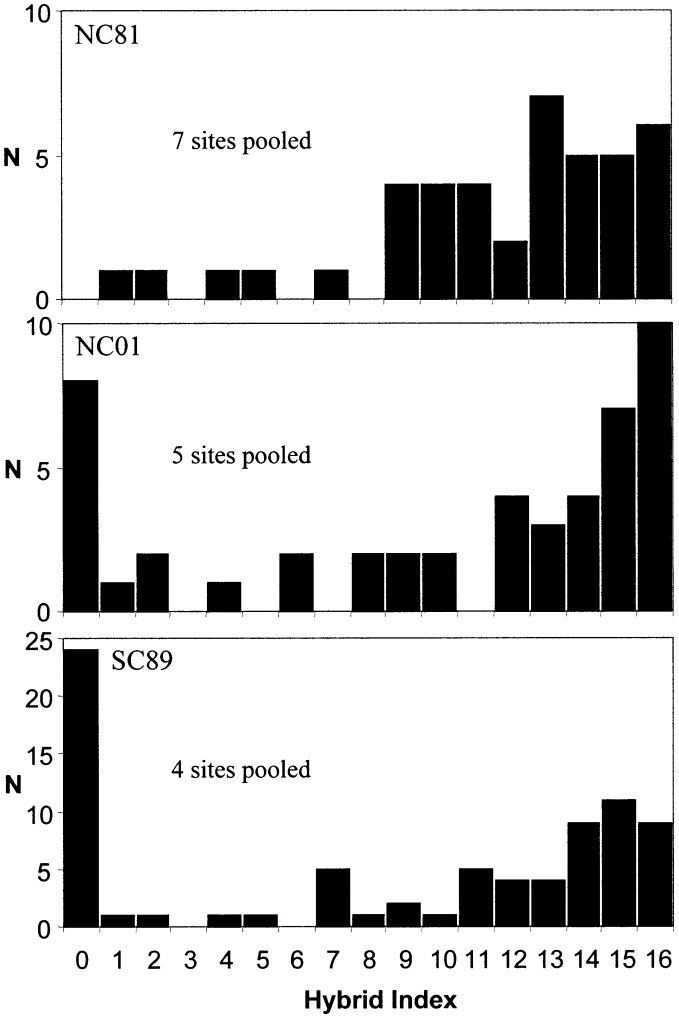

FIG. 4. Allozyme hybrid index distribution at central sites (see text) in the northern Calaveras (NC81 and NC01) and the southern Calaveras (SC89) transects across the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone.

(results not shown), but we had insufficient statistical power to fully explore stepped cline hypotheses. Clines in NC01 showed similar widths across markers (MLEs 500–960 m) and likelihood profiles suggested cline concordance ($G_{W\text{same}-W\text{diff.}} = 3.479$, 9 df, $P > 0.05$).

Southern Calaveras.—Cline centers were coincident ($G_{C\text{same}-C\text{diff.}} = 1.585$, 8 df, $P > 0.05$; see also Table 4 and Fig. 6). Cline widths were generally similar (MLEs 1460–2240) and analysis of the likelihood profiles indicated that widths were concordant for all markers ($G_{W\text{same}-W\text{diff.}} = 4.152$, 8 df, $P > 0.05$).

DISCUSSION

We have extended previous analyses of interactions in the *xanthoptica*/*platensis* contact zones (Wake et al. 1989) by adding temporal and spatial replication of transects (NC81, NC01) and conducting formal statistical analysis of clines in the context of tension zone theory. Key results across all transects were: (1) cline centers for all markers were coincident and the zones were narrow, with average width estimates of 730 m to 2000 m; (2) cline centers at the northern Calaveras transect were coincident between 1981 and 2001, suggesting stability of the hybrid zone center after four to five generations; (3) there are very few if any putative F_1s (0–2%), but a relatively high number of backcrossed individuals (57–86%), in the central portion of transects; (4) there are substantial associations of parental allele combinations across loci (linkage disequilibrium; R) across all three studies and strong heterozygote deficit at northern Calaveras, in 2001, and southern Calaveras. Both linkage disequilibrium and heterozygote deficit show maximum values near the center of the zones (R and $F_{IS} \approx 0.5$).

