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MtDNA Phylogeography of the California Newt, *Taricha torosa* (Caudata, Salamandridae)

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Thirty-six individuals of the California newt, *Taricha torosa*, representing 22 populations from throughout the range of the two currently recognized subspecies, *torosa* and *sierrae*, were examined for sequence variation in a segment (375 bp) of the mitochondrial (mt) cytochrome b gene. The maximum sequence divergence within *T. torosa* is about 9%. Phylogenetic analyses used the sister taxa *T. rivularis* and *T. granulosa* as outgroups. Eighteen haploid sequence types found in *T. torosa* were grouped by parsimony, maximum likelihood, and neighbor-joining analyses into five mitochondrial clusters: two in *torosa* (the northern and southern clusters) and three in *sierrae* (the northern, central, and southern clusters). The southern *sierrae* cluster apparently shared a most recent common ancestor with the northern *torosa* cluster. The approximate time of sequence divergence within the current species was calibrated using the known fossil record (0.8% divergence per million years or 0.01 maximum likelihood distance per million years). Phylogenetic implications of mtDNA sequence variation for evolution and biogeography of the *T. torosa* species complex are discussed.

INTRODUCTION

Mitochondrial (mt) DNA sequences have been used widely in the study of population genetic structure and phylogenetic analysis (e.g., Brown *et al.*, 1982; Smith and Patton, 1991; Moritz *et al.*, 1992). Mt genes are inherited clonally and maternally, and recombination is rare. The effective population size of mt genes is about one-fourth that of nuclear genes (Birky *et al.*, 1989), and there is no proof-reading mechanism (Wilson *et al.*, 1985); thus, the rate of evolution of mt genes is generally much higher than that of nuclear genes. Because mt codons and genes evolve at different rates, mtDNA has been used to resolve such problems as human evolution as short as several thousand years to as long as over 100 million years (Meyer and Wilson, 1990). These properties of mtDNA are advantageous for the study of population differentiation and biogeography (Avise *et al.*, 1987). However, because of its maternal inheritance and lack of genetic recombination, workers have been cautioned from using mtDNA sequences to define species boundaries (Moritz *et al.*, 1992).

The western North American newts of the genus *Taricha* form a monophyletic group that appears to be well suited to use in a study of mtDNA evolution. There are three currently recognized species (Riemer, 1958), with distribution patterns ranging from allopatric to sympatric and with different degrees of geographic and genetic differentiation (Hedgecock, 1974, 1976). The taxonomy of *Taricha* has been stable since the work of Riemer (1958), but earlier workers recognized greater diversity. *T. torosa* (the California newt) is subdivided into two subspecies: *T. t. torosa* (hereafter *torosa*) along the Pacific Coast from Humboldt County, northern California, to the Mexican border (Fig. 1: populations 12–22); and *T. t. sierrae* (hereafter *sierrae*) in the Sierra Nevada (1–11). The subspecies *torosa* is sympatric with the other recognized species, *T. granulosa* and *T. rivularis*, in coastal California north of San Francisco Bay, and with *T. granulosa* in the northern Sierra Nevada. At one time *sierrae* was considered to be a distinct species (Twitty, 1942), but it was reduced to a subspecies by Stebbins (1951), based on its morphological similarity to *T. torosa*. This subspecific status is widely accepted (Riemer, 1958; Nussbaum and Brodie, 1981; Frost, 1985), but recently Collins (1991) proposed that *sierrae* should be revived as a distinct species on the grounds of its unique morphological traits and disjunct distribution. This proposal (together with other suggested taxonomic changes) sparked controversy (Montanucci, 1992; Van Devender *et al.*, 1992; Frost *et al.*, 1992). Within the subspecies *torosa*, the southernmost populations (San Diego County) were originally recognized as a separate species, *T. klauberi*, or subspecies (Wolterstorff, 1935). The major diagnostic feature of *T. klauberi*, wart-like out-

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growths of the skin, which also can be found in some other localities outside San Diego County, was considered to be a diseased state, so Twitty (1942) synonymized it with *T. torosa*. Myers (1942) and Brattstrom and Warren (1953) also rejected taxonomic recognition of *T. klauberi*. Coates (1967) proposed that northern and southern genetic races of *torosa* could be recognized on either side of the Monterey Bay area, and his proposal was further supported by Hedgecock (1974, 1976). These genetic races have not been recognized taxonomically. Riemer (1958) systematically sampled the entire distribution of *Taricha*. His morphological studies, using mostly external characters such as proportions of different body parts and color patterns, supported the distinctiveness of the San Diego County populations from other *torosa* populations and showed much more morphological variation in *torosa* than in *sierrae*. However, Riemer (1958) did not recognize the San Diego populations of *torosa* as a distinct species or subspecies.

The phylogeographic history of the California newt is also controversial. Both Twitty (1942) and Riemer (1958) speculated that *T. torosa* is the direct descendant of a *T. granulosa*-like ancestor. Riemer (1958) further speculated that an early *T. torosa*-like stock was isolated by the Salinas Trough in the Monterey area and first entered southern California in Pliocene times. He suggested that both *T. rivularis* and *sierrae* originated from this lineage. Hedgecock (1974, 1976) postulated that *T. rivularis* diverged from a *T. granulosa*-like ancestral stock, followed by *sierrae* in northern Sierra Nevada. The northern *torosa* originated from a northern *sierrae*-like progenitor, and the southern *torosa* was thought to be more recently derived.

In this paper, we will test the previous phylogeographic hypotheses of the California newt using mtDNA sequences. Specifically we will address (1) how deeply these two subspecies are differentiated, (2) how variable the sequences are within and among populations, and (3) where the likely regions of intraspecific differentiation are and the relationships of population within each subspecies as shown from the mtDNA phylogeny. Findings from mtDNA will be compared with findings of other studies of allozyme, ecomorphology, and chromosomes in the California newt (Tan, 1993, 1994), as well as studies from some other salamanders in the same areas.

