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Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*

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Abstract

Allozymes and mitochondrial DNA sequences were used to examine the phylogeographical history of the rough-skinned newt, *Taricha granulosa,* in western North America. Nineteen populations were analysed for allozyme variation at 45 loci, and 23 populations were analysed for cytochrome *b* sequence variation. Both data sets agree that populations in the southern part of the range are characterized by isolation by distance, whereas northern populations fit the expectations of a recent range expansion. However, the northern limit of isolation by distance (and the southern limit of range expansion) is located in Oregon State by the mtDNA data, and in Washington State by the allozyme data. Nevertheless, both data sets are consistent with the known Pleistocene history of western North America, with phylogenetically basal populations in central and northern California, and a recent range expansion in the north following the retreat of the Cordilleran ice sheet 10 000 years ago. Additionally, a population in Idaho, previously considered introduced from central California based on morphometric analyses, possesses a distinct mtDNA haplotype, suggesting it could be native. The relevance of these results for Pacific Northwest biogeography is discussed.

Keywords: allozymes, glaciation, isolation by distance, phylogeography, range expansion, *Taricha granulosa*

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Introduction

Advances in population genetic and phylogenetic techniques are aiding evolutionary biologists in unraveling the complex interactions among geological change, genetic differentiation, species distribution, and species formation (Moritz *et al*. 1992; Avise 2000; Brunsfeld *et al*. 2001; Kuchta & Meyer 2001; Matocq 2002; Zamudio & Savage 2003; Mahoney 2004). Increasingly, diverse data sets are being employed in phylogeographical studies, and discordances among data sets reflective of complex evolutionary dynamics are often discovered (e.g. Ruedi *et al*. 1997; Wake & Jockusch 2000; Garcia-Paris *et al*. 2003; Ballard & Whitlock 2004). For instance, molecular markers with distinct transmission modes (e.g. maternal vs. biparental markers) may record separate histories (Jockusch & Wake 2002). In this study, we characterize patterns of genetic variation throughout the range of the rough-skinned newt, *Taricha granulosa*, using allozymes and mitochondrial DNA sequences. Our goal is to infer biogeographical history, and to compare this history with other taxa from western North America, which frequently show higher levels of diversity and population structure in the south (California to central Oregon) than the north (central Oregon to Alaska). In addition, we explore the ancestry of a disjunct population in Idaho, which earlier morphometric studies suggest was introduced from the southern portion of the range of *T. granulosa* (Nussbaum & Brodie 1971).

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Pleistocene history of western North America

At the height of the last glacial maximum, 22 000–18 000 years ago, the Laurentide and Cordilleran ice sheets covered much of Canada, southeastern Alaska, and the northern United States (Mann & Hamilton 1995). In addition to the physical presence of the glaciers, climatic change during the ice ages displaced plants and animals, restructured habitats, and radically influenced the distribution of taxa (e.g. Hewitt 1999; Brunsfeld *et al*. 2001). The Cordilleran ice sheet reached its maximum extent in western North

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America 14 500–14 000 years ago, filling the Puget Trough and Straight of Juan de Fuca of present-day Washington State (Waitt & Thorson 1983). Thereafter, the Cordilleran ice sheet rapidly retreated inland, and by 10 000 years ago large expanses of land were exposed and colonized by terrestrial organisms (Josenhans *et al*. 1995; Mann & Hamilton 1995).

At the height of the last glaciation, unglaciated regions (refugia) were present in Beringia, the Haida Gwaii region (i.e. Queen Charlotte Islands) of coastal British Columbia, Vancouver Island, and areas south of the Cordilleran ice sheet (Barnosky *et al*. 1987; Demboski *et al*. 1999; references therein). Extensive glaciers were present in the Cascade, Olympic, Siskiyou, and Sierra Nevada mountain ranges, but the Coast Ranges in the western U.S. were largely unglaciated (Burke & Birkland 1983; Guyton 1998). In present-day Washington and Oregon State, coniferous forests were located coastally, while inland regions supported tundra, sub-Alpine and Alpine parkland, and other vegetation types with no present-day analogs (Barnosky *et al*. 1987; Whitlock 1992).

