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Phylogeography and Spatial Genetic Structure of the Southern Torrent Salamander: Implications for Conservation and Management

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Abstract

The Southern torrent salamander (*Rhyacotriton variegatus*) was recently found not warranted for listing under the US Endangered Species Act due to lack of information regarding population fragmentation and gene flow. Found in small-order streams associated with late-successional coniferous forests of the US Pacific Northwest, threats to their persistence include disturbance related to timber harvest activities. We conducted a study of genetic diversity throughout this species' range to 1) identify major phylogenetic lineages and phylogeographic barriers and 2) elucidate regional patterns of population genetic and spatial phylogeographic structure. Cytochrome *b* sequence variation was examined for 189 individuals from 72 localities. We identified 3 major lineages corresponding to nonoverlapping geographic regions: a northern California clade, a central Oregon clade, and a northern Oregon clade. The Yaquina River may be a phylogeographic barrier between the northern Oregon and central Oregon clades, whereas the Smith River in northern California appears to correspond to the discontinuity between the central Oregon and northern California clades. Spatial analyses of genetic variation within regions encompassing major clades indicated that the extent of genetic structure is comparable among regions. We discuss our results in the context of conservation efforts for Southern torrent salamanders.

Genetic analyses of natural populations can become more than theoretical investigations of biological, geographic, and historical processes when threatened or endangered species are involved as they provide unique opportunities to apply the wealth of knowledge accumulated over the last century for the purposes of addressing contemporary conservation issues. For example, pure phylogeographic analyses of molecular genetic variation can provide valuable insights about specific factors that contribute to patterns of genetic diversity and divergence across a species' range (Avise 2000). Such explorations may identify geographic features that produce deep patterns of genetic divergence and may indicate timing of historically important events (e.g., Haig et al. 2004; Ripplinger and Wagner 2004; Miller et al. 2005). From a conservation perspective, these analyses can prove invaluable for defining conservation units for species management purposes (Moritz 1994a, 1994b). Likewise, genetic analyses can also provide information about dispersal abilities, reproductive strategies, and population demography (e.g., Jarne 1995; Miller et al. 2002; Mahoney 2004). In the case of endangered taxa, generation of such information can assist with understanding the basic biology and life history of species that facilitates formulation of species conservation strategies.

The Southern torrent salamander (*Rhyacotriton variegatus*) was recently found not warranted for listing under the US Endangered Species Act after the US Fish and Wildlife Service concluded there was a "lack of information [about whether] the species is threatened by low gene flow and low genetic diversity across its range" (Federal Register 60: 33785). This species is widely but patchily distributed throughout the Pacific Coast mountain range of the US Pacific Northwest, extending from Tillamook County, OR, south to Mendicino County, CA. Although they are limited primarily to the Pacific Coast mountain range, their distribution also extends eastward into the Central Cascade Range of Oregon (Figure 1; Leonard et al. 1993; Wagner et al. 2006). Mostly found in small streams

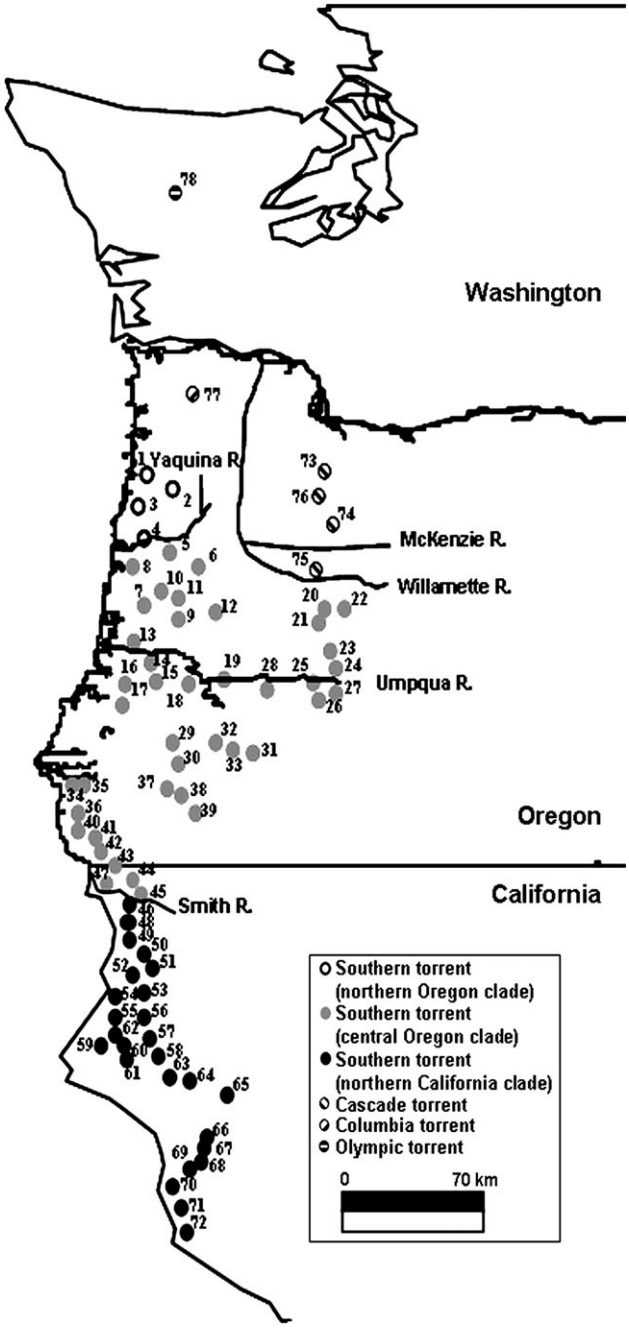

Figure 1. Map of collection locations for Southern torrent salamanders and out-groups in the western United States of America. Variation in symbols for Southern torrent salamanders indicates the 3 major phylogenetic lineages identified from cyt*b* sequence analyses (Figures 2 and 3).

