Journal of Herpetology, Vol. 40, No. 4, pp. 561–565, 2006

Limited Genetic Variation across the Range of the Red-Bellied Newt, *Taricha rivularis*

SHAWN R. KUCHTA,[1] AND AN-MING TAN[2]

Museum of Vertebrate Zoology, 3101 Valley Life Science Building, Berkeley, California 94720-3160, USA

ABSTRACT.—Early studies of genetic variation in the Red-Bellied Newt, *Taricha rivularis*, revealed only small amounts of genetic differentiation among populations. However, these studies sampled only a limited portion of the range of the species. To address this gap in our understanding, we measured genetic variation in *T. rivularis* using allozymes (45 loci) and mitochondrial DNA (mtDNA) cytochrome *b* sequences (366 base pairs). With the goal of surveying broadly for levels of genetic variation, four populations were sampled, three in the southern portion of the range, and one at the northern end of the range. Allozyme divergence throughout *T. rivularis* was low, with a maximum Nei's genetic distance of $D_N = 0.039$. MtDNA haplotype diversity was similarly low, with only two haplotypes differing by a single base pair recovered among the four populations. Relative to other salamander species in western North America, *T. rivularis* shows weak genetic differentiation among populations.

Salamander species commonly harbor substantial amounts of genetic variation within and among populations (e.g., Good, 1989; Wake and Yanev, 1986). This pattern is correlated with the low mobility and limited dispersal distances typical of many species, especially fully terrestrial, direct developing plethodontids (Avise, 2000; Wake, 2006). Indeed, many cryptic species of salamander have been uncovered by genetic surveys (e.g., Good, 1989; Jockusch et al., 2001), and high levels of genetic structure make salamanders excellent model organisms for studies of regional biogeography (Jockusch and Wake, 2002; Carstens et al., 2005; Kuchta and Tan, 2006). However, it is not always the case that salamander species exhibit elevated levels of genetic structure, as highly mobile species and species that have undergone recent range expansions are frequently relatively weakly diverged (e.g., Highton and Webster, 1976; Kuchta and Tan, 2005).

Species of Pacific Newts, genus *Taricha*, were among the first to be surveyed for levels of genetic variation (Coates, 1967; Coates and Twitty, 1967; Salthe and Kitto, 1966). These studies were motivated by the elegant work on migration and homing behavior in *Taricha* carried out by Twitty and colleagues (summarized in Twitty, 1966), which showed that species of *Taricha* were capable of migrating great distances yet were extremely philopatric to breeding sites. The expectation was that this philopatry should result in relatively low levels of genetic exchange among populations and, thus, high levels of interpopulational genetic divergence (Hedgecock, 1978). Hedgecock and Ayala (1974) examined electrophoretic variation among populations of the Red-Bellied Newt, *T. rivularis* (Fig. 1). They recorded moderate levels of genetic differentiation among three tightly clustered populations at the southern limit of the range of *T. rivularis*, with a maximum Nei's (1972) genetic distance of $D_N = 0.053$. In a second study in the same geographic region (Fig. 1), Hedgecock (1978) examined 12 populations from two separate river drainages. Significant isolation by distance (Wright, 1943) was found within both drainages, although the maximum among-population D_N was only 0.023.

We surveyed genetic variation across the range of *T. rivularis* using allozymes and mtDNA sequences. One of our populations (1) is located within the study area included in Hedgecock and Ayala (1974) and Hedgecock (1978); the other three populations are outside of this region (Fig. 1). This study was designed as a preliminary survey of levels of genetic variation among populations across the range of the species and, hence, is not a statistical phylogeographic study (e.g., Kuchta and Tan, 2005, 2006).

MATERIALS AND METHODS

Allozyme Electrophoresis.—Thirty-four individuals of *T. rivularis* from four populations were analyzed for allozyme variation (Table 1; Fig. 1). Specimens were sacrificed in 25% chlorotone, and heart, liver, and

[1] Corresponding Author. Present address: University of California Santa Cruz, Department of Ecology and Evolutionary Biology, Earth and Marine Sciences Room A316, Santa Cruz, California 95064, USA; E-mail: skuchta@biology.ucsc.edu

[2] Present address: University of Hawaii at Manoa, Pacific Biomedical Research Program, 3050 Maile Way, Honolulu, Hawaii 96822, USA.

