Herpetologica, 63(3), 2007, 332–350

CONTACT ZONES AND SPECIES LIMITS: HYBRIDIZATION BETWEEN LINEAGES OF THE CALIFORNIA NEWT, *TARICHA TOROSA*, IN THE SOUTHERN SIERRA NEVADA

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ABSTRACT: Recent phylogeographic work on *Taricha torosa* has revealed that the subspecific lineages, *T. t. torosa* and *T. t. sierrae*, are distinct evolutionary lineages that form a secondary contact zone in the southern Sierra Nevada of California. I examined the dynamics of this contact zone using two allozyme markers, mitochondrial DNA, morphometrics (head shape), and head color pattern. The subspecific lineages interbreed where they meet, and form a hybrid zone centered along the Kaweah River in Tulare County. Clines among genetic markers had similar shapes and centers, and ranged from 7–10 km wide. There is evidence of selection against hybrid genotypes in the center of the hybrid zone. Analyses of head shape and color pattern show that the two subspecies are phenotypically differentiated, and that patterns of differentiation in these characters are congruent with the genetic clines. The two subspecies constitute distinct evolutionary lineages and merit recognition as separate species: *T. torosa* (California newt) and *T. sierrae* (Sierra newt).

Key words: Alloyzme; Contact zone; Evolutionary species concept; General metapopulation lineage concept; Hybrid zone; Hybridization; MtDNA; Restriction fragment length polymorphism; *Taricha*

WHEN related but evolutionarily divergent lineages adjoin their geographic ranges after a period of isolation, a process called secondary contact, multiple outcomes are possible. At one extreme, the interacting lineages may exhibit complete reproductive isolation (Coyne and Orr, 2004). Alternatively, they may interbreed freely with no selection against hybrid genotypes, ultimately resulting in reticulate patterns of evolution (e.g., Echelle and Conner, 1989; Jockusch and Wake, 2002; Kuchta and Tan, 2006*a*). Intermediate to these endpoints are processes such as hybridization, reinforcement, and introgression (Harrison, 1993). Contact zone dynamics are of interest to evolutionary biologists because they provide insight about evolutionary processes, including species formation and the nature and extent of reproductive isolation (e.g., Alexandrino et al., 2005; Harrison and Rand, 1989; Matocq, 2002; Wake et al., 1989). Contact zones that include hybridization between interacting lineages also pose taxonomic challenges, however, and draw attention to the nebulous boundary between intra- and inter-specific differentiation.

In this paper I report on an analysis of a contact zone involving two subspecific lineages of the California newt, *Taricha torosa*: the coast range newt, *T. t. torosa*, and the Sierra newt, *T. t. sierrae* (Stebbins, 2003). Traditionally, *T. t. torosa* was thought to be distributed throughout the coast ranges of California, while *T. t. sierrae* was thought to range along the western slopes of the Sierra Nevada (Riemer, 1958; Stebbins, 1951, 2003). A recent phylogeographic study using mitochondrial DNA (mtDNA) sequences, however, has suggested that populations in the southern Sierra Nevada, once considered members of *T. t. sierrae*, are actually members of *T. t. torosa* (Tan and Wake, 1995). This unanticipated result raised the prospect that *T. t. torosa* may form a secondary contact zone with *T. t. sierrae*.

Kuchta and Tan (2006*a*) subsequently used nuclear markers (45 allozyme loci) and mitochondrial DNA (mtDNA) sequences to further investigate the biogeographic history of *T. torosa*. They verified that *T. t. torosa* and *T. t. sierra* constitute monophyletic lineages, and, based on a molecular clock calibrated using fossils related to *Taricha*, they estimated that the two subspecies diverged 7–13 myr ago. They further estimated that that *T. t.*

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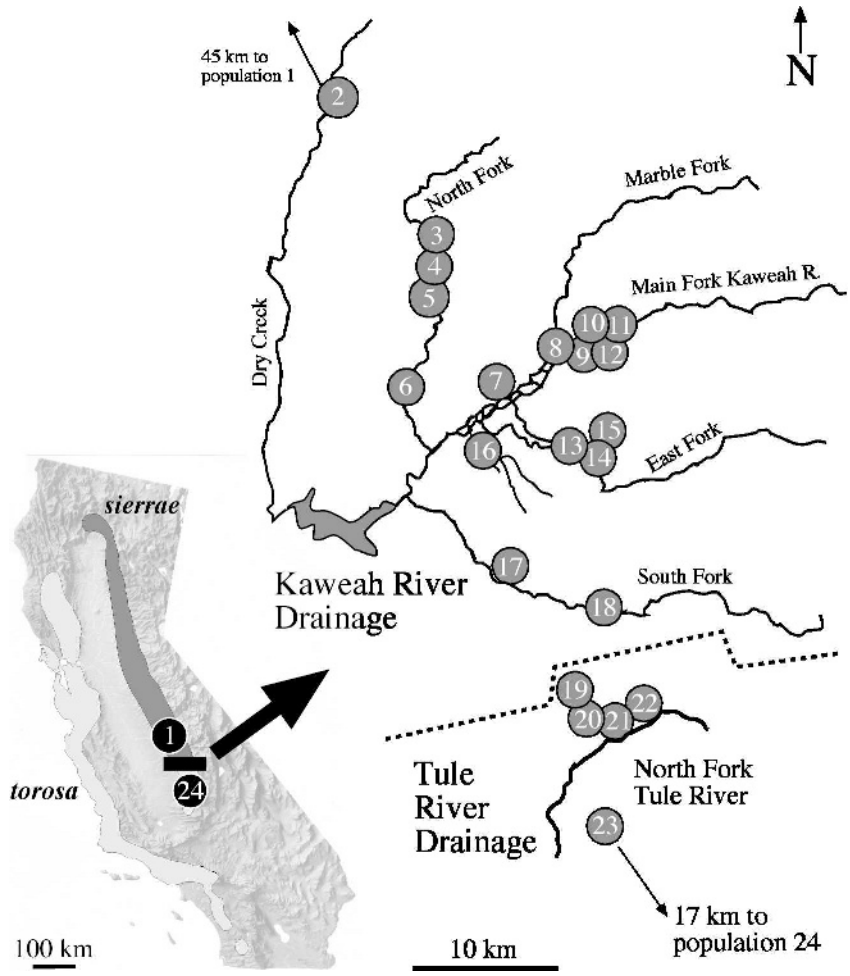

FIG. 1.—Map of California showing the range of *T. t. torosa* and *T. t. sierrae*. The inset zooms in on the hybrid zone. Population numbers correspond to Table 1.

torosa colonized the southern Sierra Nevada 1.4–1.7 Myr ago, and verified that *T. t. torosa* and *T. t. sierrae* indeed form a secondary contact in the vicinity of the Kaweah River in Tulare County (Fig. 1). MtDNA haplotypes belonging to both subspecies were found within 9 km of each other. In addition, using allozymes, the Nei's (1978) genetic distance between nearest neighbor populations of *T. t. torosa* and *T. t. sierrae* in the southern Sierra Nevada was $D_N = 0.148$, including fixed allozymic differences and no clear evidence of genetic introgression. The populations on either side of the contact zone sampled for allozymes were ~122 km apart, however.

A study of the contact zone between *T. t. torosa* and *T. t. sierrae* was thus initiated to test their evolutionary independence. It is generally agreed that lineages that do not interbreed are evolutionarily independent (Coyne and Orr, 2004). With hybridization, however, independence is more difficult to assess. In contact zones in which there is no selection against hybrid genotypes, cline width is a product of dispersal and the number of generations since contact, and thus will widen over time (Endler, 1977). Accordingly, unless it recently formed, a neutral hybrid zone is expected to be wide relative to dispersal, and to have discordant cline shapes and cline centers among loci (Barton and Hewitt, 1985;

Endler, 1977). In contrast, when there is selection against hybrids, cline width is a function of the rate of dispersal into the hybrid zone and the strength of selection against hybrid genotypes (Barton and Gale, 1993; Barton and Hewitt, 1985). Hybrid zones that fit this description are called tension zones (Key, 1968). With strong selection, tension zones will tend to be narrow, and clines among loci will tend to have concordant shapes and centers.

Thus, hybrid zone analyses can provide estimates of lineage independence. For the current study of the contact zone between *T. t. torosa* and *T. t. sierrae*, two allozyme loci and an mtDNA locus were chosen as genetic markers based on the work of Kuchta and Tan (2006*a*) In addition, analyses of head shape and head color pattern were made for comparison with the genetic data, and to determine if there are diagnostic phenotypic differences distinguishing the subspecies on either side of the contact zone.