Populations of plants and animals occupying unglaciated regions expanded their ranges following glacial retreat and the shift of climatic zones (Whitlock 1992). Range expansions are predicted to result in reduced levels of genetic variation and genetic structure, as repeated long distance dispersal of individuals (founder events) imposes a series of bottlenecks on the colonizing genomes (Hewitt 1996, 1999, 2000; Ibrahim *et al*. 1996; Hewitt & Ibrahim 2001). Unglaciated regions, in contrast, may show higher levels of genetic variation and population structure, such as isolation by distance. As *T. granulosa* currently occupies formerly glaciated regions in the north, we hypothesized those populations would harbour less genetic diversity than refugial populations. In this paper, we test this scenario using isolation by distance plots and other phylogeographical methods. Our results are compared with phylogeographical studies of other Pacific Northwest taxa.

Materials and methods

Allozyme variation

Population Sampling and Laboratory Techniques. One hundred nine individuals from 19 populations spanning the range of *Taricha granulosa* were examined for allozyme variation (Fig. 1; Table 1; Appendix). Following collection, specimens were euthanized in 25% chlorotone, and heart, liver, and 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 (Table 2), and standard methods of starch gel electrophoresis were employed (Murphy *et al*. 1996).

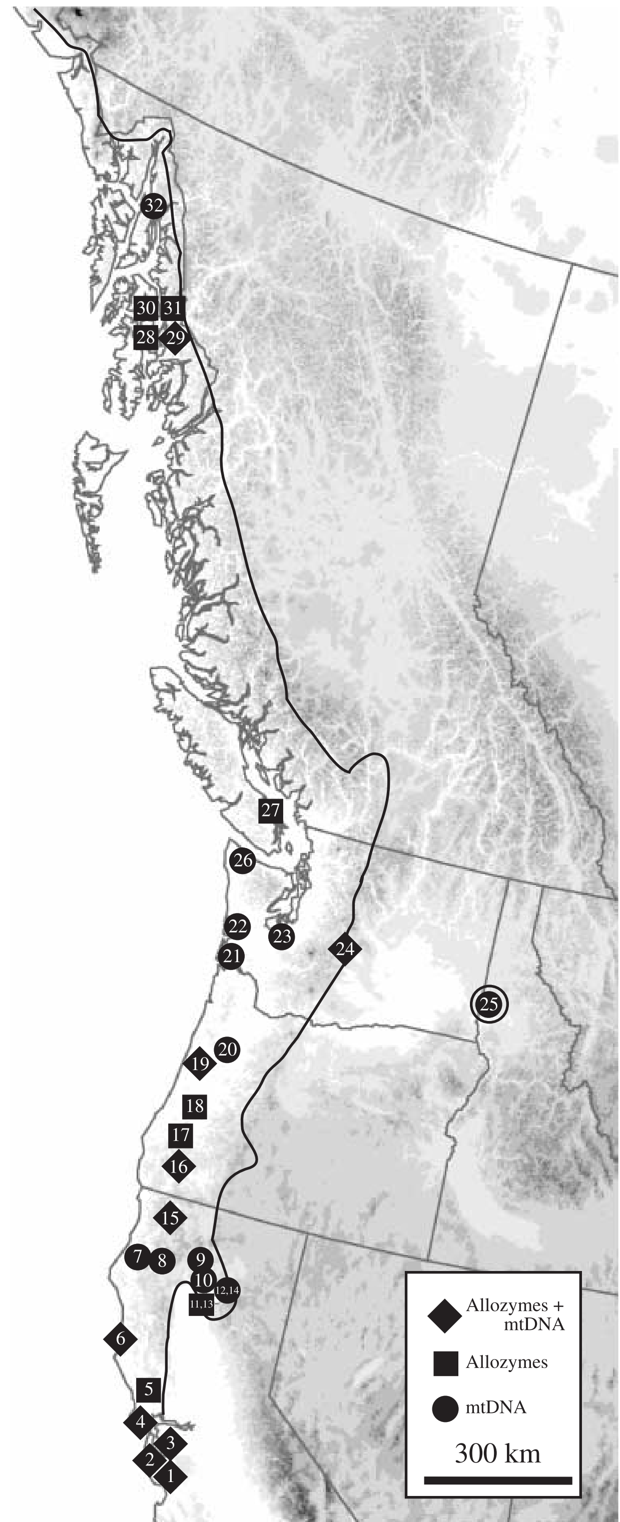

Fig. 1 Map of western North America showing the distribution of *Taricha granulosa*. Numbers correspond to sampled populations (Table 1). The legend indicates the type of data collected for each population.