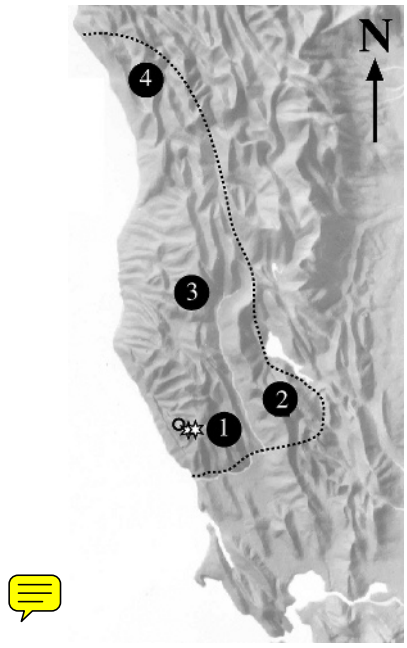

FIG. 1. Location of the Panaca Formation in Meadow Valley, southeastern Nevada.

intestine were removed and frozen at −70°C. Carcasses are stored as vouchers in the Museum of Vertebrate Zoology (MVZ), University of California at Berkeley. Thirty-four enzymatic products encoded by 45 loci were surveyed (enzyme and buffer systems are provided in table 2 of Kuchta and Tan, 2005), and standard methods of starch gel electrophoresis were employed (Murphy et al., 1996).

For each population, the percentage of polymorphic loci and the observed and expected levels of heterozygosity were calculated. Genetic distances among populations were calculated using Nei (1978) distances, which was designed for use with small samples of individuals.

Mitochondrial DNA (mtDNA) Sequence Variation.—MtDNA sequences were obtained from six individuals from three populations (Table 1). Haplotypes were amplified using the primers MVZ15 and Cytb2, and correspond to nucleotide positions 19 through 396 (from the 3′ end; Tan and Wake, 1995). Sequencing was done on manual gels, and the protocol is described in detail in Tan and Wake (1995). These sequences are short by contemporary standards but were reasonable at the time of collection (1991).

RESULTS

Allozyme Variation.—Of 45 allozyme loci examined, 9 were variable (Table 2). Population 1 (Sonoma County) was the most variable, with low frequency allozyme variants at three loci (G3PDH, PAP, PGD) that were not found in populations 2–4. However, population 1 had the best sampling (N = 14 individuals); thus, the probability of detecting low frequency allozymes is also the highest. A unique allozyme of moderate frequency (44%) was recorded in population 4 at the ESTD locus. Five loci (AAT1, ADA2, IDH1, MDH1, NADH1) have two to three allozymes segregating in multiple populations.

The percentage of polymorphic loci found within populations ranged from 4.4–17.8% (Table 2). These results are correlated with sample size, however. The population with the highest sample size (population 1; N = 14) had the highest level of polymorphism. In addition, this ''population'' combines individuals from the vicinity of Skaggs Springs (Table 1) and is not a point locality. The lowest estimate of polymorphism was from the population with the smallest sample (population 3; N = 3). Populations 2 (N = 9) and 4 (N = 8) had polymorphisms of 8.9% and 11.1%, respectively. Observed estimates of heterozygosity ranged from 4.4–6.8% (Table 2). As with polymorphism, these results are correlated with sample, with population 1 possessing the highest heterozygosity and population 3 the lowest. The observed levels of heterozygosity in populations 2 and 4 were 5.9% and 6.4%, respectively.

As a consequence of the low level of observed interpopulational variation, Nei's (1978) genetic distances among populations are low, ranging from D_N

TABLE 1. Locality information for *Taricha rivularis*. Population numbers correspond to Figure 1. Specimen identification numbers in **bold** designate specimens for which we have mtDNA sequence data and electrophoretic data; normal print designates specimens for which we have electrophoretic data only; *italics* designate specimens for which we have only mtDNA sequence data.

Pop.	County	Specific locality	Latitude/longitude	Sample size		Specimen identification number
				Allo.	mtDNA	
1	Sonoma	Stewart Point Skaggs Springs Rd., 10.1–22.6 miles east of Stewards Point, Skaggs Springs.	38.6761 N/123.2000 W 38.6931 N/123.0256 W	10 4	– –	MVZ 217829–217838 MVZ 161863–161866
2	Sonoma	Big Sulphur Creek, 13–13.7 miles east of U.S. Hwy. 101 on Geysers Road.	38.8043 N/122.8355 W	9 –	– 2	MVZ 217842–217850 *MVZ 217851–217852*
3	Mendocino	14.1 miles east of Flynn Creek Road on Orr Springs Road.	39.2429 N/123.4069 W	3	2	**MVZ 158853, 158854**, 158855
4	Humboldt	Eubank Creek Drainage, ~3 miles south of Ettersburg.	40.1070 N/123.9534 W	8	2	MVZ 219803–219810 *MVZ 219811–219812*