Systematic History of Taricha

Taricha t. torosa and *T. t. sierrae* were originally described as separate species (Twitty, 1942). This proposal was based on a number of diagnostic differences, including adult color pattern and hue, iris coloration, the extent of the tail fin in breeding males, larval pigmentation patterns, egg size and number, and egg laying behavior (Twitty, 1942; see also Kuchta, 2005, for a recent review of studies on *T. torosa*). Nonetheless, Stebbins (1951) later recommended treating these forms as a subspecies of *T. torosa*, though he did not detail his reasoning, and this taxonomy has subsequently been followed. The first comprehensive investigation of the distribution and systematics of the genus *Taricha* was conducted by Riemer (1958), who examined geographic variation in head shape, coloration, and other characters. In general, Riemer (1958) found that *T. t. torosa* and *T. t. sierrae* displayed considerable morphometric overlap, but that *T. t. sierrae* have more of their ventral pigmentation extending onto their snout, face, and eyelids than does *T. t. torosa*. Riemer (1958) did not work at the population level, however, but instead defined large geographic regions as samples and averaged within these samples. This approach is unfortunate because populations on either side of the contact zone between *T. t. torosa* and *T. t. sierrae* were grouped into his "population 1" of *T. t. sierrae*. Thus, differences between the subspecies were confounded, and the presence of *T. t. torosa* in the southern Sierra Nevada was not detected. Accordingly, Riemer (1958) recommended that *sierrae* remain a subspecies of *T. t. torosa*, noting that this taxonomy better highlights their relatedness. (For a recent phylogeographic and systematic review of the rough-skinned newt, *T. granulosa*, see Kuchta and Tan (2005), and for a recent survey of genetic variation in the red-bellied newt, *T. rivularis*, see Kuchta and Tan (2006*b*).)

Materials and Methods

Sampling and Laboratory Techniques

Allozyme electrophoresis.—Two hundred and eighteen individuals from 22 populations spanning the contact zone between *T. t. torosa* and *T. t. sierrae* were examined for variation in allozymes (Fig. 1, Table 1). Following collection, specimens were euthanized in 25% chlorotone, and heart, liver, and muscle were removed and frozen at −70 C. Carcasses are stored as vouchers in the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley. Allozyme variation was surveyed at two loci, aconitase (ACON2) and superoxide dismutase (SOD). These loci were chosen because previous work showed they possess fixed allozymic differences north and south of the contact zone: ACON2 has distinct allozymes fixed between the *T. t. torosa* and *T. t. sierrae* lineages (allozymes *a* and *b*, respectively), and SOD has distinct allozymes fixed between southern Sierran *T. t. torosa* and the central Sierran populations of *T. t. sierrae* north of the contact zone (allozymes *a* and *b*, respectively) (Appendix in Kuchta and Tan, 2006*a*). Standard methods of starch gel electrophoresis were employed for the survey of allozyme variation, and enzyme and buffer systems are provided in Table 2 of Kuchta and Tan (2005). For this study, two populations from Kuchta and Tan (2006*a*) were supplemented with additional specimens: population 1 (population 30 in Kuchta

and Tan, 2006*a*) and 24 (population 20 in Kuchta and Tan, 2006*a*). The remaining populations in the current study (2–23) are geographically intermediate to these populations (Fig. 1). Sample sizes range from 1–22 per population (mean = 9.25; Table 1).

Cytochrome b *sequence variation*.—Kuchta and Tan (2006*a*) published on 21 cytochrome *b* haplotypes collected within the region of this study (their populations 20–30). In this study, Restriction Fragment Length Polymorphisms (RFLPs) of amplified cytochrome *b* haplotypes (~778 bp; see Kuchta and Tan, 2006*a*, for PCR conditions) were used to assign an additional 180 individuals to either the *T. t. torosa* or *T. t. sierrae* mtDNA clades. These data were collected from the same individuals from which allozyme data were collected (Table 1). For the RFLP analyses, two restriction enzymes were chosen by making reference to the cytochrome *b* sequences obtained by Kuchta and Tan (2006*a*). Hha I (New England Biolabs) cut *T. t. torosa* haplotypes in two places (three fragments), but did not cut *T. t. sierrae* haplotypes, and Alu I (New England Biolabs) cut *T. t. sierrae* haplotypes in one place (two fragments) but did not cut *T. t. torosa* haplotypes. For Hha, 6 μL of PCR product were added to 1.75 μL ddH_2O, 1 μL New England Buffer #4, 1 μL BSA, and 1 μL of Hha I restriction enzyme. For Alu I, 4 μL of PCR product were added to 4 μL ddH_2O, 1 μL New England Buffer #2, and 1 μL Alu I restriction enzyme. All amplified PCR products were exposed to both enzymes in separate reactions. Reaction mixtures were incubated at 37 C for 16 h, and the enzyme was inactivated by incubation at 65 C for 20 min. Reaction products were electrophoresed on 2% Metaphore agarose gels (Bio Whittaker Molecular Applications), visualized with ethidium bromide staining, and photographed.

Genetic Analyses

Cline analyses were conducted using the computer program Analyse 1.30 (Barton and Baird, 2002). Visual inspection of the data (Fig. 2) suggested that the Main Fork of the Kaweah River is the barrier separating *T. t. torosa* and *T. t. sierrae*. Because with moderate sampling cline analyses are best carried out on a linear array of populations, all populations were first collapsed into a one-dimensional transect perpendicular to the Kaweah River to estimate the width of the hybrid zone. At each locus, sigmoidal maximum-likelihood clines were fitted to population allozyme and mtDNA haplotype frequency data, and the width of each cline estimated as the inverse of the maximum slope (Barton and Gale, 1993).

Maximum likelihood estimates of heterozygote deficit (F_{IS}) and statistical associations between parental combinations of alleles (linkage disequilibrium, D) within populations was calculated using Analyse 1.30. Estimates of D were standardized by allozyme and haplotype frequency ($R = D_{ij}/(pquv)^{1/2}$) (Barton and Baird, 2002). For both F_{IS} and R, confidence limits were calculated as ±2 log-likelihood units, and these limits were used to determine which populations had values that differed significantly from zero. Individual hybrid indices were calculated for the allozyme loci by giving all *T. t. sierrae* allozymes a value of zero and all *T. t. torosa* allozymes a value of 1, resulting in a scale from zero to four.

Color Pattern

Taricha t. torosa has a brown dorsal coloration and a yellow to orange ventral color, while *T. t. sierrae* is dark brown to chocolate dorsally with a burnt or reddish ventral coloration (Stebbins, 2003). In addition to hue, the subspecies differ in the distribution of color on their bodies (Riemer, 1958; Stebbins, 2003; Twitty, 1942), with *T. t. sierrae* having more ventral coloration extending onto its snout and upper eyelids than *T. t. torosa*. Color pattern data were collected from all specimens used in the genetic analyses (Table 1) to determine if this generalization holds true for populations on either side of the contact zone in the southern Sierra Nevada. To remove bias, all specimens for the study were randomized in a bucket prior to scoring. Color patterns scores were adapted from Riemer (1958):

(1) Eyelid coloration: the amount of ventral coloration extending onto the dorsal surface of the eyelid. *Taricha t. sierrae* tend to have eyelids that are highly infused with the ventral coloration, while

TABLE 1.—Locality information for *T. torosa* population samples. Population numbers correspond to Fig. 1. **Specimen Identification numbers in bold designate specimens for which there is mtDNA sequence, mtDNA RFLP, and electrophoretic data**; normal print designates specimens for which there is mtDNA sequence data and electrophoretic data only; italics designate specimens for which there is electrophoretic data only.