MATERIALS AND METHODS

Thirty-six individuals representing 22 populations of *T. torosa* and 4 individuals from one population each of *T. granulosa* and *T. rivularis* were used to obtain mtDNA sequences (Fig. 1 and Table 1).DNA samples were prepared by Chelex extraction with 5% (weight/volume) solution of Chelex (Bio-Rad. Cat. No. 143-2832) (Walsh *et al.*, 1991) and the conventional SDS–NaCl–

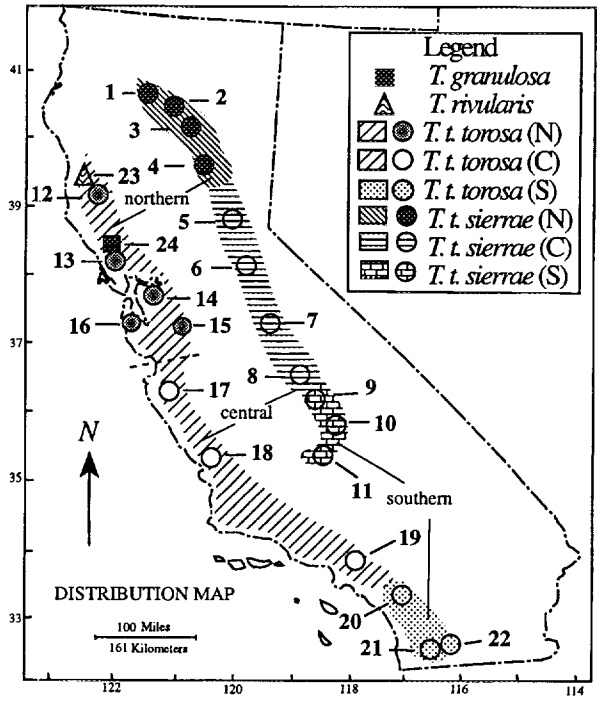

FIG. 1. Distribution and sampling map of *Taricha torosa* in California. Five diagnosable mtDNA clusters, as well as the northern and southern races (Coates, 1967), are shown. The species *T. rivularis* from Sonoma County and *T. granulosa* from Marin County are used as outgroups to root the phylogenetic trees of *T. torosa*. Population numbers are described in detail in Table 1.

ethanol method (Medrano *et al.*, 1990). For Chelex extraction, either frozen, unground tissues or ground protein extracts (<5 mg) (Tan and Orrego, 1992) were added to a 1.5-ml tube and incubated at 55°C for 2 to 18 hr before DNA amplification. The following primers were used for both double- and single-stranded PCR amplification: MVZ15, GAACTAATGGCCCACAC (AA/TT)TACGNAA; and cytochrome (Cyt) b2, AAACT GCAGCCCCTCAGAATGATATTTGTCCTCA. Cyt b2 and MVZ15 were designed as conservative primers to match many groups of vertebrates (Kocher *et al.*, 1989; Moritz *et al.*, 1992). The amplified fragment corresponds to position 16,268 (5' end) and 16,654 (3' end) of the *Xenopus laevis* mtDNA (Roe *et al.*, 1985). Including primers, the total length of this fragment is 446 bp.

PCR amplification of double-stranded products was performed in a 12.5- or 25-μl volume with 38 cycles using *Thermus aquaticus* DNA polymerase (Saiki *et al.*, 1988). Amplification was done using a Techne programmable Dri Block PHC-1 with denaturation at 93°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min. Aliquots of 5 μl of products were run on 3% NuSieve and 1% HGT–agarose minigels, from which a small plug was taken and diluted in 1:100 in m*M* Tris–0.1 m*M* EDTA.

TABLE 1

Sample Localities and MVZ (Museum of Vertebrate Zoology) or S (Wake Laboratory Salamander Catalogue) Numbers for Mitochondrial DNA Studies[a]

Pop. No.	Table 2 No.	Sp./subsp.	Abbreviation	MVZ No./S No.	Locality
1	1	*T. t. sierrae*	T.s.SH1	219831	Squaw Creek, Shasta County (Co.)
2	2	*T. t. sierrae*	T.s.SH2	172748, 172749	Bear Creek, Shasta Co.
3	2	*T. t. sierrae*	T.s.TEH	217911	Paynes Creek, Tehema Co.
4	3	*T. t. sierrae*	T.s.BUT	219091, 219092	Cherokee Creek, Butte Co.
5	4, 5	*T. t. sierrae*	T.s.ED1,ED2	197461, 197462	Big Canyon, El Dorado Co.
6	6, 7	*T. t. sierrae*	T.s.CV1,CV2	197463, 197464	West Point, Calaveras Co.
7	8, 9	*T. t. sierrae*	T.s.MP1,MP2	175404, 175405	Sherlock Creek, Mariposa Co.
8	10	*T. t. sierrae*	T.s.FRS	197474, 197475	Jose Basin, Fresno Co.
9	11	*T. t. sierrae*	T.s.TUL	219849, 219850	Camp Nelson, Tulare Co.
10	11	*T. t. sierrae*	T.s.TUL	219846	Dear Creek, Tulare Co.
11	12	*T. t. sierrae*	T.s.KRN	219098, 219099	Mill Creek, Kern Co.
12	13	*T. t. torosa*	T.t.NOR	S10762, no MVZ No.	Near Highway 20, Lake Co.
13	13	*T. t. torosa*	T.t.NOR	216138	Point Reyes, Marin Co.
14	13	*T. t. torosa*	T.t.NOR	217871, 217872	Bear Creek, Contra Costa Co.
15	13	*T. t. torosa*	T.t.NOR	217918	Pleasanton, Alameda Co.
16	13	*T. t. torosa*	T.t.NOR	217878, 217881	Bear Gulch Creek, San Mateo Co.
17	14	*T. t. torosa*	T.t.MTY	217914	Hastings, Monterey Co.
18	15	*T. t. torosa*	T.t.SLO	213094, 213096	San Simeon Creek, San Luis Obispo Co.
19	16	*T. t. torosa*	T.t.LSA	219814, 219815	Clear Creek, Los Angeles Co.
20	17	*T. t. torosa*	T.t.ORA	219817, 219818	Trabuco Canyon, Orange Co.
21	18	*T. t. torosa*	T.t.SAD	219828, 219829	Cedar Creek, San Diego Co.
22	18	*T. t. torosa*	T.t.SAD	219825	Boulder Creek, San Diego Co.
23	19	*T. rivularis*	T.r.SON	217851	Big Sulphur Creek, Sonoma Co.
24	20	*T. granulosa*	T.g.MN1	2218997	Point Reyes, Marin Co.

[a] Same abbreviations are used in other tables, figures, and in the text. All collections are from California.

Single-stranded products were prepared by asymmetric PCR (Gyllensten and Erlich, 1988) with 1:50 primer ratios in 50-μl volumes and the same reaction profiles as above. The products were assessed by minigel electrophoresis using 5-μl aliquots and washed in sterilized distilled water with three cycles of dialysis using Millipore MC 30 (Amicon Corp.). Dideoxy chain termination sequencing (Sanger *et al.*, 1977) was performed using the US Biochemicals Sequence version 2.0 kit and ^{35}S-labeled dATP. We used negative controls in all PCR amplifications to make sure the sequences were not from contaminated sources.