These observations on the genetic structure of the hybrid zones are consistent with strong selection against hybrids, which is likely to be mostly postzygotic given that significant heterozygote deficit was not detected at all diagnostic loci in any of the studies. That the centers of the zones are dominated by backcrossing, rather than F_1 individuals suggests either that opportunities for matings between parental types are rare, or that early generation hybrids suffer greater fitness reduction than do some classes of later generation backcrossed individuals.

Cline Width Comparisons

In northern Calaveras, the clines appeared to be consistently wider in NC81 than in NC01 (Table 1). This is confirmed by sigmoid cline fitting at some but not all markers (Table 4, Fig. 6; also see Results). The sharp increase in *platensis* allele frequency between approximately 100 and 900 m north of the transect center was observed in both NC81 and NC01, but in NC81, *xanthoptica* alleles penetrated into the *platensis* side of the zone farther away from the transect center (Table 1). These two observations suggest the maintenance of a barrier to gene flow in both years but with tails of introgression of *xanthoptica* alleles into *platensis* genetic background in NC81. We cannot rule out that the observed differences between NC81 and NC01 are due to sampling artifacts because fewer sites were sampled in NC01, but more individuals were collected per site near the center of the zone where most of the variation occurs. Fitting sigmoid clines to the NC81 data may have resulted in generally overestimating cline width (wider clines at *Mdh, Idh, Pep-D, Aat, Gpdh*, and *Sordh*), a tendency that was opposed in clines with higher/fixed *platensis* allele frequencies near the transect center (narrow clines at *Pgdh, Pep-B*, and color pattern). We hypothesize instead that the average width of the barrier to gene flow (cline width) was similar in NC81 and NC01 (as shown by exploratory fitting of stepped clines), and that the estimated cline width differences are an artifact caused by reduced sampling (not allowing for formal testing of stepped vs. sigmoidal clines), and introgression. Regarding the latter, comparisons between years at sites 96, 197, 494, 930, and 6025 suggest that introgression into the *platensis* side of the zone was more widespread in NC81 than in NC01. We speculate that introgression could have been reduced in NC01 due to the action of selection by pruning heterozygote genotypes away from the hybrid zone.