Pop.	County	Specific locality	Latitude/Longitude	Sample sizes			Specimen identification number
				Allo.	mtDNA sequence	mtDNA RFLP	
1	Fresno	Junction of Jose Basin Rd and Jose Basin, E. of Shaver Lake, Sierra National Forest	37.1298 N/119.3758 W	11–12	2	0	MVZ 197468–69 *MVZ 197467, 197470–197478*
2	Tulare	Near Eshom Creek Campground (10)	36.6705 N/118.9719 W	10	2	10	SRK 1945–1947
		& Dry Creek (1), Sequoia National Forest	36.7042 N/118.9574 W	1	1	—	**SRK 1948–1954** DBW 4693
3	Tulare	Burnt Point Creek, Sequoia National Park	36.5802 N/118.8912 W	10	—	10	**SRK 1945–1954**
4	Tulare	Small un-named creek midway between Burnt Point Creek and Yucca Creek, Sequoia National Park	36.5659 N/118.8928 W	2	—	2	**SRK 1792–1793**
5	Tulare	Yucca Creek, end of North Fork Road, Sequoia National Park	36.5461 N/118.8969 W	11	1	11	**SRK 1916–1928** DBW 5974
6	Tulare	North Fork Rd, 4.4 miles N. of Hwy 198	36.4914 N/118.9178 W	1	1	—	DBW 5940
7	Tulare	Sycamore Creek, along Sheppard Saddle Road, Sequoia National Park	36.4949 N/118.8503 W	11–12	3	8	**SRK 1857–1864**; DBW 4694–4695; DBW 5873; *SRK 1965*
8	Tulare	Middle Fork Kaweah River, 1/4 miles upstream of jct. with Marble Fork, Sequoia National Park	36.5125 N/118.7974 W	10	—	10	**SRK 1769–1778**
9	Tulare	Un-named creek entering Middle Fork Kaweah across from Potwisha Campground, Sequoia National Park	36.5106 N/118.7887 W	10	—	10	**SRK 1802–1804** **SRK 1850–1856**
10	Tulare	Un-named creek west of Hospital Rock, Sequoia National Park	36.5207 N/118.7779 W	10	—	10	**SRK 1820–1829**
11	Tulare	Hospital Creek, ~1 mile N. of Hospital Rock, Sequoia National Park	36.5264 N/118.7719 W	1	1	—	DBW 5862
12	Tulare	Paradise Creek, entering Middle Fork	36.5200 N/118.7600 W	5	—	5	**SRK 1805–1809**
		Kaweah (900–950 m elevation), Sequoia National Park	36.5170 N/118.7570 W	10	—	10	**SRK 1810–1819**
13	Tulare	Vicinity of Oak Grove, Mineral King	36.4531 N/118.7887 W	20	—	20	**SRK 1840–1849**;
		Road, Sequoia National Park	36.4502 N/118.8064 W	2	—	2	**SRK 1886–1895**; **JMR 17–18**
14	Tulare	Grunigan Creek, near jct. with Mineral King Road	36.4422 N/118.7703 W	13	3	10	*DBW 5890–92* **SRK 1906–1915**
15	Tulare	Squirrel Creek, ~2 miles upstream of Mineral King Rd, Sequoia National Park	36.4600 N/118.7620 W	9	—	9	**SRK 1936–1944**
16	Tulare	Salt Creek, entering Kaweah River	36.4498 N/118.8634 W	3	—	3	**SRK 1795–1797**

TABLE 1.—Continued.

Pop.	County	Specific locality	Latitude/Longitude	Sample sizes: Allo.	Sample sizes: mtDNA sequence	Sample sizes: mtDNA RFLP	Specimen identification number
17	Tulare	Cinnamon Creek, ~1/2 mile upstream of South Fork Drive	36.3747 N/118.8435	10	—	10	**SRK 1779–1787** **SRK 1801**
18	Tulare	0.4 miles downstream of South Fork Campground, South Fork Kaweah River, Sequoia National Park	36.3519 N/118.7684 W	10	—	10	**SRK 1926–1935**
19	Tulare	Kramer Creek, at jct. with Backbone Rd, Sequoia National Forest	36.2989 N/118.7908 W	12	2	10	*DBW 4691–92* **SRK 1876–1885**
20	Tulare	Un-named creek entering Kramer Creek, at jct. of Backbone Road, Sequoia National Forest.	36.2893 N/118.7839 W	10	—	10	**SRK 1896–1905**
21	Tulare	Backbone Creek, at jct. of Backbone Road.	36.2725 N/118.7629 W	10	—	10	**SRK 1830–1839**
22	Tulare	Jenny Creek, entering North Fork Tule River, Sequoia National Forest.	36.2750 N/118.7333 W	0	1	—	*DBW 4688*
23	Tulare	Rancheria Creek, off Forest Road 220, Sequoia National Forest	36.2167 N/118.7667 W	0	2	—	*DBW 4689–90*
24	Tulare	Camp Nelson, near S. Fork Tule River, Sequoia National Forest	36.1393 N/118.6126 W	13	2	—	**MVZ 219849–219850** MVZ 219851–219855

TABLE 2.—Allozyme frequencies, mtDNA haplotype frequencies, and mean color pattern scores (±1 standard deviation).

Pops:	1	2	3	4	5	6*	7	8	9	10	11*	12	13	14	15	16	17	18	19	20	21	22	23	24
ACON2																								
a	1.00	0.90	0.90	1.00	0.90	—	0.50	0.05	0.10	—	—	0.07	0.07	—	—	0.33	0.05	—	—	—	—	—	—	—
b	—	0.10	0.10	—	0.10	—	0.50	0.95	0.90	1.00	—	0.93	0.93	1.00	1.00	0.67	0.95	1.00	0.82	0.95	0.95	1.00	1.00	1.00
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.18	0.05	0.05	—	—	—
SOD																								
a	1.00	0.90	1.00	1.00	1.00	—	0.79	0.35	0.10	—	—	0.07	0.09	0.08	0.06	0.33	—	0.05	—	—	—	—	—	—
b	—	0.10	—	—	—	—	0.21	0.65	0.90	1.00	—	0.93	0.91	0.92	0.94	0.67	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00
mtDNA																								
sierrae	1.00	1.00	1.00	1.00	1.00	1.00	0.82	0.10	—	—	—	—	0.14	0.08	—	0.33	0.10	—	—	—	—	—	—	—
torosa	—	—	—	—	—	—	0.18	0.90	1.00	1.00	1.00	1.00	0.86	0.92	1.00	0.67	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Color																								
Snout	4.4	3.9	4.4	4.5	3.9	—	3.6	2.2	2.4	2.9	2	2.4	2.7	2.6	2.9	2.7	2.1	2.8	2.6	3.3	2.2	3	4	2.4
(SD)	(0.5)	(1.0)	(0.7)	(0.7)	(0.7)		(1.1)	(0.9)	(0.5)	(0.7)		(0.6)	(0.8)	(0.9)	(0.8)	(1.5)	(0.3)	(0.9)	(0.8)	(1.0)	(0.4)		(1.4)	(0.5)
Eyelid	4.7	3.9	4.9	5	4.5	—	3.4	1.4	1	1.2	1	1.2	1.5	1.5	1.0	2.0	1.0	1.1	1.3	1.5	1.1	1	2.5	1.8
(SD)	(0.7)	(1.6)	(0.3)	(0.0)	(1.01)		(1.8)	(0.5)	(0.0)	(0.4)		(0.4)	(0.8)	(1.2)	(0.0)	(1.7)	(0.0)	(0.3)	(0.5)	(0.3)	(0.3)		(2.1)	(1.2)