Table 1 Locality information for *Taricha granulosa* and the outgroup taxa, *T. rivularis* and *Notophthalmus viridescens*. Population numbers correspond to Fig. 1. Specimen identification numbers in bold designate specimens with mtDNA sequence data and electrophoretic data; normal print designates specimens with electrophoretic data only; italics designate specimens with mtDNA sequence data only. MtDNA sample sizes are in normal print for short sequences (up to 375 bp) and in bold for longer sequences (up to 778 bp)

Population	County	State	Specific locality	Latitude/ longitude	Sample size		Specimen identification number
					Allo.	mtDNA	
1	Santa Cruz	CA	Eureka Canyon Road, 5.8 miles West and 9.1 miles North of Watsonville	37.0200 N/ 121.8183 W	3	2	**MVZ 219791,** 291791 **MVZ 219793**
2	San Mateo	CA	10 miles South of Skyline on Luttonda Road	37.3114 N/ 122.3181 W	6	1	MVZ 217768, **217769** MVZ 217770–217773
3	Alameda	CA	Near Pleasanton, ~1 mile East of Palamares Road and ~4 miles South Hwy 580	37.6553 N/ 121.9862 W	10	2	MVZ *217806*, **217807**, MVZ 217808–217816
4	Marin	CA	Pond, Point Reyes National Seashore	37.9679 N/ 122.7849 W	10	2	MVZ 216143–216152 MVZ 218997–217998
5	Sonoma	CA	Spring North Mark West Creek, 400 Ft. elev. 0.2 miles South of Bodega Hwy on Wagnon Road	38.5098 N/ 122.7259 W 38.3824 N/ 122.8890 W	1 8	— —	MVZ 217745 MVZ 217780–217787
6	Mendocino	CA	1 mile North of Albion at Dark Gulch 'Drive-through Tree', Leggett	39.2386 N/ 123.7665 W 37.3114 N/ 122.3181 W	1 1	1 —	MVZ 158863 MVZ 218996
7	Humboldt	CA	Ammon Ridge, Six Rivers National Forest	40.7965 N/ 123.6574 W	—	2	Larval samples (No MVZ #)
8	Trinity	CA	Swede Creek, 1.5 miles up Shast-Trinity National Forest Road #4 off Hwy 299	40.8079 N/ 123.3475 W	—	**1**	SRK 60
9	Shasta	CA	Samwell Cave, across the McCloud Arm of Shasta Reservoir from Ellery Creek	40.9203 N/ 122.2378 W	—	**1**	SRK 1619
10	Shasta	CA	NE end of Swede Plains	40.6888 N/ 122.1202 W	—	**1**	*SRK 1968*
11	Shasta	CA	Jct. Canyon Creek and Rock Creek, ~5 miles ENE (by air) of Manton	40.4591 N/ 121.7822 W	10	—	MVZ 208319–208328
12	Shasta	CA	Rock Creek, 5.3 mi from Manton-Shingletown Road on Rock Creek Road	40.4592 N/ 121.7808 W	—	1	MVZ 217764
13	Shasta	CA	~3 miles ENE (by air) of Midway and 0.9 miles south of Ponderosa Way, South Fork Bear Creek	40.5206 N/ 121.9157 W	9	—	MVZ 217752, 217761, MVZ 215847–217848, MVZ 217775–217779
14	Tehama	CA	Forward Road, 9.1 miles East of Manton	40.4332 N/ 121.7558 W	—	1	*MVZ 217765*
15	Siskiyou Del Norte	CA	Little Grider Creek, near Jct. with Hwy 96 Low Divide Road, 3.4 miles East of Hwy 197	41.7840 N/ 123.3940 W 41.8569 N/ 124.0751 W	2 1	2 —	**MVZ 219799–219800** MVZ 222654
16	Josephine	OR	Jct. Grave and Butte Creeks, Angola Creek Road	42.6421 N/ 123.5051 W	— 8	2 —	*MVZ 219027–219028* MVZ 219029–219036
17	Lane	OR	8.5 miles South of Hwy 126 on West Fork Road	43.05 N/ 123.62 W	6	—	MVZ 218989–218994
18	Douglas	OR	Elk Creek, 3 miles East of Elkton	43.6421 N/ 123.4978 W	10	—	MVZ 219014–219023
19	Benton	OR	Junction of North Fork Alsea Road and Parker Creek	44.4557 N/ 123.5970 W	3	2	**MVZ 219055–219056** Mvz 219057
20	Benton	OR	Near Soap Creek, 4.9 miles N of Corvallis (air)	44.6641 N/ 123.2720 W	—	**2**	Brodie*
21	Pacific	WA	1 mile North of Chinook, near Chinook River	46.2844 N/ 123.9389 W	—	**2**	Brodie*
22	Greys Harbor	WA	2 miles North of Hwy 105 along Newskay Creek	46.9207 N/ 123.8293 W	—	**2**	Brodie*