TABLE 2. Allozyme frequencies in *Taricha rivularis*. Population numbers correspond to Figure 1. The following loci were invariable: AAT2, ACON1, ACON2, ADA1, ADH1, ADH2, AK, ALDH, ALDO, CAH1, CAH2, CK, EST1, EST2, G6PD, GAPDH, GDA, GDH, GLUD, GPI, HADH, IDH2, LA1, LA2, LDH1, LDH2, LGG, ME, MDH2, MPI, NADH2, ODH, PGM, SOD, SORD, XDH.

Pop:	1	2	3	4
AAT1				
a	0.250	—	—	0.500
c	0.750	0.944	1.000	0.500
d	—	0.056	—	—
ADA2				
a	—	—	—	0.500
b	0.500	0.500	1.000	0.500
d	0.500	0.500	—	—
ESTD				
b	—	—	—	0.438
c	1.000	1.000	1.000	0.562
G3PHD				
a	0.071	—	—	—
b	0.929	1.000	1.000	1.000
IDH1				
a	0.214	0.722	0.500	0.125
b	0.786	0.278	0.500	0.875
MDH1				
b	0.286	—	1.000	—
c	0.714	1.000	—	1.000
NADH1				
a	0.643	0.500	0.500	0.500
c	0.357	0.500	0.500	0.500
PAP				
b	0.786	1.000	1.000	1.000
c	0.214	—	—	—
PGD				
a	0.071	—	—	—
b	0.929	1.000	1.000	1.000
% loci polymorphic	17.8	8.9	4.4	11.1
Observed Heterozygosity (SE)	0.068 (0.030)	0.059 (0.033)	0.044 (0.031)	0.064 (0.033)
Expected Heterozygosity (SE)	0.062 (0.022)	0.035 (0.019)	0.027 (0.019)	0.052 (0.023)

= 0.009 between populations 1 and 2 to D_N = 0.039 between populations 3 and 4 (Table 3). The average D_N among populations is 0.021.

MtDNA Variation.—Sequence divergence is quite limited. Four individuals from populations 2 and 3 shared a single mtDNA haplotype, and two individuals from population 4 share another haplotype. The two haplotypes are one base pair different from each other (0.3% sequence divergence).

TABLE 3. Nei's (1978, below diagonal) and Rogers' (1972, above diagonal) genetic distances between population pairs of *Taricha rivularis*. Population numbers correspond to Figure 1.

Pop:	1	2	3	4
1	—	0.102	0.148	0.122
2	0.009	—	0.17	0.151
3	0.019	0.027	—	0.202
4	0.013	0.021	0.039	—

DISCUSSION

Taricha rivularis possesses low levels of interpopulational genetic variation. Among four populations, only nine of 45 allozyme loci were variable. Nei's (1978) genetic distance (D_N) between the northernmost population (4) and the southern-most populations (1, 2) was 0.013 and 0.021, respectively (Table 3). The maximum D_N was 0.039, between populations 3 and 4 (Table 3). These are low values for a California salamander (e.g., Wake and Yanev, 1986; Good, 1989; Jockusch and Wake, 2002), including other species of *Taricha* (Kuchta and Tan, 2005, 2006). It is unlikely that these low genetic distances are a product of sampling

error, because Nei (1978) has shown that, when large numbers of loci are examined (the current study used 45 loci), large genetic distances can be detected with very small samples (as few as one individual per population). When genetic distances are low, it is not clear that they can be accurately measured with small samples of individuals; however, it is clear that large versus small genetic distances can be reliably distinguished (Nei, 1978). In the current study, the sample size of population 3 is $N = 3$, and the other populations range from 8–14 (Table 1). The D_N between population 3 and the other populations is larger than the other estimates of genetic distance in this study, and this may be a result of the small sample size (Table 3).