* Populations 6 and 11 have incomplete data

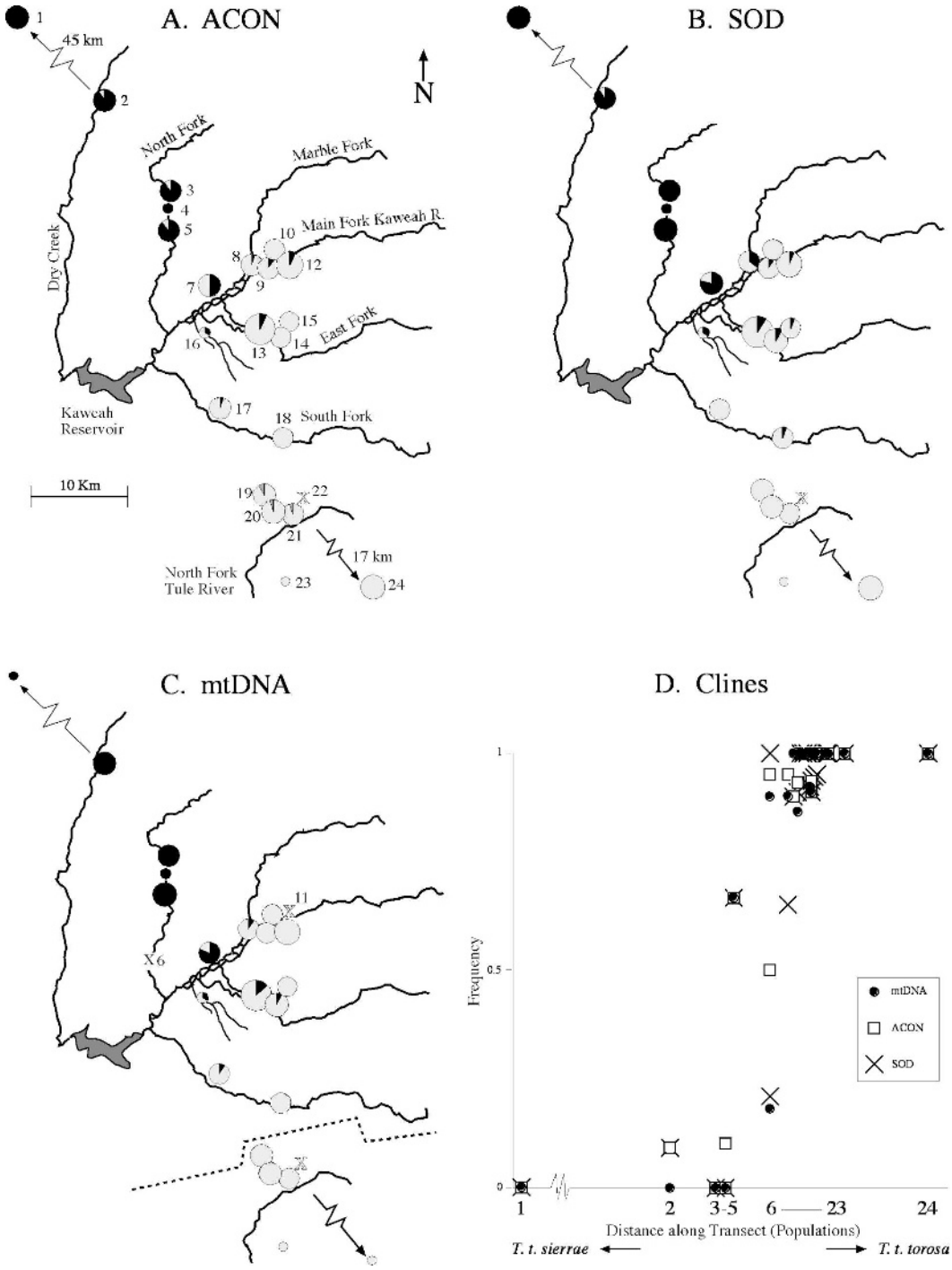

FIG. 2.—Distribution of genetic variation in the contact zone between *T. t. torosa* and *T. t. sierrae*. Black pie slices designate *T. t. sierrae* alleles, and light pie slices designate *T. t. torosa* alleles. Pie chart size is proportional to sample size, and single individuals are marked with an "X". A. Aconitase (ACON) electromorphs. B. Superoxide dismutase (SOD) electromorphs. C. mtDNA cytochrome *b* haplotypes, as determined by sequencing and RFLPs. D. Clines at all three loci. Population numbers are labeled along the X-axis, and are proportional to geographic distance; the Y-axis shows the frequency of genetic variants diagnostic of *T. t. torosa* (variation diagnostic of *T. t. sierrae* is the inverse).

T. t. torosa tend to have eyelids that match the dorsal coloration. Eyelids were scored from 1 (completely covered with the dorsal coloration) to 5 (completely covered with the ventral coloration). Riemer (1958) scored eyelids on a scale of 1–4 (Fig. 15, page 343); I added a category between scores three and four to make the spacing among categories more even.

(2) Snout: the amount of ventral coloration extending onto the snout. *Taricha t. sierrae* tend to have a large amount of the ventral coloration extending onto the snout, whereas *T. t. torosa* tend to have dorsal coloration covering this region. The snout was scored from one (extreme *T. t. torosa* pattern) to five (extreme *T. t. sierrae* pattern). Riemer (1958) had nine categories (Fig. 15, p. 343); my five categories evenly span these nine. Five categories instead of nine were used so that snout and eyelid coloration would be equally weighted.

A color index was created by adding together the Eyelid and Snout values (totals range from 2 to 10). To illustrate the relationship between color pattern and genotype, color index scores were plotted against hybrid index scores.

Morphometrics

Several measures were taken to determine if *T. t. torosa* and *T. t. sierrae* differ in head shape. The following seven measurements (the same used by Riemer, 1958) were recorded with calipers to the nearest 0.1 mm: (1) Snout to vent length (SVL), the distance from the tip of the snout to the anterior opening of the cloaca; (2) Head length, the distance from the tip of the snout to the posterior edge of the gular fold; (3) Head width, the widest line across the head, usually at the quadrate; (4) Eye width, the widest anterior to posterior portion of the eye opening; (5) Eye–eye, the distance between the eyes measured from the anterior of the eye; (6) Nostril–nostril, the distance between the interior edges of the nostril openings; (7) Eye–nostril, measured from the anterior-most opening of the eye to the nearest nostril opening.

Head shape was measured in the same individuals used in genetic and coloration studies. As with the analysis of color pattern, all specimens were randomized in a bucket prior to measuring. Morphometric data were analyzed using a principal components analysis (PCA). All variables were log transformed prior to the analysis (Bookstein et al., 1985), and five individuals with SVL < 60 mm were excluded because they were outliers. For the PCA I used a correlation matrix, meaning that all the variables were standardized to have a mean of zero and a variance of one prior to deriving the principal components. Statistical analyses were carried out using SPSS (SPSS, Inc.).

Classification Analyses

Discriminant function analyses (DFA) were used to quantify how well the morphometric results (i.e., principal components), color pattern categories, and genetic data (allozyme loci and mtDNA haplotypes) predict subspecific membership. DFA analyses were carried out using JMP 5.1.1 (SAS Institute, Inc.) and SPSS (SPSS, Inc.). In some cases, a discriminant function calculated using one sample was used to classify another sample. In other cases, the discriminant function was used to classify the same sample. In this latter case, discriminant functions are known to be overly discriminating, even though this is the most common application of DFA in classification analyses (Norman and Streiner, 2003).

Results

Genetic Transitions across the Hybrid Zone

The contact zone between *T. t. torosa* and *T. t. sierrae* qualifies as a hybrid zone, with interbreeding between the separate lineages leading to patterns of mixed ancestry in the center of the hybrid zone and introgression into the tails of the clines (Fig. 2 A, B, C). The clines for allozyme loci ACON2 and SOD have congruent shapes and centers (Fig. 2D). Population 1, at the northern limit of the study area, is fixed for allozymes that are diagnostic of *T. t. sierrae*. Population 2 has *T. t. torosa* allozymes in low frequency at both allozyme loci (10% for SOD and ACON2; Table 2) in an otherwise *T. t. sierrae* genetic background.