and headwaters associated with late-successional forests, they are impacted by timber harvest and related disturbance activities (Bury and Corn 1988a; Corn and Bury 1989; Diller and Wallace 1996). Juveniles are restricted to cold, clear, fast-flowing streams, and adults are rarely found more than a few meters from stream banks. Both adults and juveniles appear sensitive to water loss and heat shock and require low ambient temperatures (Brattstrom 1963; Nussbaum and Tait 1977; Nussbaum et al. 1983). Subsequently, removal of the forest canopy may increase mean stream temperatures and stream sedimentation, leading to extirpation of local populations (Bury and Corn 1988b; Corn and Bury 1989; Welsh 1990; Welsh and Lind 1992). As a consequence, recolonization after extirpation is thought to be low, due to these ecological factors and their apparent limited dispersal abilities (Nussbaum and Tait 1977; Nijhuis and Kaplan 1998).

In this paper, we examine patterns of phylogeographic and population genetic structure in the Southern torrent salamander cytochrome *b* gene at both regional and local spatial scales. We compare our results to previously conducted allozyme studies (Good et al. 1987; Good and Wake 1992) and discuss our results with respect to conservation issues related to the Southern torrent salamander.

Methods

DNA Isolation and Sequencing

Southern torrent salamanders were sampled from 72 localities (1–3 individuals per location, 189 in total) throughout their known range (Figure 1 and Table 1). Also included in sequence analyses were limited numbers of Cascade torrent, Columbia torrent, and Olympic torrent salamanders (*Rhyacotriton cascadae*, *Rhyacotriton kezeri*, and *Rhyacotriton olympicus*, respectively) as outgroups (Table 1 and Figure 1). Sample tissue was taken by nonlethal tail clipping (~1 cm) from hand-captured adults and was stored immediately in a cryogenic tube containing buffer solution (100 mM Tris–HC1 pH 8.0, 100 mM ethylenediaminetetraacetic acid pH 8.0, 10 mM NaCl, 0.5% sodium dodecyl sulfate) until transferred to an ultracold freezer (−80 °C).

A modified phenol–chloroform extraction procedure was used to isolate DNA (Sambrook et al. 1989) with the final extracted aqueous layer purified in a microcon-50 filter (Millipore, Billerica, MA). An ~850-bp fragment of the cytochrome *b* gene was amplified for each of the 189 individuals in the data set using the following primers designed for vertebrates: MVZ15 (5′-GAACTAATGGCCCACACWWTACGNAA-3′) and MVZ16 (5′-AAATAGGAAATATCATTCTGGTTTAAT-3′) (Kocher et al. 1989). Each polymerase chain reaction was carried out with 100 ng DNA in a 50-µl volume using 0.5 units of *Taq* Gold (Perkin Elmer, Wellesley, MA), 100 µM each deoxynucleoside triphosphate, 2 mM MgCl, and 1 µM of each primer. Thermal cycling was performed using the following parameters: an initial denaturation of 10 min at 93 °C, followed by 40 cycles of denaturation for 1 min at 93 °C, annealing for 1 min at 52 °C, and extension at 72 °C for 2 min. A final extension at 72 °C for 10 min completed the reaction. Reaction products were run on 1% agarose gels, and amplified cytochrome *b* fragments were extracted from gel slices using an ultra-free-mc 0.45 filter (Millipore). The supernatant was then transferred to a microcon-50 filter (Millipore) and washed twice with 400 µl distilled deionized water. Sequencing was performed on an Applied Biosystems (373A) sequencer. Sequencing primers included MVZ15, MVZ16, and cyt*b*2 (5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′;

Moritz et al. 1992). Bidirectional sequences from each individual were aligned by hand using the Genetic Data Environment (Smith et al. 1992). After trimming sequence ends, we obtained a final 778-bp alignment corresponding to nucleotides 28–805 of the complete cytochrome *b* gene (based on comparisons with GenBank accession number AY728210; Muller et al. 2004).

Phylogenetic Analyses of Mitochondrial Haplotypes

MEGA 2.1 (Kumar et al. 2001) was used to perform preliminary sequence data explorations and to obtain average pairwise genetic distances. In addition, we used 2 methods to infer phylogenetic relationships among Southern torrent cytochrome *b* sequences. First, we used MEGA to perform a minimum evolution (ME) search of tree space. The tree with the shortest sum of branch lengths was identified using the close-neighbor-interchange algorithm with the starting tree obtained via the neighbor-joining algorithm (Saitou and Nei 1987). We relied on Jukes–Cantor genetic distances for the ME analysis. Bootstrap support (Felsenstein 1985) for the resulting topology was obtained from 1000 replicates. Cytochrome *b* sequences from the other 3 *Rhyacotriton* species were used to root phylogenetic trees (Table 1 and Figure 1). Second, we used the computer program TCS (Clement et al. 2000) to produce a haplotype network. This procedure, based on the statistical parsimony approach of Templeton et al. (1992), calculated the genealogy of the set of haplotypes observed in this study with a 95% confidence connection limit.