DNA sequences were read and checked using the program ESEE (Cabot and Beckenbach, 1989). No insertions or deletions were detected. The sequence was aligned by comparison with the published *X. laevis* sequence (Roe *et al.*, 1985). DNA sequence divergence among populations and species was analyzed using the program Macsequence (D. A. Good, unpublished). Analysis of base pair substitutions was done using MacClade (version 3.0, Maddison and Maddison, 1992). Sequence divergences and transitions and transversions in first and third codon positions were plotted using maximum likelihood (ML) DNA distances to detect saturation of base pair substitutions.

Three major approaches to phylogenetic analyses were used: (1) the ML method using PHYLIP (version 3.4, Felsenstein, 1991) to determine the statistically most likely phylogeny under the estimated transition/transversion bias and rates of base pair substitutions at different codon positions; (2) the maximum parsimony (MP) method using PAUP (version 3.0s, Swofford, 1991) to generate the MP tree and a bootstrap analysis (Felsenstein, 1985) to estimate the statistical confidence of the MP tree; and (3) a neighbor-joining (NJ) distance tree-building method (Saitou and Nei, 1987) using PHYLIP.

Sequence divergences of different species of *Taricha* and ML DNA distances were calibrated using known fossils of *Taricha*. The estimated times of divergence and the known paleogeography of California were used to infer the historic biogeography of the California newt, under the assumption of a molecular clock.

RESULTS

Analysis of mtDNA Sequences

Of 386 bp flanked by the two primers, 375 bp are available for phylogenetic analyses. Among them, there are 47 variable sites in the sequences of *T. torosa* and 78 variable sites if the two outgroup populations of *T. granulosa* and *T. rivularis* are included. The 375-bp code for 125 amino acids is presented in Table 2. Total sequence divergence, DNA transitions, and transversions were calculated as percentage of pair-

TABLE 2

375 bp of Mitochondrial DNA Cytochrome b Gene Sequences of the *Taricha torosa* Complex[a]

Abbrev.	Genbank Accession No.	H	P	L	M	E	I	I	N	N	S	F	I	D	L	P	T	P	S	N	I	S	Y	W	W	N	P	G	S	L	29
1.T.s.SH1	L22697	CAC	CCA	CTC	ATA	AAA	ATC	ACT	AAC	AAC	TCA	TTT	ATT	GAC	CTC	CCC	ACA	CCA	TCA	AAT	ATC	TCC	TAT	TGA	TGA	AAC	TTC	GGC	TCT	CTG	87
2.T.s.SH2	L22698	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
3.T.s.BOT	L22699	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
4.T.s.ED1	L22700	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
5.T.s.ED2	L22701	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
6.T.s.CV1	L22702	NNN	NNN	NNN	NNN	NNN	NNN	...	...	...	...	...	...	...	...	..T	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
7.T.s.CV2	L22703	NNN	NNN	NNN	NNN	NNN	NNN	...	...	...	...	...	...	...	...	..T	..G	...	...	...	...	...	...	...	...	...	...	...	..C	...	
8.T.s.MP1	L22704	NNN	NNN	NNN	NNN	NNN	N..	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	
9.T.s.MP2	L22978	NNN	NNN	NNN	NNN	NNN	N..	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	
10.T.s.BRS	L22705	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	
11.T.s.TUL	L22706	NNN	NNN	NNN	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
12.T.s.KRN	L22707	NNN	NNN	NNN	NNN	NNN	N..	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
13.T.t.NOR'	L22708	NN.	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
14.T.t.MTY	L22709	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NN.	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	..C	
15.T.t.SLO	L22710	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
16.T.t.LSA	L22711	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
17.T.t.OBA	L22870	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
18.T.t.SAD	L22712	NNN	NNN	NNN	NNN	NNN	N..	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	
19.T.r.SON	L22713	...	...	..T	...	...	...	...	...	...	...	..C	...	...	...	..A	...	..G	...	...	...	...	..C	...	...	...	...	..A	..C	..T	
20.T.g.MNI	L22714	...	...	..T	...	...	..T	..C	..T	...	...	...	...	...	...	..A	..G	...	..G	...	...	...	...	...	...	...	...	...	..C	..C	

	L	G	I	C	L	I	T	Q	I	L	T	G	L	F	L	A	M	H	Y	T	A	D	T	Q	S	A	F	S	S	V	A	H	61
1.	CTG	GGA	ATC	TGC	CTG	ATC	ACA	CAG	ATC	CTC	ACA	GGC	CTA	TTC	CTA	GCT	ATA	CAC	TAC	ACA	GCA	GAC	ACC	CAA	TCA	GCA	TTC	TCA	TCA	GTA	GCT	CAC	183
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5.	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	..C	...	...	
6.	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
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14.	..A	...	G..	...	..C	...	...	...	...	..T	...	...	..C	..T	..G	...	...	...	...	...	...	...	...	..G	...	...	..T	...	...	...	..C	...	
15.	..A	...	G..	...	..C	...	...	...	...	..T	...	...	...	..T	..G	...	...	...	...	...	...	...	...	..G	...	...	..T	...	...	...	..C	...	
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	I	C	R	D	V	N	Y	G	W	L	V	R	N	I	H	A	N	G	A	S	L	F	F	I	C	I	Y	L	H	I	G	R	93
1.	ATC	TGT	CGA	GAT	GTA	AAC	TAT	GGC	TGA	CTA	GTA	CGA	AAC	ATC	CAC	GCC	AAC	GGG	GCC	TCA	CTA	TTC	TTT	ATC	TGC	ATC	TAC	CTG	CAC	ATT	GGA	CGC	279
2.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
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6.	...	...	...	..C	..T	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	
7.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	
8.	...	...	...	..C	..T	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
9.	...	...	...	..C	..T	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
10.	...	...	...	..C	..T	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
11.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	..A	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	..G	...	
12.	...	..C	...	...	...	...	..C	...	...	...	..G	..C	...	...	...	..A	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	...	...	
13.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	..G	...	
14.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	..G	...	
15.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	..G	...	
16.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	..A	...	..C	..G	...	
17.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	...	...	..C	...	..T	
18.	...	..C	...	...	...	...	..C	...	...	...	..G	...	...	...	...	...	...	...	...	T..	..T	...	...	...	...	...	...	...	...	...	..C	...	..T	
19.	..T	..C	...	...	...	...	..C	...	...	...	...	..G	..T	..T	...	...	...	...	...	...	...	...	...	..C	...	...	...	..T	..A	...	..C	...	..T	
20.	...	..C	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	..C	..C	..T	..C	...	...	...	...	...	...	...	..C	...	..T	