Comparisons should be made cautiously between cline

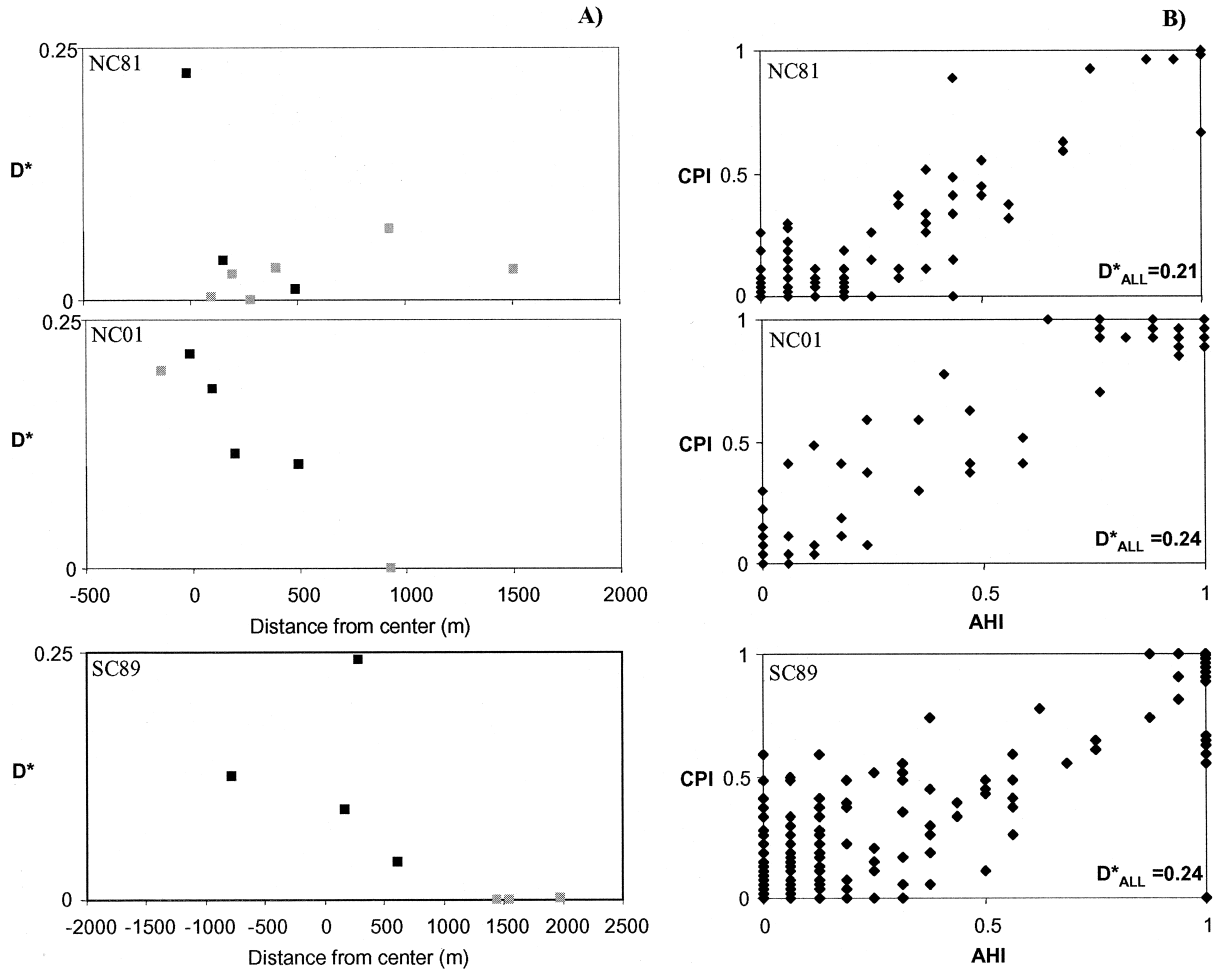

FIG. 5. Association of the allozyme hybrid index (AHI) and the color pattern index (CPI) in the northern Calaveras (NC81 and NC01) and the southern Calaveras (SC89) studies of the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone. (A) Disequilibrium measure *D** (see text) across sites, where significant and nonsignificant values of *D** are represented, respectively, by black and gray shaded squares. (B) Plot of AHI against CPI for all individuals.

widths estimated for southern and northern Calaveras transects because of the distinct scales at which the central portion of the hybrid zones were sampled (more sparsely sampled in SC89 than either NC81 or NC2001) and the possibility that the sampling transects are unequally aligned with the direction of maximum genetic change.

Cline Width and Linkage Disequilibrium: Evidence for and Measurement of Selection