Spatial Analyses of Regional Phylogeographic Structure

Sequence data from each phylogenetic clade were used to infer the presence and extent of genetic structure using spatial autocorrelation analysis (Sokal and Oden 1978). Most forms of spatial autocorrelation rely on the calculation of average measures of dissimilarity or covariance for all pairs of observations that fall within user-defined sets of distance classes. Through the use of randomization procedures, specific sets of distance classes can be identified that are significantly smaller or greater than random expectations. For example, identifying significantly small genetic dissimilarities or significantly large covariances in distance classes encompassing relatively short physical distances between individuals provides evidence for both nonrandom patterns of genetic diversity and the spatial scale at which the nonrandom pattern occurs (Manel et al. 2003).

Spatial autocorrelation analyses were performed using the computer program ALLELES IN SPACE (Miller 2005). The measure of autocorrelation used for analysis (A_y) was quantified as the average genetic distance between pairs of individuals that fell into distance class *y*. Analyses were initially performed using 10 distance classes and were subsequently repeated using 20 and 30 distance classes to ensure that the arbitrary choice of distance class size had no effect on analysis outcomes. A randomization procedure consisting of 5000 replicates was used to identify distance classes where average genetic distances were significantly larger or smaller than random expectations.

Results

Phylogenetic Analyses

There was substantial haplotype variation among Southern torrent salamander populations at regional and local scales. Nucleotide sequences (778 bp) were characterized by 122 polymorphic sites (80 parsimony informative) and relatively low average pairwise genetic distances between haplotypes (mean Jukes–Cantor distance = 0.027, standard error = 0.003). Consequently, use of more complicated nucleotide substitution models (with their associated greater variances) to account for multiple hits at nucleotide sites was not necessary for phylogeny reconstruction (Nei and Kumar 2000, p. 112). In total, 52 unique haplotypes were detected among the 189 individuals and 72 locations sampled (Table 1). Individuals sampled from the same location generally bore identical haplotypes (Table 1). When more than one haplotype was detected at a site (sites 32, 35, and 70; Table 1), nucleotide differences between haplotypes were very small (<0.5%).

Our phylogenetic analyses produced similar results for both inferential methods used. ME analyses yielded a single tree with a sum of branch lengths of 0.458 (Figure 2). Bootstrap analyses revealed 3 well-supported haplotype clades that corresponded to distinct geographic regions (Figure 1). A northern Oregon clade, supported by 100% of bootstrap replicates, contained 3 haplotypes detected at 4 populations. A second clade comprising haplotypes detected in central Oregon was supported by 77% of bootstrap replicates. In total, 34 unique haplotypes detected in 42 populations were included in the central Oregon lineage. Finally, a haplotype lineage comprising 15 unique haplotypes from 26 northern California populations was supported by 98% of bootstrap replicates. This inference was largely supported by the haplotype network generated from our data (Figure 3). In this analysis, 3 groups of haplotypes were recovered that could not be joined into a single composite network via statistical parsimony with 95% confidence. The constituents of these groups were identical to those contained within major clades revealed by ME analyses. Three additional steps beyond the 12 calculated to achieve a 95% parsimony probability were required to join the central Oregon and northern California clades. A total of 21 steps were required to join all 3 clades into a single network.

Regional Spatial Phylogeographic Structure

Due to small sample sizes, no spatial autocorrelation analyses were performed on individuals from the 4 northern Oregon clade locations. However, analyses performed on individuals from the central Oregon and northern California clades clearly indicated the presence of strong regional phylogeographic structure (Figure 4). In analyses performed using 10 distance classes, the central Oregon data set yielded highly significant values of A_y that were smaller than random expectations over the 2 shortest distance classes (encompassing geographic distances up to ~63 km; $P < 0.001$). The third distance class of this data set (~63–94 km) was significantly smaller than random at the $\alpha = 0.05$ level ($P = 0.023$).

Table 1. Localities of Southern torrent salamander populations surveyed in this study. Sample sizes (*n*) from each location, haplotype codes (Figure 2), and GenBank accession numbers are also provided