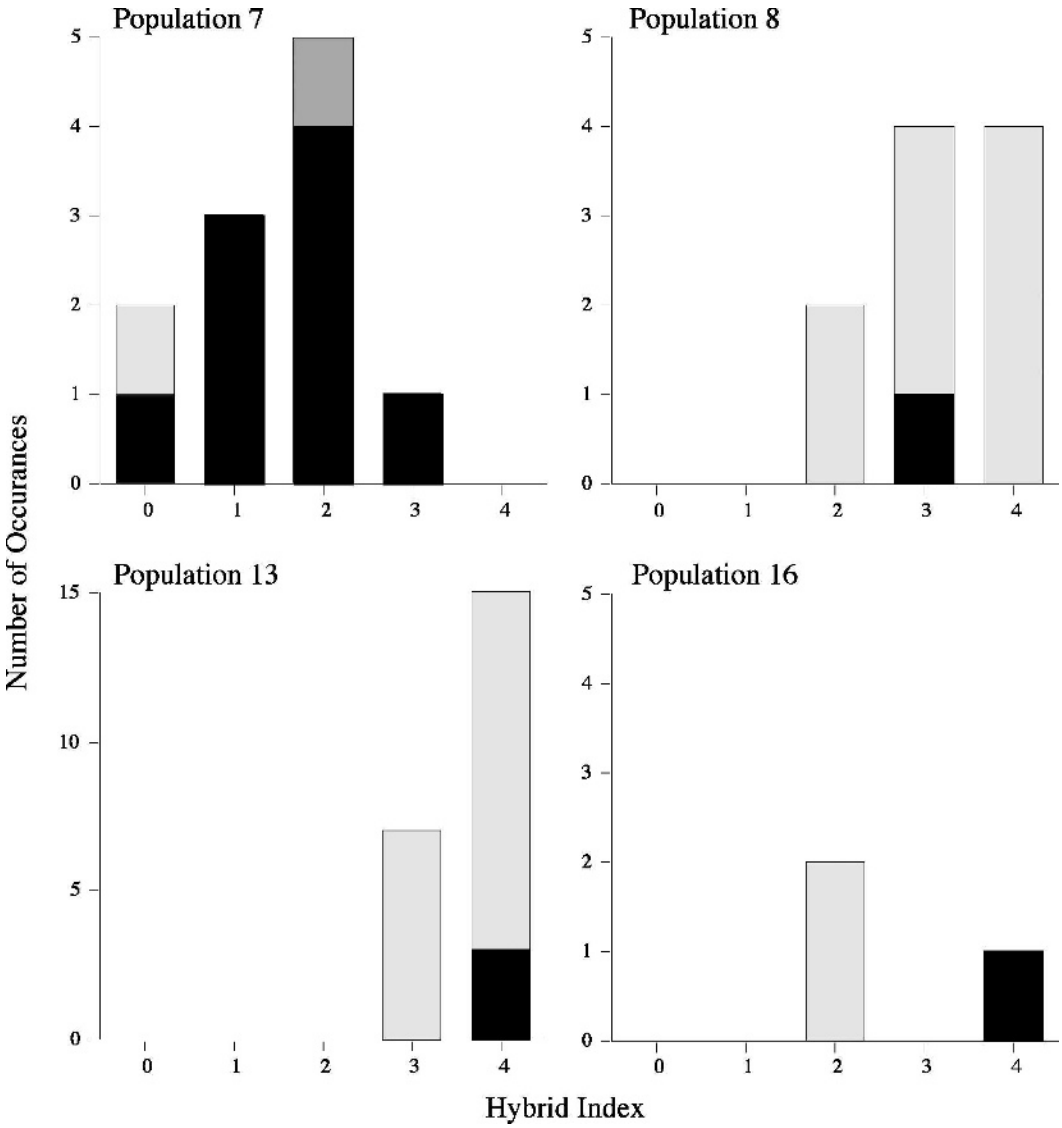

FIG. 3.—Histograms of allozyme hybrid index scores by population. The four most genetically heterogenious populations are shown; many other populations have introgressed allozymes present at low frequency (Fig. 2; Table 2). Hybrid index scores of zero indicate individuals with only *T. t. sierrae* allozymes, and scores of 4 indicate individuals with only *T. t. torosa* allozymes. Within the histograms, black bars represent individuals with *T. t. sierrae* mtDNA haplotypes, and light grey bars represent individuals with *T. t. torosa* mtDNA haplotypes. The dark grey bar in population 7 designates one individual for which the mtDNA haplotype could not be obtained. In addition, another individual from population 7 (not shown in this figure) has a *T. t. torosa* mtDNA haplotype and *T. t. sierrae* allozymes at the SOD locus, but the ACON2 allozymes could not be determined.

No *T. t. torosa* allozymes for SOD were found in populations 3–5, but ACON2 has low frequency *T. t. torosa* allozymes in populations 3 and 5 (10% in each). Populations 7 and 16, located 5 km apart on either side of the Kaweah River, are the most highly genetically admixed (Fig. 2). Population 7 has a 50:50 ratio of *sierrae:torosa* allozymes at the ACON2 locus, and a 5:13 ratio at the SOD locus (Fig. 3A). Population 16 consists of three individuals: two are heterozygotes at both nuclear loci for *T. t. sierrae* and *T. t.*

TABLE 3.—Results of a principal components analysis of log-transformed variables. The values are the correlations between the morphometric variables and the first two principle components.

	PC1	PC2
Eye Width	0.685	0.681
Eye–Nostril	0.784	−0.312
Head Width	0.837	−0.069
Head Length	0.789	−0.095
Nostril–Nostril	0.715	−0.058
Eye–Eye	0.853	−0.238
SVL	0.850	0.183
Eigenvalues:	4.37	0.67
% of total variance	62.41	9.55

torosa allozymes, and have a *T. t. torosa* mtDNA haplotype, and one is homozygous for *T. t. torosa* allozymes at both allozyme loci, yet has a *T. t. sierrae* mtDNA haplotype (Fig. 3D). Population 8 possesses predominantly *T. t. torosa* allozymes, with *T. t. sierrae* allozymes present at moderate frequency at the SOD locus (35%) and low frequency at the ACON2 locus (5%) (Fig. 3B). This represents a considerable shift in frequency over a short distance: populations 7 and 8 are separated by 5 km. East of population 8, populations 9 and 12 have *T. t. sierrae* allozymes present at low frequency (7–10% at both loci), and population 10 is fixed for *T. t. torosa* allozymes. To the south, populations 13–15 show low levels of introgression of *T. t sierrae* allozymes at the SOD locus (6–9%) (Table 2). Similarly, for ACON2 population 13 (the largest sample at 22 individuals) possesses introgressed *T. t. sierrae* allozymes at low frequency (7%) (Fig. 3C), but populations 14–15 lack *T. t. sierrae* allozymes altogether. The southern extent of *T. t. sierrae* allozymes is population 17 at the ACON2 locus (5%), and population 18 at the SOD locus (5%). A unique, low frequency allozyme (5–18%) is was found in populations 19–21. The distance from population 2 to 18 (the widest separation of populations showing allozymic introgression) is 40 km. The distance between population 1 and 2 is 45 km, however, so the limit of northward introgression of *T. t. torosa* allozymes is unclear, though the frequency of *T. t. torosa* allozymes in population 2 is low (Fig. 2). The widths of the clines at ACON2 and SOD were estimated as 10.1 and 10.0 km, respectively.

MtDNA haplotypes are not as broadly introgressed as the allozyme loci (Fig. 2C, D). All specimens from the North Fork of the Kaweah River (populations 1–6) are assignable to *T. t. sierrae*, without evidence of introgression of *T. t. torosa* mtDNA haplotypes. The northernmost haplotype of *T. t. torosa* ancestry is found in population 7, which has one *T. t. torosa* haplotype in the sample (Fig. 3A, Table 2). In contrast, populations 8, 13 and 16, located east and south of population 7, have predominantly *T. t. torosa* haplotypes, with *T. t. sierrae* haplotypes present at low to moderate frequencies (10%, 14% and 33%, respectively; Fig. 2C, 3B–D). The southernmost extent of *T. t. sierrae* haplotypes is population 17 (10%), near the South Fork Kaweah (Fig. 2C). Further south, populations are fixed for the *T. t. torosa* haplotype. The widest separation of populations showing mtDNA introgression is between populations 8 and 17, a distance of 16 km. The width of the mtDNA haplotype cline is 7.5 km.

For both the allozyme and mtDNA data, populations 7, 8, 13, and 16 were the most genetically heterogeneous (Fig. 2, Table 2). A DFA was conducted on the genetic data (allozymes and mtDNA haplotypes) to quantify how well individuals from these populations could be assigned to subspecies. First, a DFA was conducted using all the populations except populations 7, 8, 13, and 16. All individuals from populations 1–5 were assigned to *T. t. sierrae* ($n = 35$), and all individuals from the remaining populations were assigned to *T. t. torosa* ($n = 145$). Second, the discriminant functions were used to classify individuals from populations 7, 8, 13 and 16. All the individuals in populations 8 and 13 were classified as *T. t. torosa*. Therefore, these populations, though having higher frequencies of *T. t. sierrae* alleles than other populations of *T. t. torosa* in this study, were treated as *T. t. torosa* in further analyses. In contrast, in population 7 seven individuals were classified as *T. t. sierrae*, and two were classified as *T. t. torosa*, and in population 16 one individual was classified as *T. t. sierrae*, and two were classified as *T. t. torosa*. These two populations were thus treated as admixed in further analyses.

Genotypic Disequilibrium and Hardy-Weinberg Equilibrium

Dispersal of parental types into the hybrid zone followed by selection against hybrid genotypes will generate statistical associations among loci, as measured by standardized linkage disequilibrium (R). Away from the center of the contact zone, all estimates of linkage disequilibrium were $R = 0$. In contrast, significant R was detected in population 16 between SOD and ACON2 (estimate = 1.0; range = 0.23–1). In addition, in population 8, cytonuclear disequilibrium between SOD and the mtDNA haplotypes was estimated at $R = 0.445$, and between SOD and ACON2 $R = 0.31$; neither of these estimates is statistically significant, however. Linkage disequilibrium was not detected in populations 7, though this populations is genetically admixed. However, in population 7 heterozygote deficiency (F_{IS}) at the ACON2 locus was estimated at 0.09 (range = 0–0.62), though again this estimate did not differ significantly from zero. Away from the center of the contact zone in population 2, F_{IS} at the SOD locus was significant (estimate = 1.0; range = 0.22–1.0); in this case one individual was homozygous for the allozyme diagnostic of *T. t. torosa*, while all other individuals in the sample were homozygous for the *T. t. sierrae* allozyme. No other populations had estimates of F_{IS} that differed from zero.