Given that *xanthoptica* probably invaded the foothills of the Sierra Nevada in cooler and/or wetter periods of the Holocene (Stebbins 1949; Wake and Yanev 1986; Moritz et al. 1992; Wake 1997), it is reasonable to assume that the hybrid zone with *platensis* formed at least several hundred generations ago and is now at equilibrium. Given the assumption of migration-selection equilibrium, the effective selection acting against hybrids can be estimated from hybrid zone tension model theory using the formula $s^* = 8\sigma^2/w^2$, where σ is the standard deviation of distance between parent and offspring per generation and w is cline width (Bazykin 1969). We obtained an approximate estimate of the dispersal parameter of $\sigma = 35\text{m.gen}^{-1/2}$ from a capture-marking-release-recapture study undertaken in ''pure'' *platensis* territory to the northeast of the southern Calaveras transect (Staub et al. 1995). Substituting this direct estimate of σ in the above equation with average w of 2000 m and 738 m for the two NC studies, respectively, and 1734 m for SC89, would return s^* values of 0.2%, 1.8%, and 0.3%, respectively. However, these ecological estimates may substantially underestimate dispersal and, thus, lead to underestimates of selection strength. Obtaining estimates of s^* using a direct dispersal estimate for *platensis* only may be problematic. Dispersal is likely to vary across the hybrid zone because *xanthoptica* and *platensis* are ecologically diverged, occupy distinct habitats and may have distinct dispersal behavior. At NC, for example, one of us (J. Alexandrino) observed that the roaming of salamanders across roads on rainy nights is common for *xanthoptica* but rare for *platensis* individuals. Moreover, direct estimates of σ are likely to be underestimates because long-distance migration and historical extinction-recolonization events are unlikely to be taken into account (Barton and Hewitt 1982, 1985; Szymura and Barton 1986; Barton

TABLE 4. Cline center and width estimates with the respective two log-likelihood units (2u) confidence limits obtained by fitting maximum-likelihood curves (MLE) to the data for the northern Calaveras (NC81 and NC01) and southern Calaveras (SC89) studies of the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone.

		Northern Calaveras				Southern Calaveras	
		NC81		NC01		SC89	
Marker	Estimate	Center	Width	Center	Width	Center	Width
	2u min.	−600	310	−90	620	−190	1760
Pgdh	MLE	−30	580	40	950	20	2240
	2u max.	80	1300	160	1520	220	2900
	2u min.	−190	2150	−90	270	−190	1230
Mdh	MLE	180	3230	10	540	−10	1620
	2u max.	480	5060	160	1380	170	2160
	2u min.	−830	2540	−90	600	−240	1450
Idh	MLE	0	4130	50	960	−30	1870
	2u max.	570	7220	180	1620	170	2500
	2u min.	−910	1000	−70	500	−310	1130
Pep-D	MLE	−220	2230	40	770	−90	1460
	2u max.	60	4000	150	1250	110	1940
	2u min.	−370	2050	−50	380	−270	1120
Aat	MLE	50	3130	50	590	−90	1480
	2u max.	370	4810	140	980	80	1960
	2u min.	−240	410	−100	570	−190	1890
Pep-B	MLE	−50	800	30	870	40	2400
	2u max.	80	1340	140	1440	260	3120
	2u min.	−580	2000	−80	460	−210	1320
Gpdh	MLE	−150	2870	40	730	−20	1710
	2u max.	140	4580	160	1210	150	1920
	2u min.	−910	2180	−50	580	−250	1610
Sordh	MLE	−160	3230	60	880	−40	2090
	2u max.	210	6020	170	1420	160	2720
	2u min.	—	—	−120	290	—	—
MtDNA	MLE	—	—	0	500	—	—
	2u max.	—	—	190	920	—	—
	2u min.	−240	30	−130	260	−430	1370
Color pattern	MLE	−20	390	−10	590	−80	2240
	2u max.	60	900	110	1220	240	3620