Table 2—*Continued*

	G	L	Y	Y	G	S	T	M	F	K	E	T	W	N	I	G	V	I	L	L	F	L	V	M	A	T	A	F	V	G	P	P	125
1.	GGC	TTA	TAC	TAC	GGC	TCT	TAC	ATA	TTC	AAA	GAG	ACC	TGA	AAC	ATT	GGC	GTC	ATC	CTG	CTC	TTC	TTA	GTA	ATG	GCC	ACC	GCC	TTT	GCA	GGG	TAN	NNN	375
2.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	NNN	NNN	NNN	NNN	NNN	
3.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	G	NNN	NNN	
4.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	NNN	NNN	NNN	NNN	NNN	
5.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	NNN	NNN	NNN	
6.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	
7.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	NN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	V	
8.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	T	A	.	.	.	.	.	T	.	T	GTC
9.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	C	T	A	.	.	.	.	.	.	NNN	NNN	NNN
10.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	T	A	.	.	.	NNN	NNN	NNN	NNN	NNN	NNN
11.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	NNN
12.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	C	.	.	.	.	.	.	.	G	.	.	NNN
13.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	C	.	.	.	.	.	.	.	G	.	.	NNN
14.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	C,G	.	.	A	.	.	.	NNN	NNN	NNN	NNN	NNN
15.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	C	T	.	.	.	C	C	.	.	.	.	.	.	.	N	NNN	NNN	NNN
16.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	C	.	.	.	.	.	.	.	G	.	.	N	NNN
17.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	T	.	.	.	C	C	.	.	.	.	.	.	.	G	.	.	C	GTC
18.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	T	.	.	.	C	C	.	.	.	.	.	.	.	G	.	.	C	GNC
19.	.	.	.	.	A	C	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	C	.	.	A	.	.	.	.	N	NNN	NNN	NNN
20.	.	T	.	.	T	.	C	.	.	G	.	A	.	.	.	.	T	.	T	T,A	.	C	G	.	A	.	.	.	T	.	C	G	.	C	GTC

[a] Same population abbreviations as in Table 1 are used. Amino acid abbreviations are IUPAC standards. "." is the identical sequence as above, for both base pairs and amino acids. "N" denotes uncertain nucleotides; "?" denotes uncertain amino acids; variable amino acids are in boldface.

[b] Individuals from different populations (Fig. 1: 12–16) of the northern *T. t. torosa* race (Coates, 1967) have identical sequence.

wise sequence differences. The distribution of transitions and transversions over all sites shows that transitions were more abundant than transversions and that transversion changes were not evenly distributed among sites. There were more transversions at sites 90–105 than at other sites, where the only amino acid substitution in *Taricha* occurred (Table 2). Among transitions, changes between T and C predominate; among transversions, there are more changes between A and C. The sequence divergence between the two currently recognized subspecies of *T. torosa* (excluding populations 9–11 from the southern Sierra Nevada, which has a *torosa*-like sequence) is about 7–9% (populations 9–11 diverged from 1–8 by 7.2–9.4%), but is greater with reference to both outgroups (*T. rivularis,* 9.7–11.5%; *T. granulosa,* 10.8–12.9%). Only 0.6–2.5% sequence divergence was measured between populations 9–11 and *torosa* populations. Sequence divergence between the populations of *T. granulosa* and *T. rivularis* is 10.7%.

The mtDNA sequences of the *T. torosa* complex have approximately four times as many transitions as transversions and 10 and 100 times more changes in the first and third codon positions compared with the most conserved second position. These biases are used to formulate different weightings for calculating ML DNA distances and in building the ML and parsimony trees. The plot of ML DNA distances over DNA divergences, transitions, and transversions is used to evaluate the degree of sequence saturation. If saturation occurred, the rates of DNA substitutions would not be linear with the increase of ML DNA distances, but would show a decrease at high ML distances. The degree of codon saturation is used to estimate the reliability of using these sequences to infer phylogeny. Our results suggest that even for third position transitions, saturation is not a problem for the sequence and taxa studied (Fig. 2).

Hillis (1991) proposed using the g_1 statistic (Sokal and Rohlf, 1981; a measure of skewness of tree distribution) for determining how much phylogenetic information is contained in sequence data. The g_1 statistic for 10,000 random trees in 10 repeats has an average of -0.449 with a standard deviation of 0.02. Hillis and Huelsenbeck (1992) suggested that the data contain useful phylogenetic information ($P < 0.01$) for 15–25 taxa and 50 variable characters if $g_1 = -0.24$ or lower. The g_1 statistic for sequence data of the *T. torosa* complex is much lower than this value, and these sequences may well contain useful phylogenetic information.

Base Pair and Amino Acid Variation

We sequenced two individuals for most populations (Table 1). Most individuals from the same population have identical DNA sequences, such as all coastal *torosa* populations north of Monterey Bay (12–16). Intrapopulational variation is found in *sierrae* in the central Sierra Nevada (Table 2). Although some of these intrapopulational differences are unique, others are shared with neighboring populations. Furthermore, most of

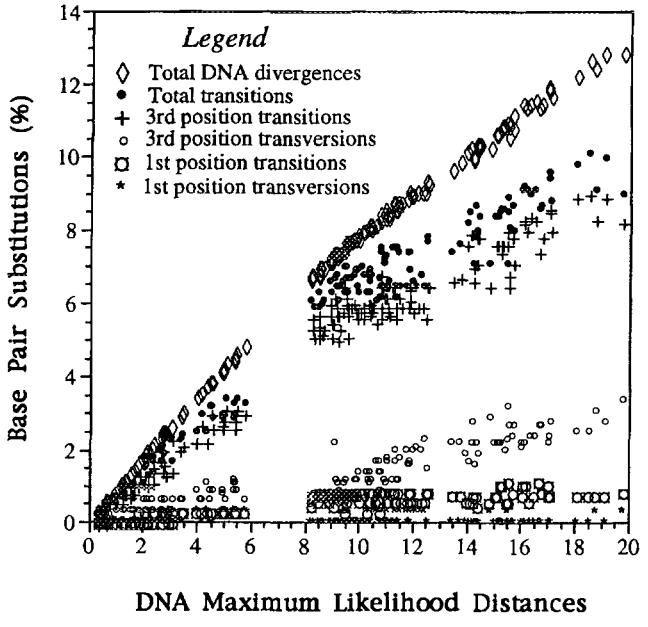

FIG. 2. Plot of sequence divergences, transitions, and tranversions over maximum likelihood (ML) DNA distances. Most sequence divergences are from transitions, especially third position transitions, and these changes are not saturated in the *T. torosa* complex.

these variations occur in basal populations, as inferred from phylogenetic analyses of mtDNA sequences. Since intrapopulational variation is generally much smaller than interpopulational variation, one individual per population is adequate for the level of analysis in this study.

One amino acid substitution, at position 32 of the 125 amino acids translated from the mtDNA sequence, diagnoses the *sierrae* populations 1–8 and *torosa* from Orange County (20) as isoleucine, the *sierrae* populations 9–11 as alanine, and all other *torosa* populations and the outgroups *T. rivularis* and *T. granulosa* (12–24, excluding 20) as valine.