Color Pattern

A scatter plot of snout coloration against eyelid coloration is depicted in Fig. 4A. Two clusters are present, corresponding to populations of *T. t. sierrae* and *T. t. torosa*. Of the two most admixed populations, population 7 is located intermediate between the *T. t. torosa* and *T. t. sierrae* clusters, and population 16 is located on the edge of the *T. t. torosa* cluster. Population 23 also occupies an intermediate position in the scatterplot, though genetically it is pure *T. t. torosa* (Fig. 2). The sample size of this population is quite small, however ($n = 2$).

A discriminant function analysis was used to quantify how effective color pattern (snout and eyelid scores) is at assigning individuals to subspecies. Admixed populations 7 and 16 were first excluded from the analysis. Out of 149 individuals of *T. t. torosa* examined, 142 (95.3%) were correctly assigned to *T. t. torosa*, and out of 41 *T. t. sierrae* examined, 38 (92.7%) were correctly assigned to *T. t. sierrae*. Thus, the pattern of coloration on the head of *T. t. torosa* is effective at distinguishing between the subspecies. When these discriminant functions were applied to admixed populations, however, they suggested the admixed populations were of mixed ancestry: in population 7, 7 of 10 (70%) individuals were applied to *T. t. torosa*, and in population 16, 2 of 3 (66%) individuals were assigned to *T. t. torosa*. In many cases, individuals in populations 7 and 16 that were classified as one subspecies with the genetic data were classified as a different subspecies using the color pattern data (5 of 11 comparisons). This result lends support to the interpretation of these populations as admixed.

The correlation between color pattern and genetic composition is further explored in a plot of color index against hybrid index (Fig. 4B). Individual values and population averages are plotted. In general, low average scores in the color index (dark eyelids and snout, as found in *T. t. torosa*) correlate with high hybrid indices (genetically *T. t. torosa*), and vice versa. However, the correlation between color and hybrid indices is not perfect at the individual level, which is to be expected when there is hybridization. For example, one individual is shown to have a hybrid index score of 4 (pure *T. t. torosa*) but a color pattern of 9 (*T. t. sierrae*). Nevertheless, on average populations of *T. t. torosa* and *T. t. sierrae* away from the center of the contact zone have color patterns that are correlated with genotype. In contrast, admixed populations 7 and 16 are intermediate in their hybrid index/color index scores.

Morphometrics

Principal Components Analysis (PCA) was used to explore the clustering patterns of populations according to head shape. The first component accounted for 62% of the variation, and had large, positive factor loadings with all of the variables (Table 3). This axis is thus interpreted as primarily a size axis (Bookstein et al., 1985). The second component accounted for 10% of the variation. Eye

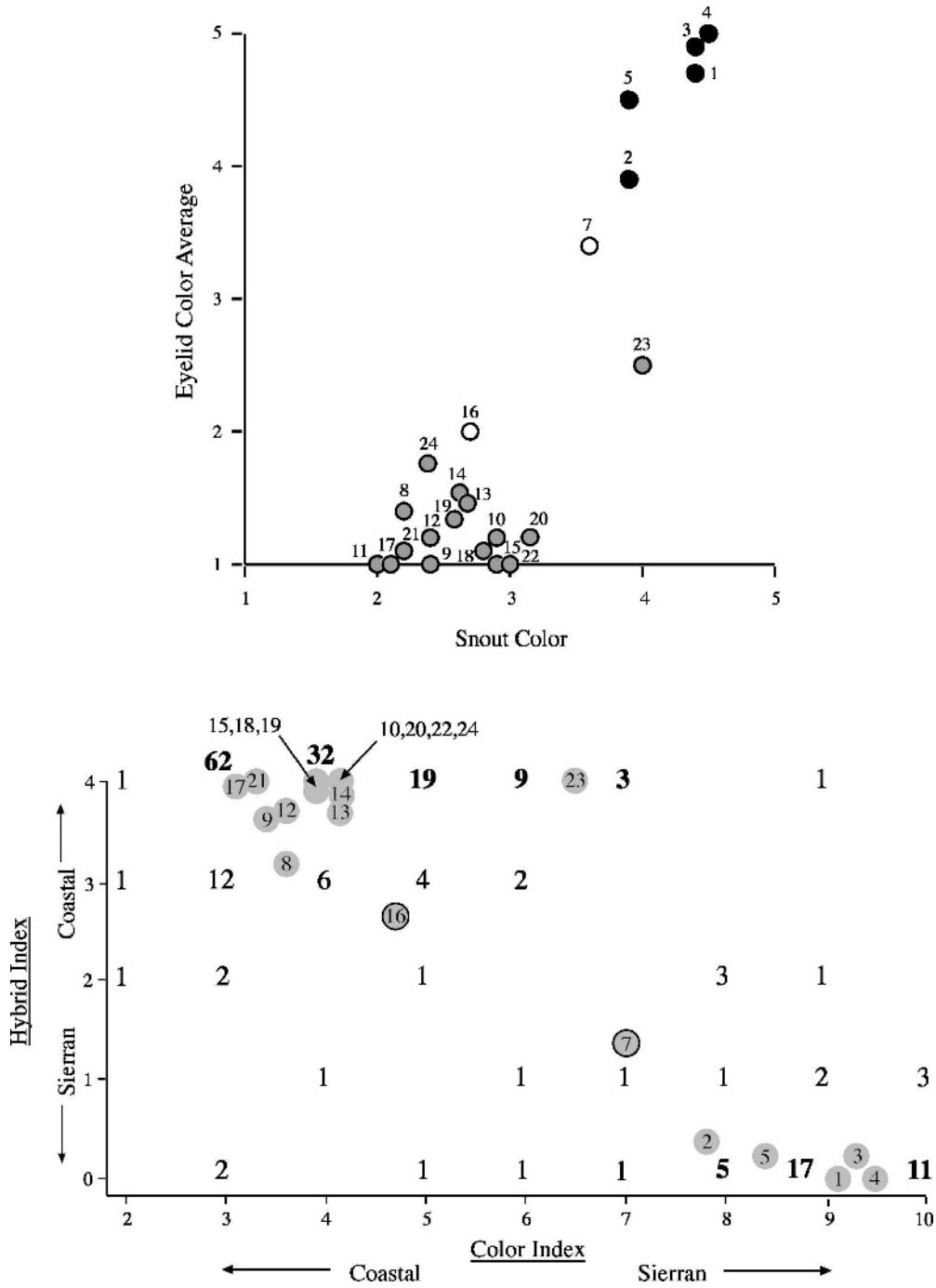

FIG. 4.—A. Relationship between snout color and eyelid color, averaged by population. Black circles represent populations of *T. t. sierrae*, grey circles represent populations of *T. t. torosa*, and open circles represent the two admixed populations (Fig. 3). B. Relationship between color hybrid index and allozyme hybrid index. Color scores lower than two do not exist. Numbers in grey circles are the average values of populations (Table 2); they have been slightly arranged for visibility. Grey circles with a black outline indicate admixed populations. Numbers not in circles are the number of individuals with that combination of scores. For each color index score, the hybrid index with the largest number of individuals is in boldface; this highlights the trend in the data.