Site	*n* (haplotype)	Longitude, latitude	County, state	GenBank accession number
1. E. Little Nestucca	3 (H1)	−123.892, 45.123	Tillamook, OR	AY753838
2. W. Little Nestucca	2 (H1)	−123.819, 45.107	Tillamook, OR	AY753838
3. Ball Mountain	3 (H2)	−123.940, 44.920	Tillamook, OR	AY753839
4. Siletz	3 (H3)	−123.941, 44.656	Lincoln, OR	AY753840
5. Salmon Ck	2 (H4)	−123.728, 44.587	Lincoln, OR	AY753841
6. Mary's Peak	3 (H5)	−123.551, 44.495	Benton, OR	AY753842
7. Alsea Area Trib.	3 (H6)	−123.546, 44.306	Benton, OR	AY753843
8. Risley Ck	2 (H7)	−124.064, 44.411	Lincoln, OR	AY753844
9. Bear Ck Trib.	3 (H8)	−123.790, 44.349	Benton, OR	AY753845
10. Mossy Falls	3 (H9)	−123.749, 44.350	Benton, OR	AY753846
11. Little Lobster Ck	2 (H10)	−123.704, 44.310	Benton, OR	AY753847
12. Heidi Ck	3 (H10)	−123.461, 44.252	Lane, OR	AY753847
13. Madera's Grave	2 (H9)	−123.928, 44.218	Lane, OR	AY753846
14. Mapleton	2 (H11)	−123.856, 43.920	Lane, OR	AY753848
15. Kentucky Falls	3 (H12)	−123.820, 43.890	Lane, OR	AY753849
16. Elliot SF #1	2 (H13)	−124.026, 43.589	Douglas, OR	AY753850
17. Elliot SF #2	2 (H13)	−124.034, 43.492	Douglas, OR	AY753850
18. Bear Ck	3 (H14)	−123.618, 43.320	Douglas, OR	AY753851
19. No name	3 (H15)	−123.440, 43.480	Douglas, OR	AY753852
20. Goodman #1	3 (H16)	−122.676, 43.831	Lane, OR	AY753853
21. Goodman #2	3 (H16)	−122.696, 43.831	Lane, OR	AY753853
22. Patterson Mountain	3 (H16)	−122.616, 43.776	Lane, OR	AY753853
23. M. Bryce Ck	3 (H17)	−122.681, 43.642	Lane, OR	AY753854
24. Rainbow Mine	3 (H18)	−122.656, 43.573	Lane, OR	AY753855
25. N. Scaredman	2 (H19)	−122.794, 43.397	Douglas, OR	AY753856
26. W. Scaredman	2 (H19)	−122.754, 43.368	Douglas, OR	AY753856
27. E. Scaredman	2 (H19)	−122.794, 43.368	Douglas, OR	AY753856
28. Scott Mountain	3 (H19)	−123.063, 43.348	Douglas, OR	AY753856
29. Cow Creek	3 (H20)	−123.632, 42.904	Douglas, OR	AY753857
30. Ollala Ck	3 (H20)	−123.546, 44.306	Douglas, OR	AY753857
31. Canyon Ck	3 (H21)	−123.257, 42.876	Douglas, OR	AY753858
32. Shoestring #1	2 (H22, H23)	−123.396, 42.905	Douglas, OR	AY753859, AY753860
33. O'Shea Ck	3 (H24)	−123.316, 42.877	Douglas, OR	AY753861
34. Elk #1	2 (H25)	−124.327, 42.702	Curry, OR	AY753862
35. Elk #2	2 (H26, H27)	−124.365, 42.710	Curry, OR	AY753863, AY753864
36. Qoutsana	3 (H28)	−124.236, 42.485	Curry, OR	AY753865
37. N. Galice	3 (H29)	−123.694, 42.539	Douglas, OR	AY753866
38. Galice	3 (H30)	−123.631, 42.543	Douglas, OR	AY753867
39. Limpy Ck	3 (H31)	−123.439, 42.423	Douglas, OR	AY753868
40. Pistol R.	3 (H32)	−124.313, 42.284	Curry, OR	AY753869
41. Little Redwood	2 (H32)	−124.143, 42.145	Curry, OR	AY753869
42. Chetco R.	3 (H33)	−124.173, 42.130	Curry, OR	AY753870
43. Winchuck R.	3 (H34)	−124.101, 42.024	Curry, OR	AY753871
44. L. Division Rd.	3 (H35)	−124.025, 41.870	Del Norte, CA	AY753872
45. M. Fork Smith R.	3 (H36)	−124.012, 41.770	Del Norte, CA	AY753873
46. S. Fork Smith R.	3 (H37)	−123.887, 41.550	Del Norte, CA	AY753874
47. Dominie Ck	2 (H38)	−124.130, 41.963	Del Norte, CA	AY753875
48. Miller Rellium	2 (H37)	−124.054, 41.748	Del Norte, CA	AY753874
49. Hunter Ck	1 (H39)	−124.029, 41.575	Humboldt, CA	AY753876
50. Turwer Ck #1	3 (H39)	−123.950, 41.590	Humboldt, CA	AY753876
51. Turwer Ck #2	3 (H39)	−123.970, 41.590	Humboldt, CA	AY753876
52. Omagar	3 (H40)	−123.974, 41.455	Humboldt, CA	AY753877
53. Morek Ck	3 (H41)	−123.826, 41.269	Humboldt, CA	AY753878
54. McDonald Ck	3 (H42)	−124.091, 41.221	Humboldt, CA	AY753879
55. Mitsui Ck	3 (H43)	−124.052, 40.978	Humboldt, CA	AY753880
56. Wire Grass	2 (H42)	−123.902, 41.020	Humboldt, CA	AY753879
57. Cannon Ck #1	2 (H44)	−123.847, 40.714	Humboldt, CA	AY753881
58. Cannon Ck #2	3 (H44)	−123.888, 40.711	Humboldt, CA	AY753881
59. Jacoby Ck	2 (H44)	−124.034, 40.817	Humboldt, CA	AY753881