The MP Trees and Bootstrap Analyses

Using the branch and bound option in PAUP, we performed the following analyses: (a) Transversions were weighted 4 times transitions, and the first and second codon positions 3 and 10 times the third position (as calculated from the data set). Twenty equally most parsimonious trees, each with 215 steps, were found. The majority rule consensus tree is shown in Fig. 3B. (b) Transversions were weighted 4 times transitions, and all codon positions were weighted equally. The same 20 MP trees (with 182 steps) and consensus tree as in Fig. 3B were obtained. (c) If all characters were treated as unordered and weighted equally, 64 equally parsimonious trees were obtained, with 116 steps, a consistency index (CI) of 0.759, and a retention index (RI) of 0.882. The CI and RI were not calculated for other parsimony analyses because of the use of step matrices. Among the 64 trees, there are 10 trees with topology identical to Fig. 3B. Others resolved *sierrae* populations 7 and 8 (Fig. 1) more basal or were unresolved.

Bootstrap analyses were done under two conditions: (a) transversions were weighted 4 times transitions, with equal weight for each codon position (Fig. 3B); and (b) transversions were weighted 4 times transitions, but the first and second codon positions were weighted 3 and 10 times the third position. The same bootstrap tree resulted, with only slightly different bootstrap values. The two mtDNA lineages in *T. torosa* were strongly supported (99%), but there was less support for groupings within each lineage (< 97%).

The ML Tree and the NJ Distance Tree

The ML tree under the estimated transition and codon biases as described above is similar to the NJ tree using ML DNA distances (Table 3; Fig. 3A). The major difference between them is that in the ML tree, *sierrae* populations 1–8 are ordered in such a way that the northernmost populations are more basal, while the NJ tree groups populations 1–4 together as a sister group to populations 5–8 (Fig. 1).

DISCUSSION

Phylogenetic Implications of mtDNA Variation

The mtDNA sequences studied display the general characteristics noted in other vertebrates (Wilson *et al.*, 1985; Moritz *et al.*, 1992). The cyt b gene shows different rates of base substitution at different codon positions, with transitions being more common than transversions. However, saturation is not a problem in the *T. torosa* complex, probably because the recency of divergence, and phylogenetic trees derived from MP, ML, and NJ are highly congruent with minor discrepances, as represented by the NJ tree (Fig. 3A).

Even though the population genetic structure within each mtDNA lineage is less well supported (<97%) by bootstrap analysis, five mtDNA clusters appear to be supported from the NJ tree. The term cluster is defined here as one or a group of populations that meet the following three criteria. First, they are either monophyletic or paraphyletic, but not polyphyletic. Second, they have low genetic divergence within each cluster compared to differences between clusters. Third, they are geographically continuous and have the capacity for gene flow within them.

Within the currently recognized *torosa* there are two clusters: southern populations from San Diego to Orange counties (Fig. 1, 20–22; Table 2, sequences 17–18) and more northern populations (12–19; sequences 13–16). There are three clusters in *sierrae:* northern populations from Shasta to Butte counties (1–4; sequences 1–3; paraphyletic in ML trees), central popula-

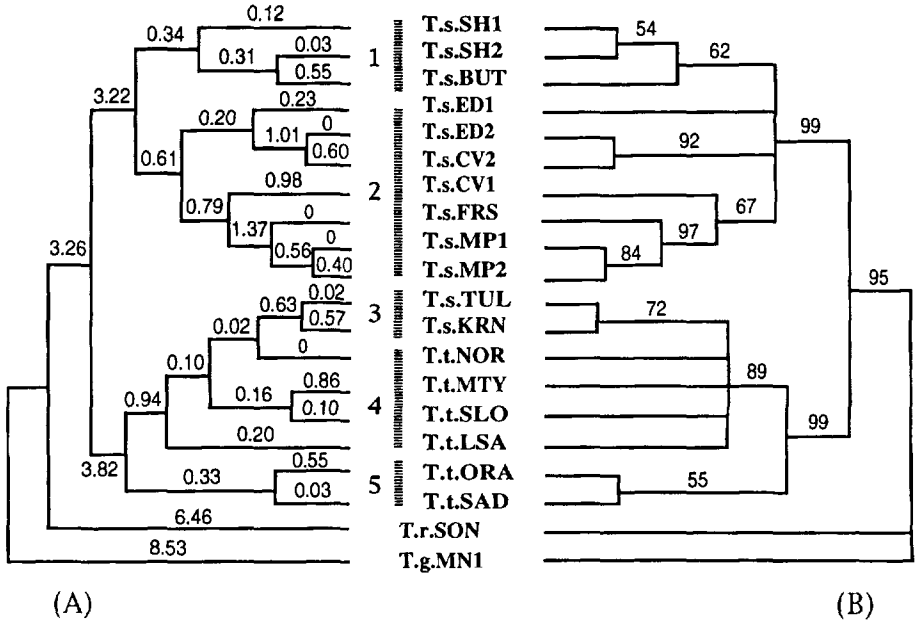

FIG. 3. The neighbor-joining (NJ) tree (A) and bootstrap tree (B). Tree A is constructed using ML DNA distances with PHYLIP. Tree B is constructed by weighting transversions 4 times transitions, and each codon position equally using PAUP. The majority-rule consensus tree of the MP trees discussed in the text is identical to the tree B. Population abbreviations are defined in Table 1. The five groups revealed from the NJ tree are: (1) the northern *sierrae* cluster; (2) the central *sierrae* cluster; (3) the southern *sierrae* cluster; (4) the northern *sierrae* cluster; and (5) the southern *torosa* cluster.

tions from El Dorado to Fresno counties (5–8; sequences 4–10), and southern populations from Tulare to Kern counties (9–11; sequences 11–12), which apparently derived from a coastal *torosa*-like progenitor.

The general linear fashion of base pair substitution as a function of increasing sequence divergence, both for transitions and for transversions, suggests that the mtDNA sequences are evolving as a function of divergence times in the *T. torosa* complex (Fig. 2). Therefore we used the assumption of a molecular clock to hypothesize the biogeographic history of the *T. torosa* species complex. The long branch lengths of the two major *T. torosa* mtDNA lineages and much shorter branch length within each lineage suggest a pattern of early separation of the lineages and more recent differentiation of populations and clusters in each lineage.