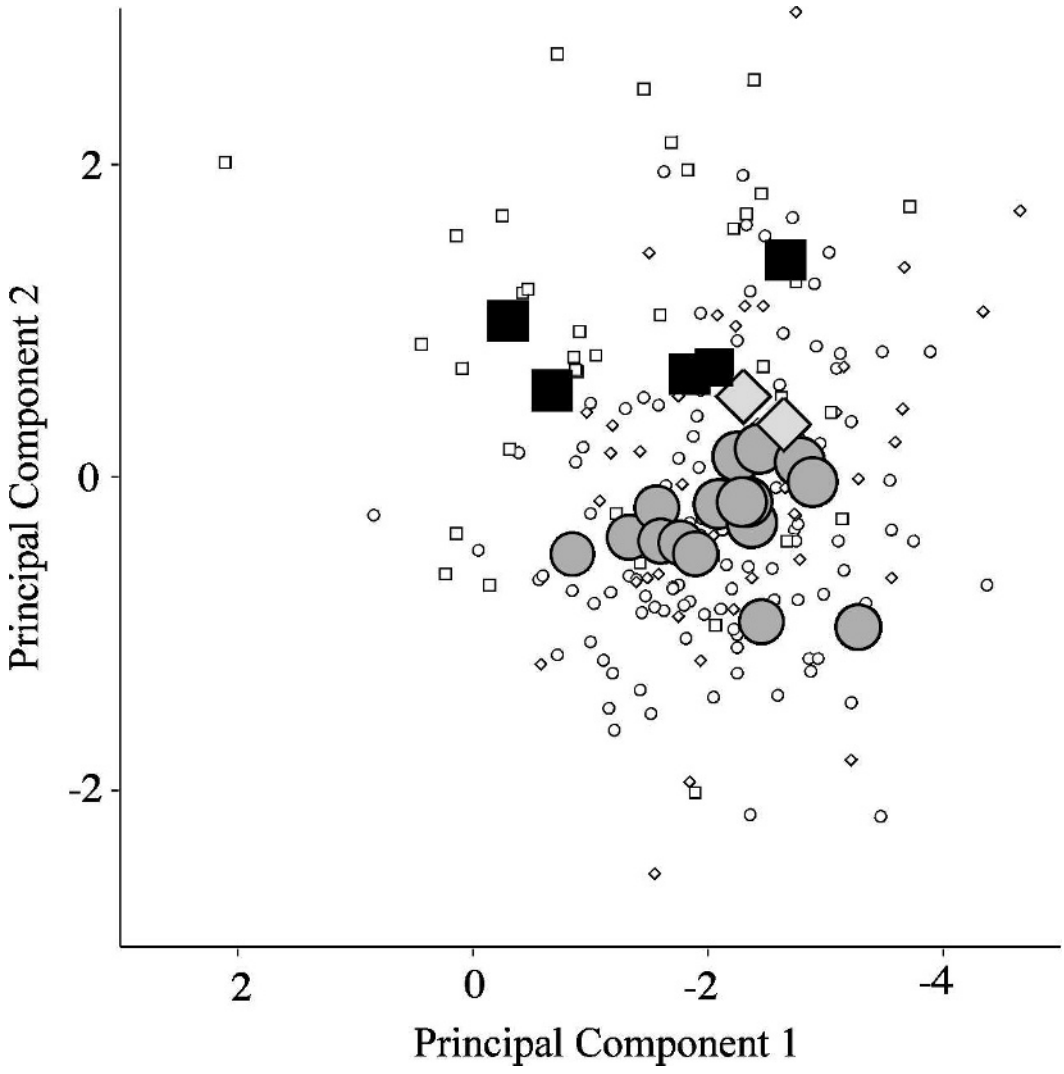

FIG. 5.—A. Scatterplot of the 1st and 2nd axes of a principal components analysis of morphometric data. Population numbers correspond to Table 1. Squares designate individuals of *T. t. sierrae* (large black squares show the population averages), circles designate populations of *T. t. torosa* (large grey circles show the population averages), and triangles designate the genetically admixed populations (large, light-colored triangles show population averages).

Width was the variable most strongly correlated with the second axis ($r = 0.681$), with eye–nostril and eye–eye making lesser contributions ($r = -0.312$ and -0.238, respectively) (Table 3). Figure 5 shows a scatter plot of all the populations in the study along the first two PC axes. Populations that are genetically *T. t. sierrae* have high scores along the second axis, whereas populations of *T. t. torosa* have low scores along the second axis. Of the genetically admixed populations, population 7 has an intermediate score on the second axis, and populations 16 is located on the edge of the cluster of *T. t. torosa* populations. These results are analogous to the results of the color pattern analysis (Fig. 4A, B). Thus, *T. t. torosa* and *T. t. sierrae* are largely distinguishable on morphometric grounds when population averages are employed.

A discriminant function analysis was conducted on the first two components of the

TABLE 4.—Discriminant function analysis of morphometric variables (PCA1, PCA2) and color pattern elements (Snout, Eyelid). The grouping variable was subspecies (*T. t. torosa, T. t. sierrae*).

Variable	Standardized coefficient[1]	Loadings[2]
PCA 1	−0.294	−0.189
PCA 2	0.201	0.248
Snout	0.219	0.431
Eyelid	0.875	0.915

[1] Standardized canonical discriminant function coefficients. Larger values have more weight in the discriminant function.
[2] The correlation between each variable and the discriminant function

PCA. As with the analysis of color pattern, admixed populations 7 and 16 were excluded. Of 147 individuals of *T. t. torosa* examined, 117 (79.5%) were correctly assigned to *T. t. torosa*, and of 40 *T. t. sierrae* examined, 27 (67.5%) were correctly assigned to *T. t. sierrae*. Thus, relative to color pattern, head shape is ineffective at distinguishing between individuals of *T. t. torosa* and *T. t. sierrae*, despite the pattern of population means in Figure 5.

Classification Analysis

When all color pattern and morphometric (i.e., PCA) variables were combined into a single DFA, the results were nearly identical to the analysis of color pattern alone: 37 of 40 (92.5%) *T. t. sierrae* were correctly assigned, and 142 of 147 (96.6%) of *T. t. torosa* were correctly assigned. These results suggest that morphometrics add very little to discrimination between the subspecies. Indeed, the standardized coefficient and factor loading of eyelid coloration (0.875 and 0.915, respectively) greatly exceeded the other variables, all of which had measures between 0.2 and 0.3 (Table 4).

DISCUSSION

Contact Zone Characteristics

The current study examined hybrid zone dynamics between *T. t. torosa* and *T. t. sierrae* with the goal of determining whether they are maintaining their evolutionary independence. The results indicated that the secondary contact between *T. t. torosa* and *T. t. sierrae* is a hybrid zone centered on the Main Fork of the Kaweah River in Tulare County (Fig. 1, 2). There the two lineages interbreed, including admixture in the center of the hybrid zone and introgression into the tails of the clines (Fig. 2A–C). One mtDNA locus and two nuclear allozyme loci had congruent cline centers (Fig. 2D), with populations 7 and 16 being the most genetically admixed (Fig. 3). In the center of the hybrid zone, significant linkage disequilibrium was detected in population 16, suggesting selection against hybrid genotypes. There is also evidence of linkage disequilibrium in population 8, and of heterozygote deficit in population 7, though these estimates lack statistical support. Estimates of linkage disequilibrium and inbreeding in the center of the hybrid zone suggest this may be a tension zone, though more sampling is necessary to make this determination with confidence. Away from the center of the contact zone populations possess introgressed allozymes and mtDNA haplotypes at low frequency.

Analyses of color pattern and head shape have patterns that are similar to the genetic data. Populations on either side of the hybrid zone are phenotypically diverged and manifest differences that are consistent with earlier descriptions of subspecific differentiation (Riemer, 1958; Twitty, 1942), while the two most genetically admixed populations (7 and 16) possess intermediate phenotypes (Fig. 4, 5). Head color pattern was highly effective at assigning individuals to the subspecies, but head shape was less discriminating. Unlike the genetic data, however, color patterns do not appear to have introgressed across the hybrid zone.

There is a discordance in the extent of introgression into the tails of the clines by allozymes and mtDNA. The distance separating populations with introgressed allozymes at ACON2 and SOD is 35–40 km (minimum, because the extent of northward introgression is unclear), while the distance separating introgressed mtDNA haplotypes is 16 km (Fig. 2). It is not uncommon for mtDNA to show divergent patterns relative to nuclear markers (e.g., Ballard and Whitlock, 2004; Jockusch and Wake, 2002; Moritz et al., 1992). This is likely because mtDNA is haploid, maternally inherited, and may be weakly linked to the nuclear genome, and consequently demographic factors impact mtDNA differently than nuclear loci (Ballard and Whitlock, 2004). For instance, genetic drift is a stronger force on mtDNA because it has

a lower effective population size than nuclear loci. Sex-biased dispersal can also generate discordances. Contrary to the results of this study, Ballard and Whitlock (2004) have observed that mtDNA may be more likely to introgress than nuclear alleles, and this is a common finding in hybrid zone studies (e.g., Marshall and Sites, 2001; Martinsen et al., 2001). The cause of the reduced introgression of mtDNA haplotypes between *T. t. torosa* and *T. t. sierrae* is unclear, but could be driven by higher philopatry to reproductive sites in females than males. Discordances between nuclear and mtDNA have also be recorded between populations of *T. torosa* in southern California (Kuchta and Tan, 2006*a*), and in the rough-skinned newt, *T. granulosa*, following range expansion (Kuchta and Tan, 2005).

Though the mtDNA and allozymes differ in their levels of introgression, they have overlapping cline centers and similar widths (where width is defined as the inverse of the maximum slope in the cline; Barton and Gale, 1993). The width of the mtDNA cline was estimated at 7.5 km wide, and the clines of the allozyme loci ACON2 and SOD were estimated as 10 km and 10.1 km wide, respectively. These widths should be viewed as maximum estimates. First, it was assumed that the hybrid zone runs perpendicular to the river, and if the cline takes some other orientation the cline widths will be overestimated. Second, wide sampling will also cause an overestimation of hybrid zone width, and the populations nearest to the center of the hybrid zone (7 to 8, and 7 to 16) are ~5 km apart.