Table 1. Continued

Site	*n* (haplotype)	Longitude, latitude	County, state	GenBank accession number
60. M. Trib.	3 (H42)	−124.019, 40.843	Humboldt, CA	AY753879
61. Dry Ck	2 (H42)	−124.019, 40.843	Humboldt, CA	AY753879
62. Black Dog	2 (H45)	−124.018, 40.858	Humboldt, CA	AY753882
63. Goodman Prairie	3 (H46)	−123.888, 40.711	Humboldt, CA	AY753883
64. Graham Ck	2 (H47)	−123.847, 40.714	Humboldt, CA	AY753884
65. University Hills	2 (H46)	−123.472, 40.650	Trinity, CA	AY753883
66. Ten Mile	3 (H48)	−123.598, 39.753	Mendocino, CA	AY753885
67. Fox Ck	3 (H48)	−123.594, 39.741	Mendocino, CA	AY753885
68. Elder Ck	3 (H48)	−123.617, 39.736	Mendocino, CA	AY753885
69. Skunk Ck	3 (H48)	−123.615, 39.738	Mendocino, CA	AY753885
70. Chadbourne	2 (H49, H50)	−123.761, 39.628	Mendocino, CA	AY753886, AY753887
71. Dark Gulch	3 (H51)	−123.773, 39.236	Mendocino, CA	AY753888
72. M. Alder Ck	3 (H52)	−123.639, 39.005	Mendocino, CA	AY753889
73. *Rhyacotriton cascadae*	3 (H54)	−122.059, 45.122	Clackamas, OR	AY764249
74. *Rhyacotriton cascadae*	3 (H55)	−122.434, 44.594	Linn, OR	AY764250
75. *Rhyacotriton cascadae*	2 (H56)	−122.640, 43.914	Lane, OR	AY764251
76. *Rhyacotriton cascadae*	2 (H57)	−122.162, 45.136	Clakamas, OR	AY764252
77. *Rhyacotriton kezeri*	2 (H58)	−123.519, 45.794	Tillamook, OR	AY764253
78. *Rhyacotriton olympicus*	2 (H59)	−124.276, 48.044	Clallam, WA	AY764254

Likewise, in analyses of the northern California data set, the first 3 distance classes (up to ~92 km) were all significantly small ($P < 0.001$). Qualitatively similar results were obtained when $z = 20$ and $z = 30$ distance classes were used for analyses (Figure 4). Thus, in total, both geographic regions contain strong patterns of phylogeographic structuring that were relatively comparable between clades.

Discussion

Phylogeography of Southern Torrent Salamanders

Vicariance, geography, and factors related to climate change can influence genetic structure of populations across a species' range by restricting gene flow or allowing range expansion and colonization of new areas (Templeton et al. 1995; Bernatchez and Wilson 1998). These factors, combined with limited vagility of some species may contribute to population fragmentation. Patterns of divergence also may be the result of phylogeographic barriers. In our analyses, divergences among 3 major clades of Southern torrent salamanders may correspond to potential phylogeographic barriers as the geographic range of each clade appears to be constrained by major rivers (Figure 1).

The Yaquina River appears to be a geographic barrier between the northern Oregon and central Oregon clades (Figure 1). Likewise, a sharp discordance between the northern California and central Oregon clades occurs in the region around the Smith River (Figure 1). Although the efficacy of rivers as barriers to dispersal of salamanders has been questioned (Highton 1972), our results are comparable to other molecular genetic analyses conducted on salamanders from the Pacific Northwest. For example, strong patterns of mitochondrial divergence have been observed on either side of the Columbia River for the Larch Mountain salamander (*Plethodon larselli*; Wagner et al. 2005), while historical changes in the position of the Columbia River main stem have also been hypothesized as a potential factor that constrains the species' distribution and influences patterns of mitochondrial sequence diversity in the Oregon Slender salamander (*Batrachoseps wrighti*; Miller et al. 2005). In torrent salamanders, prior investigations suggest that dispersal across rivers (that correspond to species boundaries) is minimal. For example, the northern extent of the Southern torrent salamander's distribution (as encompassed by the northern Oregon clade in this study) is in the vicinity of the Little Nestucca River, where it is parapatric with the Columbia torrent salamander to the north. Allozyme analyses previously determined that no hybridization occurred along this contact zone and also indicated that no gene flow occurred across the river (Good and Wake 1992). Likewise, analyses of a contact zone between Southern torrent salamanders and Cascade torrent salamanders on either side of the Willamette River showed reciprocal monophyly of both mitochondrial and allozyme alleles (Wagner et al. 2006). Thus, despite their primarily aquatic life cycle, torrent salamanders may actively avoid warmer, higher order rivers that experience more direct sunlight due to the width of the rivers itself. Alternatively, mortality associated with predation or downstream displacement of individuals due to strong water currents could also reduce movements of individuals across large river channels.