The salamandrids in North America have a relatively good fossil record. The earliest fossil traces back to Upper Oligocene times, about 25 million years ago (MYA; van Frank, 1955). Fossil evidence also suggests that by middle Miocene, *Notophthalmus* was well differentiated morphologically and biogeographically from its sister taxon *Taricha* (Estes, 1981). About 18% sequence divergence is observed between *Taricha* and *Notophthalmus* (Tan, 1993). Assuming a constant molecular clock, we estimate about 0.7% sequence divergence per million years. Assuming a level of 10% codon saturation between *Taricha* and *Notophthalmus*, approximately 0.8% sequence divergence per million years is estimated. This estimate is similar to the calibration by Spolsky *et al.* (1992). There is an average of 0.26 mtDNA ML distance between these two genera. Codon saturations and transition biases were taken into consideration for this distance. Assuming that 0.26 ML mtDNA distance corresponds to an intergeneric divergence of about 25 million years, a rate of about 0.01 ML mtDNA distance per million years is estimated. Using these calibrations, the species of *Taricha* are estimated to have diverged 15 MYA, and *torosa* and *sierrae* (excluding the southern *sierrae*) to have diverged 9 MYA (Table 3). The divergent times between clusters are smaller, with a range from 2 to 5 MYA.

Intraspecific Analysis of mtDNA Sequence Variation

The recognition of five clusters of populations, each with a definable geographic distribution, suggests that historic vicariances have occurred in the *T. torosa* species complex. Our phylogenetic analyses demonstrated that the southern *sierrae* cluster has a mtDNA lineage derived from the coastal *torosa* (there is only 0.6–2.5% sequence divergence between them compared to 7.2–9.4% between southern and central/northern *sierrae*) and that the deepest divergence in the *T. torosa* complex occurred within what is currently recognized as *sierrae* in the Sierra Nevada (Table 3).

The southern and central populations of *torosa* display more differentiation than the northern populations. All northern populations studied (12–16) have

TABLE 3

Maximum Likelihood DNA Distances (below Diagonal), Percentage of Sequence Differences (below Bars) and Third Position Transitions (above Bars) of the *T. torosa* Complex[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	—	0.3	1.1	1.1	2.5	2.4	2.4	2.8	2.9	2.8	5.2	5.1	5.2	5.7	5.2	5.4	5.7	5.4	6.8	8.6
		/0.3	/1.1	/1.1	/2.5	/2.7	/2.7	/4.2	/4.6	/3.6	/7.2	/7.3	/6.8	/7.9	/6.8	/7.0	/6.7	/6.8	/9.9	/11.9
2	2.90	—	0.6	0.8	1.9	2.1	2.1	3.2	3.2	3.1	5.4	5.2	5.3	6.0	5.6	5.6	5.8	5.5	7.2	8.1
			/0.6	/0.8	/1.9	/2.4	/2.4	/4.4	/4.7	/3.9	/7.4	/7.6	/7.0	/8.2	/7.2	/7.2	/6.9	/7.0	/10.3	/11.4
3	1.16	0.58	—	0.8	1.9	2.1	2.1	3.1	3.1	3.1	5.9	5.7	5.8	6.6	6.0	6.0	6.3	6.0	7.1	7.7
				/0.8	/1.9	/2.4	/2.4	/4.6	/4.9	/3.9	/7.8	/8.0	/7.4	/8.8	/7.7	/7.7	/7.4	/7.4	/10.1	/10.9
4	1.18	0.88	0.88	—	1.1	1.2	1.2	2.3	2.3	2.2	6.0	5.8	5.9	6.6	6.1	6.1	6.1	5.8	6.7	7.5
					/1.1	/1.5	/1.5	/3.5	/3.8	/3.1	/8.0	/8.1	/7.5	/8.8	/7.8	/7.8	/7.2	/7.3	/9.7	/10.8
5	2.67	2.09	2.05	1.18	—	1.2	0	2.9	2.9	2.8	5.9	6.3	5.8	6.6	6.0	6.0	6.6	6.3	7.7	7.7
						/1.5	/0.3	/4.3	/4.6	/3.6	/7.8	/8.6	/7.4	/8.8	/7.7	/7.7	/7.7	/7.7	/10.7	/10.9
6	3.00	2.65	2.65	1.63	1.62	—	1.2	1.5	1.5	1.5	6.3	6.6	6.0	6.5	6.3	6.0	6.9	6.6	7.9	6.9
							/1.8	/2.4	/2.7	/2.1	/8.7	/9.3	/8.1	/9.0	/8.4	/8.1	/8.4	/8.4	/10.8	/11.4
7	3.00	2.64	2.65	1.62	0.32	1.97	—	2.7	2.7	2.7	6.3	6.6	6.0	6.6	6.3	6.0	6.9	6.6	7.9	6.9
								/4.2	/4.5	/3.9	/8.8	/9.4	/8.2	/9.1	/8.5	/8.2	/8.5	/8.5	/11.5	/10.9
8	4.87	5.00	5.31	3.93	4.92	2.66	4.89	—	0	0	6.2	5.9	5.6	6.0	6.0	5.9	6.1	5.9	6.6	8.4
									/0.3	/0.3	/9.0	/9.0	/8.2	/8.8	/8.3	/8.5	/8.1	/8.1	/10.3	/12.5
9	5.34	5.40	5.71	4.31	5.32	3.03	5.31	0.30	—	0.6	0	5.7	5.7	6.0	6.0	6.0	6.0	5.7	6.6	8.3
										/0.6	/9.1	/9.1	/8.6	/9.1	/8.6	/8.9	/8.3	/8.3	/10.6	/12.9
10	4.10	4.43	4.44	3.42	4.09	2.31	4.51	0.30	0.62	—	6.0	5.8	5.9	6.0	6.1	6.1	6.1	5.8	6.7	8.1
											/8.3	/8.4	/7.8	/8.5	/8.1	/8.1	/7.5	/7.6	/10.0	/11.7
11	8.90	9.22	9.84	10.06	9.79	11.40	11.37	11.83	12.26	10.71	—	0.3	0.3	0.9	0.6	0.6	1.7	1.4	8.1	9.1
												/0.6	/0.6	/1.5	/0.8	/0.8	/2.5	/2.0	/11.0	/12.7
12	9.14	9.48	10.11	10.35	10.92	12.50	12.46	11.93	12.38	11.03	0.59	—	0.6	1.2	0.9	0.8	1.4	1.1	7.7	9.0
													/1.1	/2.1	/1.4	/1.4	/2.5	/2.0	/10.9	/12.9
13	8.25	8.54	9.13	9.33	9.09	10.42	10.39	10.57	11.28	9.94	0.58	1.20	—	0.6	0.3	0.3	1.4	1.1	7.7	8.7
														/0.9	/0.3	/0.3	/1.9	/1.4	/10.2	/12.0
14	9.87	10.29	11.24	11.20	11.18	11.83	11.79	11.48	12.08	10.98	1.62	2.31	0.96	—	0.9	0.6	2.1	1.8	8.5	7.9
															/1.2	/0.9	/3.0	/2.4	/11.5	/11.2
15	8.44	8.93	9.64	9.73	9.59	10.89	10.86	10.75	11.30	10.35	0.90	1.54	0.29	1.28	—	0.5	1.1	0.9	7.9	9.0
																/0.5	/1.6	/1.1	/10.4	/12.3
16	8.62	8.92	9.51	9.72	9.47	10.42	10.39	11.00	11.73	10.34	0.88	1.51	0.28	0.96	0.58	—	1.6	1.4	7.9	8.4
																	/2.2	/1.7	/10.4	/11.5
17	8.16	8.44	9.03	8.84	9.39	10.76	10.73	10.25	10.71	9.42	2.71	2.77	2.04	3.35	1.78	2.34	—	0.3	7.9	8.3
																		/0.6	/10.4	/11.5
18	8.16	8.45	9.07	8.86	9.44	10.78	10.75	10.36	10.73	9.47	2.11	2.13	1.50	2.64	1.22	1.81	0.58	—	8.0	8.4
																			/10.3	/11.5
19	13.68	14.32	14.17	13.39	15.07	15.66	16.71	14.79	15.47	14.17	15.42	15.45	13.93	16.26	14.38	14.36	14.27	14.04	—	7.7
																				/10.7
20	16.97	16.13	15.26	15.17	15.21	16.62	15.48	18.69	19.72	17.04	18.47	19.04	16.97	15.63	18.00	16.45	16.01	16.00	14.99	—