Interpretation of the hybrid zone widths estimated in this study is complicated by the fact that very little work has been done on the ecology of *Taricha* in the Sierra Nevada. Studies of *Taricha* in coastal California can provide a rough estimate of the dispersal potential of Sierran *Taricha*, however. For example, Trenham (1998) recovered migrating *T. t. torosa* up to 3.2 km from their known breeding sites, and also documented that adults regularly moved between alternative breeding sites separated by up to 1.3 km. In addition, Twitty (1966) demonstrated that red-bellied newts (*T. rivularis*) are capable of migrating up to 8 km over land to breeding sites, including crossing ridges separating drainages. These data strongly suggest that *T. t. sierrae* are also capable of long distance movements of up to a few km.

Given the migratory life history and demonstrated dispersal capabilities of *Taricha*, the hybrid zone widths estimated in this study are narrow. For example, Barton and Hewitt (1985; see also Hewitt, 1988) summarized a large number of hybrid zone studies and found that many hybrid zones are greater than 100 times wider than the estimate of dispersal (σ, the standard deviation of the distance moved between parents and offspring per generation). In contrast, but similar to the results of the current study, the classic tension zone between the Fire-bellied toads *Bombina bombina* and *B. variegata* is 6 km wide. In this system strong selection against hybrid genotypes is well documented, and field estimates of $\sigma = 0.43$ km per generation (Szymura, 1993; Szymura and Barton, 1986; Yanchukov et al., 2006).

Studies on the salamander *Ensatina eschscholtzii* provide a second illuminating comparison. Alexandrino et al. (2005) have documented strong selection against hybrids in two tension zones between *E. e. xanthoptica* and *E. e. platensis* in the foothills of the central Sierra Nevada. At both contact zones, clines for mtDNA, allozymes, and color pattern were concordant, and cline width estimates varied from 720 m to 2 km. This hybrid zone is thus about 1/5th as wide as the current study, however, unlike *Taricha, Ensatina* are nonmigratory and have extremely limited dispersal capability (field estimates of $\sigma = 0.035$ km per generation; Alexandrino et al., 2005; Staub et al., 1995). These hybrid zones stand in stark contrast to a third hybrid zone in the central Sierra Nevada between two unrelated lineages of *E. e. platensis* (Wake and Schneider, 1998). At this contact zone, clines widths vary from 1 km to 200 km and cline centers are highly discordant, suggesting that selection against hybrids is weak or nonexistent and that the lineages are in the process of fusing together (Alexandrino et al., 2005; Wake and Schneider, 1998). Thus, the *Ensatina* complex contains good examples of both tensions zones and neutral fusion. Relative to dispersal potential, the hybrid zone

between *T. t. torosa* and *T. t. sierrae* is most like the tension zones between *E. e. xanthoptica* and *E. e. platensis*

A Suture Zone in the Southern Sierra Nevada?

The hybrid zone between lineages of *Taricha* in the southern Sierra Nevada is not an isolated case. Many taxa exhibit phylogeographic disjunctions there, and a growing body of evidence suggests that the southern Sierra may be a "suture zone" (Remington, 1968) where multiple species form a cluster of secondary contacts due to a shared biogeographic history (e.g., Calsbeek et al., 2003; Feldman and Spicer, 2006; Lapointe and Rissler, 2005; Rissler et al., 2006). In many cases populations in the southern Sierra Nevada are more closely related to populations in coastal or southern California than to other populations in the Sierra Nevada. This is the case with southern Sierran *T. t. torosa* (Kuchta and Tan, 2006*a*). Other examples include the California mountain kingsnake (*Lampropeltis zonata*; Rodríguez-Robles et al., 1999), southern alligator lizard (*Elgaria multicarinata*; Feldman and Spicer, 2006), and mountain yellow-legged frog (*Rana muscosa*; Macey et al., 2001). However, while the pattern of phylogeographic concordance in the southern Sierra Nevada is highly suggestive of a common biogeographic history, no specific, convincing geological or climatic explanation has yet been advanced and tested. In addition, the dynamics of secondary contact zones between divergent phylogeographic lineages in species other than *T. torosa* are largely unexplored (but see Jackman and Wake, 1994).

Contact Zones and Species Limits

Contact zone studies provide much useful information for systematists. Historically, researchers studying contact zones have favored the Biological Species Concept (Mayr, 1942) because interbreeding is the most salient feature of hybridizing lineages (Barton and Hewitt, 1985, 1989; Harrison, 1990, 1993; Hewitt, 1988). However, because studies of contact zones provide an estimate of evolutionary independence, they are also valuable for adherents of alternative species concepts not specifically centered around reproductive isolation. The species concept I employ is the General Metapopulation Lineage Concept (GMLC), which defines species as segments of metapopulation lineages (de Queiroz, 2005; see also de Queiroz, 1998, 1999). The GMLC is quite similar to the Evolutionary Species Concept (Simpson, 1961; Wiley, 1978), except that it incorporates the perspective that all the prominent species *concepts* in fact share the same conceptualization of what kind of entity a species is. That is, under the GMLC all the prevalent species concepts are seen as either variations on the theme of species as evolutionary lineages, or focus on the *criteria* most appropriate for diagnosing an evolutionary lineage. The GMLC thus represents a unifying approach to the species problem—it will not end systematic disputes, but it does help to clarify the battle lines. Disagreement persists in part because the time-extended nature of species formation complicates lineage diagnosis, and in part because the criteria used to diagnose evolutionary lineages, such as reproductive isolation, monophyly, and adaptive divergence, evolve at different rates and in different orders in separate lineages (Sites and Marshall, 2004). These problems are well exemplified by many species complexes in salamanders (e.g., Jockusch and Wake, 2002; Mead et al., 2001; Tilley and Mahoney, 1996; Wake, 1997; Wake and Schneider, 1998).

In evaluating the taxonomic status of *T. t. torosa* and *T. t. sierrae*, I am primarily interested in determining whether the subspecies constitute separate metapopulation-level lineages, and, if so, whether these lineages are maintaining their evolutionary distinctiveness. Kuchta and Tan (2006*a*) showed that the two subspecies are reciprocally monophyletic using phylogenies inferred from both mtDNA and allozyme data, and used a molecular clock to estimate that the lineages diverged 7–13 myr ago. In addition, Twitty (1942, 1966) and Riemer (1958) have documented many phenotypic and ecological differences between *T. t. torosa* and *T. t. sierrae*. There is thus good evidence that *T. t. torosa* and *T. t. sierrae* are distinct evolutionary lineages. The current study found that the clines among three genetic loci were narrow relative to the dispersal potential of *Taricha*, with similar shapes and centers. There is also

some evidence of selection against hybrids, suggesting tension zone dynamics, though more sampling is required to establish this with confidence. Clines in head shape and color pattern were similar to the genetic clines. In sum, despite hybridization, the two lineages appear to maintaining their independence as distinct metapopulation-level evolutionary lineages.

In light of this evidence, I propose that the original taxonomy of Twitty (1942) be followed and *T. torosa* and *T. sierrae* be recognized as separate species. The type locality of *T. torosa* is San Francisco Bay (Rathke, in Eschscholtz, 1833: 12), and the type locality of *T. sierrae* is Cherokee Creek, Butte County (Twitty, 1942). The border between the species follows the Main Fork of the Kaweah River, though individuals found near the center of the hybrid zone may defy taxonomic diagnosis.

Acknowledgments.—I thank D. Graber and Sequoia National Park for providing access to park resources and supporting this study, and the California Department of Fish and Game for collecting permits. I thank the Museum of Vertebrate Zoology (University of California, Berkeley) for access to collections, and the members of "Wake-Lab" for help in the field, including E. Rosenblum, A. van der Meijden, and D. Wake. S. Baird very generously assisted with the use of his program Analyse 1.30. In addition, K. McGowan contributed to the collection of color pattern data, A. Ballerini helped with the RFLP analyses, and R. Diaz participated in the gathering of morphometric data: I thank them all for their efforts and companionship. This manuscript benefited from critical comments by A. Corl, T. Devitt, T. Haff, A. Kazzaz, M. Mulks, D. B. Wake, K. Zamudio, and two anonymous reviewers. This research was funded by NSF Doctoral Dissertation Improvement Grant DEB 0072869 to D. B. Wake and S.R.K., and an NSF Predoctoral Fellowship supported S.R.K. as a graduate student. S.R.K. currently is supported by NSF grant DEB 0317182 to B. Sinervo and S.R.K. I thank the NSF for its generous support. Finally, but most of all, I thank D. Wake, my Ph. D. thesis advisor, for his guidance and encouragement.

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Accepted: 15 May 2007
Associate Editor: Maureen Kearney