The genetic discontinuity between the northern and central Oregon clades is a phenomenon observed in several other plant and animal species (Soltis et al. 1997; Brunsfield et al. 2001). For example, chloroplast DNA (cpDNA) analyses of red alder (*Alnus rubra*) and stink currant (*Ribes bracteosum*) demonstrated strong patterns of genetic differentiation along a north–south axis in an area close to where similar patterns are

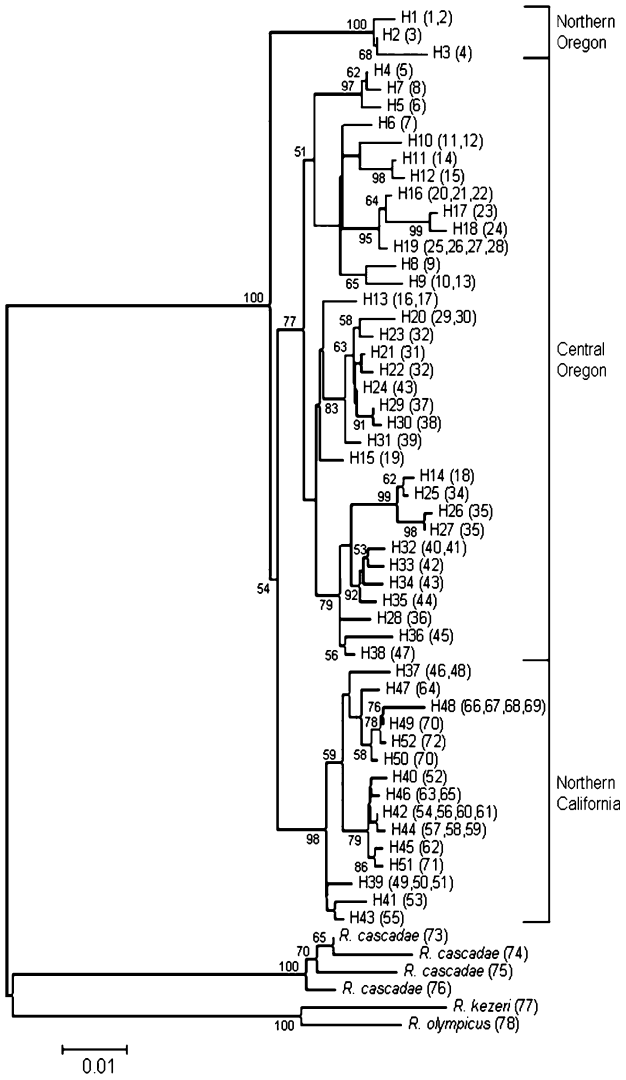

Figure 2. ME phylogenetic tree illustrating relationships of 52 Southern torrent salamander cytochrome *b* haplotypes observed at the 72 locations examined. Bootstrap values (1000 bootstrap replicates) >50 are provided.

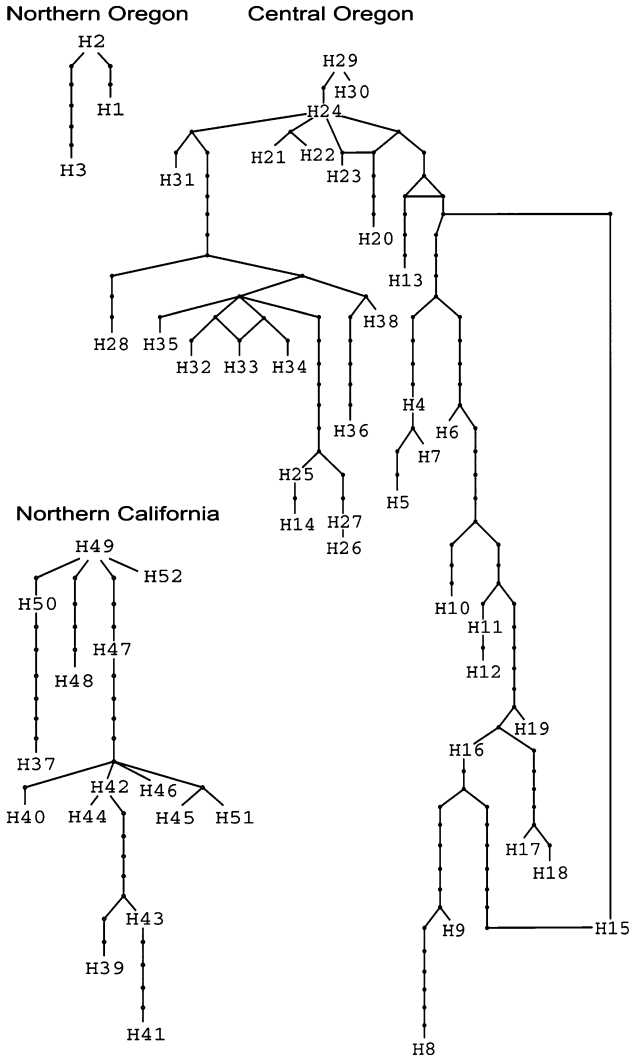

Figure 3. Haplotype network of 52 cytochrome *b* haplotypes. An additional 3 steps beyond the 12 calculated to achieve a 95% parsimony probability was required to join the central Oregon and northern California clades. A total of 21 steps were required to join all 3 clades into a single network.