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Table 1 *Continued*

Population	County	State	Specific locality	Latitude/ longitude	Sample size		Specimen identification number
					Allo.	mtDNA	
23	Pierce	WA	32 miles SW of Dupont (air)	47.0540 N/ 122.6608 W	—	**2**	Brodie*
24	Kittitas	WA	Taneum Canyon	47.0833 N/ 120.8333 W	2	2	Larval samples (No MVZ #)
25	Latah	ID	Near town of Moscow (approximate locality)	46.733 N/ 116.9990 W	—	**2**	Brodie*
26	Clallam	WA	Beaver Lake, 4 miles North of Sappho on Burnt Mountain Road	48.1130 N/ 124.2455 W	—	**2**	Brodie*
27	—	B.C.	Gabriola Island, along Pilot Bay Road behind Camp Miriam, British Columbia, Canada	49.1700 N/ 123.8000 W	3	—	MVZ 186261 –186263
28	Wrangell-Petersburg	AK	Etolin Island, Alexander Archipelago	56.19 N/ 132.48 W	4	–	MVZ 219773 –219776
29	Wrangell-Petersburg	AK	Highbush Lake, Wrangell Island, Alexander Archipelago	56.3292 N/ 132.0809 W	3	2	**MVZ 219779–219780;** MVZ 219781
30	Wrangell-Petersburg	AK	Falls Creek, Mitkof Island, Alexander Archipelago	56.6839 N/ 132.9213 W	5	—	MVZ 219768–219772
31	Wrangell-Petersburg	AK	Figure Eight Lake, near Stikine River, Stikine-LeConte Wilderness Area	56.7009 N/ 132.2767 W	3	—	MVZ 219767; MVZ 219777–219778
32	Juneau	AK	Tee Harbor (possible introduction from Shelter Lake, on Shelter Island near Tee Harbor)	58.410 N/ 134.757 W (58.418 N/ 134.870 W)	—	**2**	Brodie*
Outgroup species: *Taricha rivularis*							
33	Sonoma	CA	Big Sulphur Creek, 13–13.7 miles East U.S. Hwy. 101 on Geysers Road	38.8043 N/ 122.8355 W		1	MVZ 217851
Outgroup species: *Nothophthalmus viridescens*							
34	Brunswick	NC	Temporary Lake 2 miles NW of Southport	33.9736 N/ 78.0614 W	—	2	MVZ 161852–161853
35	Alachua	FL	River Styx Crossing, Hwy. 346	29.5169 N/ 82.2219 W	—	**1**	MVZ 205719

*Tissues provided by E. D. Brodie Jr (Utah State University) and E. D. Brodie III (Indiana University) Specimens will be donated to the University of Texas, Collection of Vertebrates.

Regional Diversity. Patterns of regional diversity were explored using multidimensional scaling (MDS) and isolation by distance (IBD) plots. Multidimensional scaling of Nei (1978) genetic distances were used to explore the relationship between geography and genetic differentiation. MDS is a class of ordination techniques that displays the complex relationships among populations in a small number of dimensions (Lessa 1990). When interpopulational variation is a function of distance alone, the MDS of the first two dimensions produces a clustering pattern similar to a geographical map of the populations (Jackman & Wake 1994; Tilley & Mahoney 1996). Note that genetically similar clusters do not necessarily identify historical relationships (clades) (de Quieroz & Good 1997). MDS was performed using the program STATISTICA (StatSoft Inc.). Data were input as a matrix of Nei's genetic distances (Nei 1978), but Rogers' distances (1972) provide equivalent results. Scree plots were used to determine the number of dimensions required to sufficiently accommodate the variation.