[a] The same series numbers as in Table 2 are used. Note that they are different from the population numbers in Fig. 1. All maximum likelihood DNA distances are multiplied by 100.

identical sequences. No sequence variation was found in different populations from the southernmost range—the San Diego County (21–22). However, each of the other central and southern populations (17–20) has unique base pair substitutions. The populations in San Diego and Orange (20) counties share more sequences with the outgroup, *T. granulosa,* than do other populations of *torosa* and *sierrae.* However, there is considerable mtDNA variation in the populations from Orange and San Diego counties, as shown in low bootstrap value (55%, Fig. 3B) and different branch lengths (Fig. 3A). The Orange County population has an amino acid substitution that is not found in the San Diego and other *torosa* populations, but is shared with northern and central *sierrae* populations. The greater differentiation of the southern than of the northern *torosa* suggests a longer independent history. This interpretation is consistent with the phylogenetic trees that place the southern *torosa* basal to both the northern *torosa* and the southern *sierrae* clusters.

There are three populations in the central *sierrae* cluster (with the highest intracluster divergence—up to 4.9%) in which the two individuals sequenced have different haploid DNA sequences. There is 2.4% divergence within the population of *sierrae* from Calaveras County (6), 1.5% within the population from El Dorado County (5), and 0.3% within that from Mariposa County (7). One individual in the El Dorado County population clusters with one individual from the more southern Calaveras population, while the other Calaveras individual is basal to the southernmost central *sierrae* populations. We hypothesize that the direction of dispersal within *sierrae* is suggested by the phylogeny as summarized in Figs. 3 and 4. We interpret the pattern to indicate that in the central *sierrae* cluster, dispersal is from north to south,

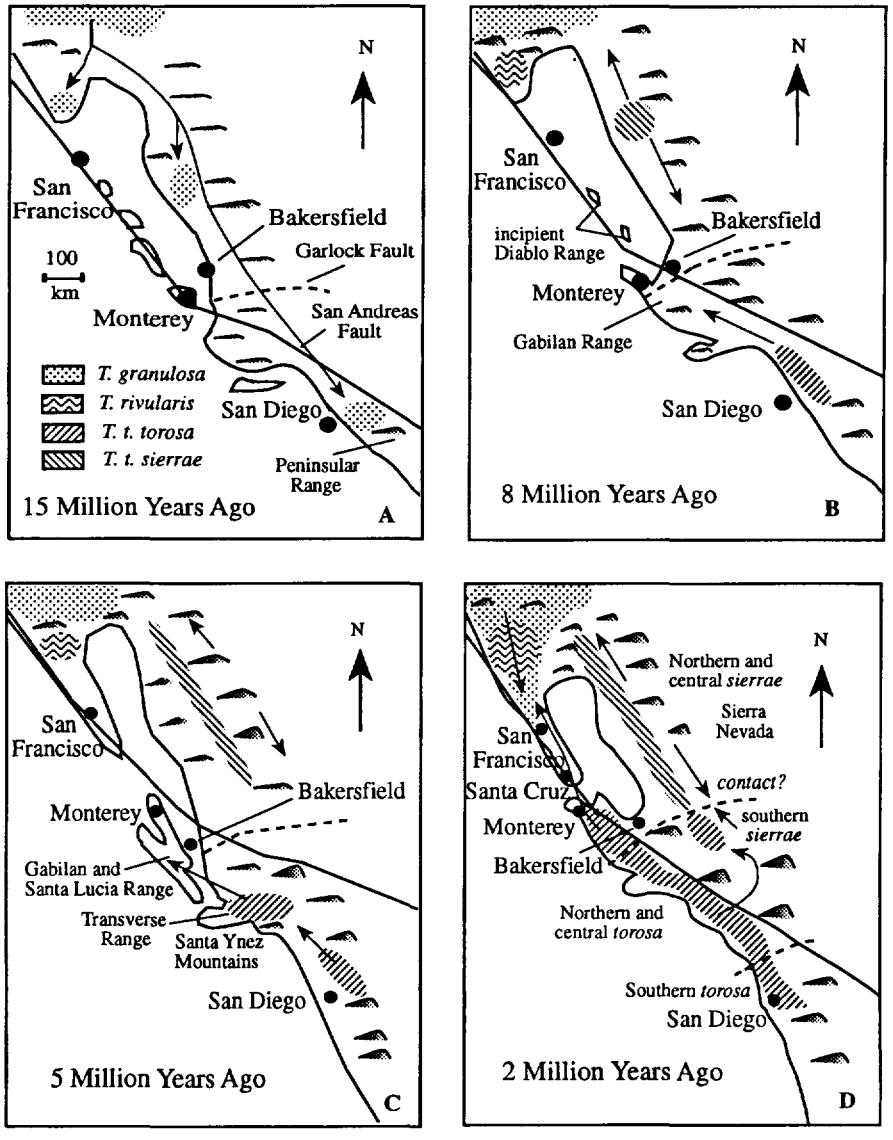

FIG. 4. Biogeographic history of the California newt, *Taricha torosa*, inferred from mtDNA sequences. Paleogeographic maps are modified from Yanev (1980).

and in the northern *sierrae* cluster, from south to north.