and Gale 1993). Better σ estimates can be obtained indirectly using values of linkage disequilibria (R) given that we observed significant associations between unlinked diagnostic loci. Assuming that (1) linkage disequilibrium (LD) is explained by the dispersal of parental allele combinations in to the hybrid zone, (2) the effect of epistasis in producing LD is small, and (3) selection is not too strong, LD at the center is $R = 4\sigma^2/w^2r$, where $r = 0.5$ for unlinked loci (Szymura and Barton 1986, 1991). Because w depends itself on a ratio of migration to selection, the maximum LD is directly proportional to the selection coefficient s^* (for $r = 0.5$, $s^* = R$; Mallet and Barton 1989). In spite of considerable uncertainty given all the assumptions, the method provides a good approximation of the magnitude of linkage disequilibrium (and selection) even in the presence of epistasis and when selection is strong (Barton and Shpak 2000). Substituting our estimates of R at or near each transect center (0.46, 0.70, and 0.75 for NC81, NC01, and SC89, respectively) and average w as above, gives values for σ of 480 m, 218 m and 531 m, respectively. Therefore, to maintain the observed clines would require selection against heterozygotes at a single locus $s^* = 8\sigma^2/w^2$ of, respectively, 46%, 70%, and 75%. These values should therefore be treated as an indication that strong selection against hybrids is involved in the hybrid zone between *xanthoptica* and *platensis*.

The high density of salamanders observed at the center of *xanthoptica-platensis* hybrid zones seems at odds with strong selection against all hybrid genotypes when most of the individuals observed here were of hybrid origin. In combination with the paucity of F_1s in our sample, this suggests that selection plays some role against the first few generations of *xanthoptica-platensis* hybrids, but also that hybrid counterselection decreases in subsequent backcrossed generations of hybrids that become established in central locations of the hybrid zone. Because parental individuals are less common at the zone center it becomes less likely for F_1s to be formed, which may also explain our observations. Many other hybrid zone studies in both plants and animals (e.g., *Iris* plants, *Bombina* toads, and *Sceloporus* lizards) have shown that although F_1 hybrids usually have lower fitness than parental forms, hybrid genotypes need not be equally selected against and some hybrid genotypes could even be favored (see review in Arnold 1997 and references therein).

Our population genetic analysis implies that strong selection is maintaining the sharp parapatric boundary between *xanthoptica* and *platensis*, but does not tell us what kind of selection is operating because cline shapes generated by endogenous and exogenous selection are indistinguishable (Kruuk et al. 1999). Many hybrid zones are in fact under a mixture of endogenous and exogenous selection with clines that will be rooted to the environmental boundary because of linkage disequilibrium and pleiotropy between traits in-