observed in the Southern torrent salamander (Soltis et al. 1997). Likewise, the perennial piggyback plant (*Tolmiea menziesii*) demonstrates a particularly striking pattern in this region as it corresponds to not only an area of cpDNA divergence but also a transition from diploid individuals in the southern area of the species' range to tetraploid individuals in northern areas (Soltis et al. 1989). More recently, a strong north–south genetic discontinuity has also been observed in central Oregon in the red tree vole (*Phenacomys longicaudus* = *Arborimus longicaudus*) (Miller et al. 2006). Soltis et al. (1997) convincingly argued that such patterns were due to Pleistocene glaciation events. In this scenario, contemporary discontinuities may have been produced as a result of secondary contact between lineages previously isolated in separate northern and southern glacial refugia. Alternately, these discontinuities may be a consequence of northern range expansions from unglaciated southern refugia after glacial retreat. Although our data do not specifically allow us to distinguish between these 2 scenarios, we suggest that comparable types of historical glacial processes likely produced patterns of genetic structure observed for the central and northern Oregon clades of the Southern torrent salamander. Furthermore, we suggest that the Yaquina River may currently act as a barrier to dispersal that effectively reinforces the effects of this historical process.

Interestingly, the geographic region around the Smith River (corresponding to the division between the central Oregon and northern California clades in our study) corresponds to an area of phylogeographic importance for a variety of taxa. Jackman (1998) described species-level divergence within the genus *Aneides* and found a zone of hybridization

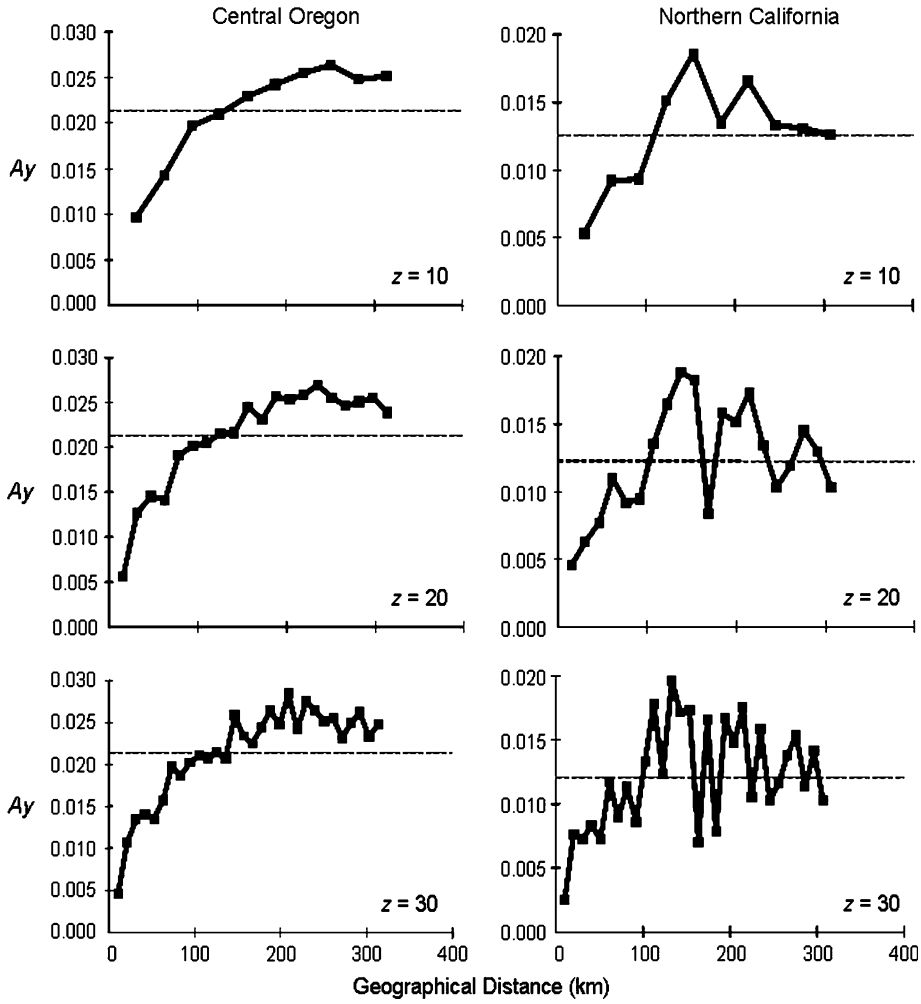

Figure 4. Results of spatial autocorrelation analyses of Southern torrent salamander cytochrome *b* haplotypes found in the central Oregon and northern California clades. Analyses were performed using $z = 10$, 20, and 30 distinct distance classes. A_y quantifies the average pairwise genetic distances of haplotypes that fall within the boundaries specified for distance class *y*. Horizontal lines indicate the average value of A_y for a data set. Results of these analyses suggest that the extent of spatial phylogeographic structure in Southern torrent salamanders occurs in the order of ~100–150 km.

occurring directly south of the Smith River's south fork between clouded salamanders (*Aneides ferreus*) and the newly identified wandering salamander (*Aneides vagrans*). Similarly, taxonomically differentiated species have been recognized among tree voles (*Phenacomys* sp.), with a chromosomal inversion occurring between the Oregon and California populations in the northern California coastal region (Johnson and George 1991). Finally, Dunn's salamander (*Plethodon dunni*) is found only directly north of the Smith River drainage, with its distribution extending slightly into the northern California coastal region (Petranka 1998).