There are paradoxes with respect to the distribution, the pattern of morphology, and the mtDNA sequence variation in the *T. torosa* complex. The external morphology, especially the color pattern, of the southern *sierrae* is more similar to that of populations to the north than to that of coastal *torosa*. Riemer (1958) failed to recognize the southern *sierrae* as distinct. There is no evident geographic gap within *sierrae* as currently recognized, and we have not yet found a contact zone between the two forms of *sierrae*. However, the southern *sierrae* is geographically disjunct relative to coastal *torosa*. Within the subspecies *torosa*, the Orange County population (20) has an amino acid substitution that differentiates it from other *torosa* mtDNA lineages. The Orange County population is more similar in morphology to northern and central populations of *torosa* than to the San Diego *torosa* or southern *sierrae* populations. However, allozyme variation clusters the Orange County population with the northern and central *torosa* populations, while the San Diego *torosa* and southern *sierrae* populations are in separate clusters (Tan, 1993). The northern *sierrae* cluster has a derived chromosomal NOR type, which tends to be more derived northward. In *granulosa*, the northernmost Alaska and the southern range populations in central coastal California have different derived NOR types. The northern *torosa* and southern *sierrae* populations have a plesiomorphic NOR type, shared with the out-

group species *rivularis,* while the southern *torosa* populations have a derived NOR type (Tan, 1994). The distribution of NOR patterns in *Taricha* supported our interpretation of the mtDNA variation that the northern *sierrae* and Alaska *granulosa* (Tan and Wake, submitted) populations are more recently derived. However, a simple explanation that NOR types are all geographically recently derived is not feasible.

Biogeographic Implications of Variation in mtDNA Sequences

The common ancestor of *Taricha* and *Notophthalmus* may have invaded the New World from the Old World via the Bering Strait some 25–30 MYA (van Frank, 1955; Wake and Özeti, 1969). Fossil evidence suggests that by middle Miocene, *Notophthalmus* was well differentiated from *Taricha* (Estes, 1981). A vicariance event subsequently isolated *Notophthalmus* to the east, and it remains completely isolated from *Taricha* by the arid and mountain formations of central North America. *T. granulosa* has the largest number of plesiomorphic traits in extant species of *Taricha* (Twitty, 1942; Tan, 1993). Molecular dating suggests that the common ancestral stock of *rivularis* and *torosa* diverged from a *granulosa*-like ancestor about 15 MYA. This common ancestor dispersed southward along the rising Sierra Nevada. The basal *torosa* lineage in San Diego and Orange counties may be a remnant of the basal stock that reached its southernmost limit. This southern origin of *torosa* was followed by northward invasions, which resulted in two mtDNA clusters: (1) the northern and central *torosa* and (2) the southern *sierrae.* The northern and central *sierrae* mtDNA lineage possibly originated as a remnant of the original southward dispersal in the central Sierra Nevada (present-day El Dorado and Calaveras counties, where the highest intrapopulational variation is detected; these populations are basal in the phylogenetic trees), and later dispersal occurred to the north and south, resulting in the central and northern *sierrae* clusters. The central *sierrae* populations expanded southward and may contact the southern *sierrae* cluster somewhere in present-day southern Fresno and northern Tulare counties.

Coastal and inland Sierran populations are known for several species of salamanders, but in each case a somewhat different biogeographic scenario has been postulated. In the plethodontid *Ensatina* the blotched subspecies occur in the Sierra Nevada and in southern mountains as far as San Diego County, while unblotched subspecies range along the coast from San Diego County into Canada (Stebbins, 1949; Jackman and Wake, 1994). The history of the blotched form is similar to that postulated for the southward invasion of the *T. granulosa*-like ancestor that gave rise to both *sierrae* and *torosa.* In the plethodontid *Batrachoseps* the southern Sierran range is an area of extensive differentiation and speciation (Brame and Murray, 1968) and the distribution of *B. nigriventris* along the southern coastal region and through the transverse ranges into the southern Sierra Nevada (Yanev, 1980) is reminiscent of the invasion of *torosa* into the region to give rise to southern *sierrae.* In her treatment of the evolutionary history of the salamander genus *Batrachoseps,* Yanev (1980) discussed the paleogeography of California in detail. We modified her paleogeographic maps and superimposed our interpretation of the biogeographic history of the *T. torosa* complex on them (Fig. 4).

Our biogeographic scenario of the *T. torosa* complex agrees with earlier hypotheses that *granulosa* represents the most basal divergence in *Taricha* (Twitty, 1942; Riemer, 1958; Coates, 1967; Hedgecock, 1974; Tan, 1993), agrees with Hedgecock (1974) that *rivularis* diverged earlier than *torosa* and *sierrae,* and agrees with Riemer (1958) and Coates (1967) that *torosa* had a southern origin. This scenario postulates that about 15 MYA (in middle Miocene times), a *granulosa*-like common ancestor invaded along the Sierra Nevada from the north to the south, to the present-day San Diego area (Fig. 4A). *T. t. torosa* arose in the southern coastal zone of the San Diego area and *T. t. sierrae* in the central Sierra Nevada area (about 8 MYA) (Fig. 4B). Later (about 5 MYA), the San Diego area *torosa* populations invaded north, as far as the present-day Monterey area. Ancestral *sierrae* populations in the central Sierra Nevada invaded both northward and southward (Fig. 4C). About 2 MYA (Fig. 4D), *torosa* invaded the Sierra Nevada from the south into present-day Kern and Tulare counties and differentiated morphologically. More recently, when the central California inland sea subsided and the Monterey area became connected to the north, *torosa* invaded northward to its present distribution. This scenario envisions the present sympatry of *torosa* with *granulosa* and *rivularis* in central and northern California, and of *sierrae* with *granulosa* in northern California, as secondary and relatively recent. We predict that a secondary contact will be found in the southern Sierra Nevada between the central *sierrae* and what are currently known as the southern *sierrae.*

CONCLUSIONS

The phylogenetic and biogeographic history of the *Taricha torosa* complex is characterized by geographic fragmentation as well as both southward and northward dispersals. The following clusters are supported from mtDNA sequence studies:

(1). *T. t. sierrae* in northern Sierra Nevada (from Shasta to Nevada counties);

(2). *T. t. sierrae* in central Sierra Nevada (from El Dorado to Fresno counties);

(3). *T. t. sierrae* (independently derived relative to 1 and 2, above) in southern Sierra Nevada (from Tulare to Kern counties);

(4). *T. t. torosa* in southern coastal California (from San Diego and Orange counties);

(5). *T. t. torosa* in central coastal California (from Los Angeles north to central and northern California).

If our scenario is correct, the currently, but inappropriately, recognized taxon *sierrae* is diphyletic. This possibility will be evaluated in detail elsewhere.

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