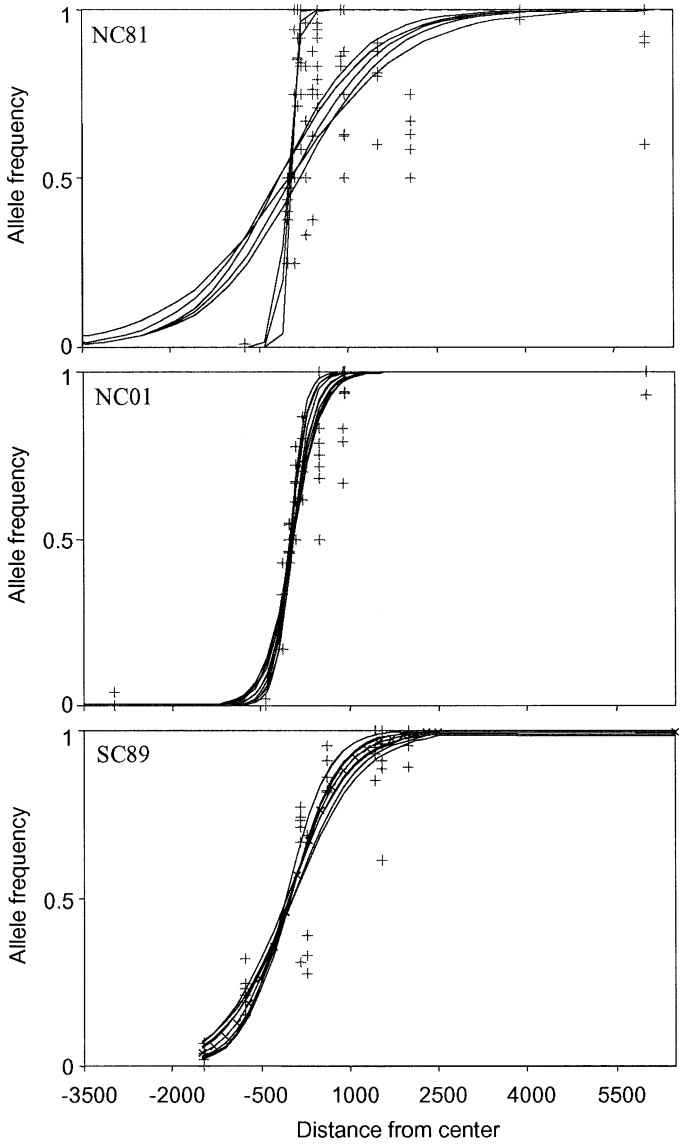

FIG. 6. Maximum-likelihood cline fitting of marker frequency data (see text for details) across the northern Calaveras (NC81 and NC01) and the southern Calaveras (SC89) transects of the *Ensatina eschscholtzii xanthoptica–platensis* hybrid zone. The original allele frequency data are also represented (dark gray symbols).

fluenced by both exogenous and endogenous selection (e.g., the fire-bellied toads *Bombina bombina* and *B. variegata*; Szymura and Barton 1986, 1991; Vines et al. 2003). Unfortunately, no experimental information is available on levels of reproductive isolation between *xanthoptica* and *platensis*. Exogenous selection could be important given that the two forms are ecologically diverged and the hybrid zone coincides with a transition between open oak woodland forests of the Sierra Nevada foothills (*xanthoptica* habitat) and Sierran montane dense forest vegetation (*platensis* habitat) and preliminary data (J. R. Macey, unpubl. data) suggests habitat partitioning within the contact zone. Moreover, the two forms are thought to have distinct predator avoidance strategies: *xanthoptica*, a case of Mullerian mimicry with the newt *Taricha torosa*, while *platensis* is thought to be cryptic through disruptive coloration (Stebbins 1949; Kuchta 2005). Indeed, if divergent predator avoidance strategies underlie selection against hybrids, then frequency-dependent selection is a possibility (e.g., *Heliconius* butterflies; Mallet et al. 1990; Mallet 1993).

Evolutionary Implications

Ring species are taxa with continuous intergradation of forms in a circular fashion but incompatibility between terminal populations (see review by Irwin et al. 2001). Two distinct ring species complexes have recently been given some attention: while studies by Irwin et al. (2001) showed that the Asian greenish warbler *Phylloscopus trochiloides* ring species conforms to a true ring species (smooth intergradation along the ring with reproductively isolated terminal forms), Liebers et al. (2004) recently reported that the ring in the herring gull complex (*Larus argentatus*) is not yet closed. In *Ensatina* spp., rather than a smooth transition around the ring, the changes are broken into a series of genetically and/or phenotypically discrete taxa (Wake 1997), which is to be expected given the large spatial (relative to dispersal of the organism) and temporal scales and environmental diversity over which the complex evolved. Though no longer considered to be a ring united by gene flow, the varying histories of allopatric divergence and divergent selection on phenotypes make the *Ensatina* complex a fascinating system in which to study the relationship between genetic divergence and speciation (Wake 1997). Hybrid zone analyses add an important dimension to the interpretation of ring species especially when they are genetically subdivided. This is the first study to estimate linkage disequilibrium and selection against hybrid genotypes in hybrid zones of the ring species *E. eschscholtzii*. The values of 46–75% for selection operating at this hybrid zone were higher than the 32% estimated for hybrid zones between distinct races of *Sceloporus grammicus* complex (Sites et al. 1995; Marshall and Sites 2001) or the 17–22% between the toads *Bombina bombina* and *B. variegata* (Szymura and Barton 1986, 1991). With the level of selection against hybrids observed for *Ensatina*, introgression of both negatively selected and neutral alleles is unlikely. However, in the absence of strong premating isolation, positively selected alleles could still cross this barrier (Piálek and Barton 1997).