Estimates of polymorphism and heterozygosity were correlated with sample size. The fraction of polymorphic loci within populations ranged from 4.4–17.7% (average across populations = 10.6%), and observed heterozygosity ranged from 4.4–6.8% (average = 5.9%). These values are lower than was found in earlier studies: Hedgecock and Ayala (1974) reported an average polymorphism of 34% and an average expected heterozygosity of 10.9%, and Hedgecock and Ayala (1978) reported an average polymorphism of 20.9% and an average heterozygosity of 6.4%. Additionally, Hedgecock an Ayala (1974) found a maximal D_N of 0.053, which, despite the limited sampling range, is larger than the largest D_N in the current study of 0.039; Hedgecock (1978) reported a maximal D_N of 0.023. The causes of these differences are probably related to sample size and the loci used in the study. The sample sizes in Hedgecock and Ayala (1974) and Hedgecock (1978) are all large, ranging from 16–40 (average = 35.0). In particular, large samples are more likely to discover low frequency allozymes and, thus, increase estimates of polymorphism. In addition, the allozymes used in the current study are relatively slowly evolving: 36 of 45 loci (80%) were monomorphic in the current study (Table 2), whereas 10 of 18 loci (56%) were monomorphic in Hedgecock and Ayala (1974), and 28 of 40 loci (70%) were monomorphic in Hedgecock (1978). The results of Hedgecock (1978) are more similar to the results of the current study than is Hedgecock and Ayala (1974), probably because the former study used a larger sample of loci. Finally, the loci and laboratory techniques used in the current study are quite similar to those used by Wake and Yanev (1986) in their study of the salamander *Ensatina eschscholtzii*; this is because the two studies were conducted in the same laboratory. Highton (1998; see also Wake and Schneider, 1998) has argued that the study of Wake and Yanev (1986) includes a number of slowly evolving loci and has resulted in relatively low estimates of genetic distance and other measures of diversity (despite the large amounts of genetic structure found in *E. eschscholtzii*), and this would also be true of the current study.

Similar to the allozyme data, mtDNA haplotype variation was also quite limited, with only two haplotypes differing by a single base pair detected. One possible explanation for this pattern is that the cytochrome *b* region used in the current study is evolving too slowly to recover phylogeographic structure. This explanation is unlikely, however, because the same fragment of cytochrome *b* was useful in documenting phylogeographic structure in *Taricha granulosa* (Kuchta and Tan, 2005) and *Taricha torosa* (Tan and Wake, 1995; Kuchta and Tan, in press). Thus, the lack of recovered variation is a result of either historical or demographic factors, such as high gene flow among populations or a recent range expansion.

In summary, contrary to the prediction of substantial interpopulational genetic structure as a consequence of high levels of philopatry to breeding sites (Twitty, 1966; Hedgecock, 1978), the current study indicates that genetic variation is quite limited across the range of *T. rivularis*. There is a sampling gap in the current study, because only one population was sampled in the northern end of the range (Fig. 1). Although it seems unlikely that there is substantial genetic variation in the region located between populations 3 and 4, or along the narrow coastal-to-inland axis, the current study cannot exclude this possibility. The lack of genetic differentiation between the southern populations (2–4) and the northern population (1) suggests that gene flow among populations of *T. rivularis* may be higher than expected; indeed, the demonstrated migratory capabilities of *T. rivularis* are considerable, up to a few kilometers (Twitty, 1966). Alternatively, although the distributional range of *T. rivularis* is restricted (Fig. 1), it may be that populations have recently expanded from a refugial source to occupy the current distribution and, therefore, are out of genetic equilibrium. For example, the effects of range expansion strongly influenced patterns of genetic variation in the Rough-Skinned Newt, *T. granulosa* (Kuchta and Tan, 2005), and the Coast Range populations of the California Newt, *T. torosa* (Kuchta and Tan, 2006). Distinguishing between high gene flow and range expansion as a cause of reduced variation in *T. rivularis* will require dense population sampling and statistical phylogeographic methods (Avise, 2000; Kuchta and Tan, 2005, 2006).

Acknowledgments.—SRK is supported by National Science Foundation grants DEB 0317182 to B. Sinervo and SRK and DEB 0072869 to D. B. Wake and SRK. The following people helped in the field: J. Cadle, E. Jockusch, J. Lynch, S. Marks, and D. B. Wake. In addition, we thank D. B. Wake for his guidance and support. Special thanks to the California Department of Fish and Game for collecting permits. Mitochondrial DNA sequences are submitted to GENBANK under the accession numbers DQ196301–196306.

Literature Cited

Avise, J. C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Carstens, B. C., S. J. Brunsfeld, J. R. Demboski, J. M. Good, and J. Sullivan. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogenetic framework. Evolution 59:1639–1652.