Regional Population and Phylogeographic Structure

Thirty-five of the 52 haplotypes detected in this study were unique to specific locations (Table 1). The remaining 17 haplotypes were generally found in geographically proximate locations (Table 1 and Figure 1). This pattern is consistent with other genetic analyses of salamanders from the northwestern United States of America (e.g., Jockusch and Wake 2002; Mahoney 2004; Miller et al. 2005) and suggests little contemporary gene flow among populations. Supporting this inference are studies of movement patterns in this species along streamside habitats that reveal very small linear movement per individual (0.08 m/month or 0.003 m/day; Welsh and Lind 1992). Likewise, studies of the Cascade torrent salamander (*R. cascadae*) also suggest limited movement with a mean distance moved per day of 0.36 m and an average linear movement per individual of 2.4 m over a 3-month period (Nijhuis and Kaplan 1998).

Our analyses of phylogeographic structure via spatial autocorrelation also provided evidence for strong population genetic structure and low contemporary gene flow among populations (Figure 4). Interestingly, despite the variety of

factors that can contribute to phylogeographic structure over a given geographic region, our analyses suggested comparable spatial extents of phylogeographic structure for both central Oregon and northern California clades (although heterogeneity among A_y values was more appreciable in the northern California data set, presumably due to the smaller sample sizes). In general, the extent of genetic structure revealed by spatial autocorrelation analyses is obtained by identifying the transition point where autocorrelation coefficients switch from values that are less than average to ones that are greater than average (Clark and Richardson 2002; Diniz-Filho and Telles 2002). In both data sets, transitions between values of A_y that were less than random expectation to ones that were greater than average occurred at distance classes encompassing physical distances in the order of 100–150 km (Figure 4). Furthermore, values of A_y did not change linearly with geographic distance, which would indicate the presence of genetic clines (Bertorelle and Barbujani 1995). Instead, patterns presented in Figure 4 more closely resemble "stabilizing profiles" suggested by Diniz-Filho and Telles (2002). This suggests that patterns observed in our analyses likely reflected the influence of minor subclades revealed in phylogenetic analyses (Figure 2) and, furthermore, may indicate the presence of minor or relatively recent vicariance in this species.

Conservation Implications

Currently, the Southern torrent salamander is protected by federal lands reserved for conservation of Northern spotted owls (*Strix occidentalis caurina*) under the Northwest Forest Plan (US Forest Service and US Bureau of Land Management 1994). This conservation strategy may not adequately provide for the maintenance of genetic diversity found in Southern torrent salamanders across their range. Therefore, management efforts could focus on reexamining their status with respect to conservation unit designations. Recognition of conservation units will help focus management efforts under the Northwest Forest Plan and for future evaluation of the population status. For example, strategic management of separate geographic regions could effectively minimize logistical issues associated with managing a widespread species throughout its entire range. Such efforts could instead focus on the most critically imperiled populations or regions and allow for management designed to address local threats to species.

Our genetic data specifically lend themselves to identification of putative management units given the identification of major phylogenetic lineages that occupy nonoverlapping geographic regions corresponding to northern Oregon, central Oregon, and northern California (Figures 1–3). Furthermore, we note that previously conducted allozyme analyses of Southern torrent salamanders (Good et al. 1987; Good and Wake 1992) revealed strong patterns of population structure in this species that largely corresponded to the central Oregon and northern California clades identified in this study. Thus, our data for the northern California region specifically meet the ESU (i.e., evolutionary significant unit) criteria of Moritz (1994b), which define ESUs as having reciprocally monophyletic mitochondrial lineages and significant differences in nuclear alleles among populations. Evidence for assigning ESU designations for the other groups, specifically the northern Oregon and central Oregon clades, is less clear. Our data indicate reciprocal monophyly of mitochondrial alleles among the sampled populations; however, it is possible there is introgression among clades along the Yaquina River through male-mediated gene flow. Therefore, until evidence clearly demonstrates significant differentiation of nuclear alleles between the northern Oregon and central Oregon clades, we conservatively suggest that these regions could be recognized as separate management units as defined by Moritz. However, given the deep divergence of the northern Oregon clade (Figure 2), we expect that any nuclear data generated to address patterns of genetic structure at this mitochondrial discontinuity will ultimately support ESU designations for these regions.

Our analyses of regional population and spatial phylogeographic structure provide further information that may assist with the development of conservation strategies for Southern torrent salamanders. Although conventional thoughts on species management reflect the idea that conservation efforts should focus on maintaining gene flow among populations to avoid loss of diversity, such goals may not be practical (or even feasible) for taxa with low dispersal rates and extreme differentiation among populations. However, our autocorrelation analyses suggested that the extent of spatial phylogeographic structuring occurs over physical distances of ~100–150 km (Figure 4). This indicates that haplotypes discovered less than ~100 km apart are on average statistically correlated, and in contrast, pairs of haplotypes selected from areas in excess of ~100–150 km apart are statistically independent. Thus, if the primary goal becomes one of conserving phylogenetic diversity within regions, management efforts could focus initially on identification of high-priority habitats separated by the physical distances identified by our analyses (Clark and Richardson 2002; Diniz-Filho and Telles 2002). In doing so, resource managers may minimize the amount of redundancy associated with their efforts to conserve genetic diversity while simultaneously ensuring that a majority of genetic diversity is maintained across this fragmented species' range.

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