Midway down the *Ensatina* ring, levels of isolation between the subspecies *xanthoptica* and *platensis* could be regarded as sufficient to recognize them as distinct species, were they not embedded in the phenotypic ring complex (Wake and Schneider 1998). Some authors have suggested that several species should be recognized within *Ensatina* (Frost and Hillis 1991; Graybeal 1995; Highton 1998). The question is not how many units we can discern with our diverse lenses, as biodiversity in this case is fractal, but rather what are the circumstances in which the potential for genetic introgression is negligible (Hey 2001). In this context, the *Ensatina* ring species provides an unusual opportunity to compare forms and levels of interaction at contact zones between units with varying levels of ecological and genetic divergence. At present, only rough comparisons can be established: at the transition of the two ecomorphologically

similar Sierran lineages of *platensis*, clines for three diagnostic allozyme loci and mtDNA are noncoincident, and cline width appears to range from 1 km for mtDNA to about 200 km for the *Ldh-2* locus (Jackman and Wake 1994; Wake and Schneider 1998; J. Alexandrino unpubl. data). By contrast, hybridization in the south of the ring between the unblotched *E. e. eschscholtzii* and the blotched *E. e. klauberi* is rare or, at one site, nonexistent, suggesting complete species formation (Wake et al. 1986, 1989). Indeed, interactions around the ring (e.g., *xanthoptica-oregonensis*, *xanthoptica-eschscholtzii*, *oregonensis-platensis*, *platensis-platensis*, *oregonensis-oregonensis*) appear to be wide, producing morphological intergradation and genetic introgression. On the contrary, hybridization across the ring between unblotched and blotched forms (e.g., *xanthoptica-platensis* and *eschscholtzii-klauberi*) produces genetic barriers that further contribute to isolation and evolutionary independence (Stebbins 1949; Brown 1974; Wake et al. 1986, 1989; Wake 1997). With genetic (allozyme and mtDNA) studies revealing deep historical subdivisions and episodes of isolation and recontact within as well as between adjacent subspecies, it has become clear that phenotypic cohesion of subspecies is more a function of selection than consistent gene flow (Wake 1997; Wake and Schneider 1998). Regarding the factors that result in genetic isolation of lineages, the available evidence for *Ensatina*, and in particular the evidence here for strong disequilibrium and narrow clines, versus broad and nonconcordant clines between previously isolated lineages within an ecotype (*platensis*, Wake and Schneider 1998), is consistent with the prediction that postzygotic incompatibilities develop faster in the presence of divergent ecological selection (Gavrilets 2003). We suggest that comparative studies of contact zones between historically isolated lineages with varying levels of ecomorphological divergence will shed light on the relative roles of isolation versus divergent selection in speciation (Turelli et al. 2001).

Acknowledgments

This work was supported by the Fundação para a Ciência e a Tecnologia (SFRH/BPD/3597/2000 postdoctoral grant to JA) and the Museum of Vertebrate Zoology (Carl K. Koford fund award to JA for fieldwork). This work is LBNL-54692 and was performed under the auspices of the U.S. Department of Energy, Office of Biological and Environmental Research, under contract No. DE-AC03-76SF00098 with the University of California, Lawrence Berkeley National Laboratory. Licensing to collect was provided by the California Department of Fish and Game. For field assistance in collecting salamanders and mapping the transect we especially thank T. Tunstall. A. Lancaster, A. Hugall, D. McCauley, E. Rosenblum, and the Moritz family also helped with setting pitfall traps and collecting. We acknowledge C. Graham; C. Oliveira; J. McKenzie; M. Mahoney; M. Soares; M. Stöck; N. Belfiore; S. Kuchta; T. Pappenfuss, and, generally, J. McGuire's, C. Moritz's and D. B. Wake's research groups for support, helpful discussions, and comments on earlier versions of the manuscript. The manuscript was improved with comments by R. Harrison and anonymous reviewers.

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