COATES, M. 1967. A comparative study of the serum proteins of the species of *Taricha* and their hybrids. Evolution 21:130–140.

COATES, M., AND V. C. TWITTY. 1967. A genetic analysis of differences in disk-electrophoretic patterns of serum proteins within the salamander genus *Taricha*. Proceedings of the National Academy of Sciences USA 58:173–180.

GOOD, D. A. 1989. Hybridization and cryptic species in *Dicamptodon* (Caudata: Dicamptodontidae). Evolution 43:728–744.

HEDGECOCK, D. 1978. Population subdivision and genetic divergence in the Red-Bellied Newt, *Taricha rivularis*. Evolution 32:271–286.

HEDGECOCK, D., AND F. J. AYALA. 1974. Evolutionary divergence in the genus *Taricha* (Salamandridae). Copeia 1974:738–747.

HIGHTON, R. 1998. Is *Ensatina eschscholtzii* a ring species? Herpetologica 54:254–278.

HIGHTON, R., AND T. P. WEBSTER. 1976. Geographic protein variation and divergence in populations of the salamander *Plethodon cinereus*. Evolution 30:33–45.

JOCKUSCH, E. L., AND D. B. WAKE. 2002. Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. Biological Journal of the Linnaean Society 76:361–391.

JOCKUSCH, E. L., K. P. YANEV, AND D. B. WAKE. 2001. Molecular phylogenetic analysis of slender salamanders, genus *Batrachoseps* (Amphibia: Plethodontidae), from central coastal California with descriptions of four new species. Herpetological Monographs 15:54–99.

KUCHTA, S. R., AND A.-M. TAN. 2005. Isolation by distance and post-glacial range expansion in the Rough-Skinned Newt, *Taricha granulosa*. Molecular Ecology 14:225–244.

———. 2006. Lineage diversification on an evolving landscape: phylogeography of the California Newt, *Taricha torosa* (Caudata: Salamandridae). Biological Journal of the Linnaean Society.

MURPHY, R. W., J. W. SITES, D. G. BUTH, AND C. H. HAUFLER. 1996. Proteins: isozyme electrophoresis. *In* D. M. Hillis, C. Moritz, and B. K. Mable (eds.), Molecular Systematics, pp. 51–120. Sinauer Associates, Inc., Sunderland, MA.

NEI, M. 1972. Genetic distance estimates between populations. American Naturalist 106:283–292.

———. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–90.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. Austin, Texas: University of Texas Publication 7213:145–153.

SALTHE, S. N., AND G. B. KITTO. 1966. Electrophoretic patterns of dehydrogenases in salamanders of the genus *Taricha*. Copeia 1966:130–132.

TAN, A. M., AND WAKE, D. B. 1995. MtDNA phylogeography of the California Newt, *Taricha torosa* (Caudata, Salamandridae). Molecular Phylogenetics and Evolution 4:383–394.

TWITTY, V. C. 1966. Of Scientists and Salamanders. W. H. Freeman, San Francisco, CA.

WAKE, D. B. 2006. Problems with species: patterns and processes of species formation in salamanders. Annals of the Missouri Botanical Garden 93:8–23.

WAKE, D. B., AND C. J. SCHNEIDER. 1998. Taxonomy of the plethodontid salamander genus *Ensatina*. Herpetologica 54:279–298.

WAKE, D. B., AND K. P. YANEV. 1986. Geographic variation in allozymes in a ''ring species,'' the plethodontid salamander *Ensatina eschscholtzii* of western North America. Evolution 40:702–715.

WRIGHT, S. 1943. Isolation by distance. Genetics 28:114–138.

Accepted: 15 August 2006.

Journal of Herpetology, Vol. 41, No. 2, p. 346, 2007

Erratum

In the article, ''Limited Genetic Variation across the Range of the Red-Bellied Newt, *Taricha rivularis*,'' by Shawn R. Kuchta and An-Ming Tan, which appeared in Volume 40, Issue 4 (December 2006), pp. 561–565, the caption for Figure 1 was incorrect. The caption should have stated, ''Map of north-central California showing the distribution of *Taricha rivularis*. The San Francisco Bay area is located at the southern limit of the map. Numbers correspond to sampled populations in the current study (Table 1). The two stars, along with population 1, show the sampling localities of Hedgecock and Ayala (1974). The isolation by distance study of Hedgecock (1978) took place between the open circle and population 1.''