The accumulation of genetic differentiation with increased geographical separation, as a result of restricted dispersal relative to geographical range, was first explored by Wright (1943), who termed the phenomenon Isolation by Distance (IBD). We tested for IBD by plotting Nei's (1978) unbiased genetic distance (D_N) among pairwise population comparisons against geographical distance. This approach has been successfully employed by many researchers, especially herpetological systematists, to document IBD (e.g. Tilley *et al*. 1978; Good & Wake 1992; Jackman & Wake 1994; Tilley & Mahoney 1996; de Quieroz & Good 1997; Tilley

Table 2 Enzyme and buffer systems used in electrophoresis

Enzyme system	Abbreviations**	No. of loci scored	E. C. number	Buffer system
Aspartate aminotransferase	AAT1, AAT2	2	2.6.1.1	1, 2
Aconitase	ACON1, ACON2	2	4.2.1.3	1
Adenosine deaminase	ADA1, ADA2	2	3.5.4.4	2
Alcohol dehydrogenase	ADH1, ADH2	2	1.1.1.1	3
Adenylate kinase	AK	1	2.7.4.3	3
Fructose-bisphosphate aldolase	ALDO	1	4.1.2.13	3
Aldehyde dehydrogenase	ALDH	1	1.2.1.3	3
Carbonic anhydrase*	CAH1, CAH2	2	4.2.1.1	3
Creatine kinase	CK	1	2.7.3.2	3
Esterase	EST1, EST2	2	3.1.1.-	5
Esterase D*	ESTD	1	3.1.-.-	3
Glycerol-3-phosphate dehydrogenase	G3PHD	1	1.1.1.8	1
Glucose-6-phosphate dehydrogenase	G6PD	1	1.1.1.49	3
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1	1.2.1.12	3
Guanine deaminase	GDA	1	3.5.4.3	2
Glucose dehydrogenase	GDH	1	1.1.1.47	3
Glutamate dehydrogenase	GLUD	1	1.4.1.-	1
Glucose-6-phosphate isomerase	GPI	1	5.3.1.9	3
Hydroxy acid dehydrogenase	HADH	1	1.1.99.6	3
Isocitrate dehydrogenase	IDH1, IDH2	2	1.1.1.42	1
L-leucyl-L-alanine (peptide)	LA1, LA2	2	3.4.11.1	4
Lactate dehydrogenase	LDH1, LDH2	2	1.1.1.27	1
L-leucylglycyglycine (peptide)	LGG	1	3.4.11.4	4
Malate dehydrogenase	MDH1, MDH2	2	1.1.1.37	1
Malic enzyme	ME	1	1.1.1.40	1
Mannose-6-phosphate isomerase	MPI	1	5.3.1.8	2
NADH dehydrogenase	NADH1, NADH2	2	1.6.99.3	1
Octanol dehydrogenase	ODH	1	1.1.1.73	3
L-phenylalanyl-L-proline (peptide)	PAP	1	3.4.11.5	4
Phosphogluconate dehydrogenase	PGD	1	1.1.1.44	2
Phosphoglucomutase	PGM	1	5.4.2.2	1
Superoxide dismutase	SOD	1	1.15.1.1	3
Sorbitol dehydrogenase	SORD	1	1.1.1.14	3
Xanthine dehydrogenase	XDH	1	1.2.3.2	3

*Fluorescent.
**Abbreviations after Shacklee *et al*. 1990.
Buffer Systems:
1. Tris-citrate II, pH 8.0 (Selander *et al.* 1971).
2. Tris-citrate III, pH 6.7 (Selander *et al*. 1971).
3. Tris-borate-EDTA, pH 9.1 (Turner 1973).
4. Lithium-borate/Tris-citrate, pH 8.2 (Selander *et al.* 1971).
5. Tris-citrate/borate, pH 8.7 (Selander *et al.* 1971).

1997; Highton & Peabody 2000; Highton & Peabody 2000; Mead *et al.* 2001). Mantel tests were used to test for a significant association between genetic and geographical distance, and reduced major axis (RMA) regression was used to estimate regression statistics. Hellberg (1994) showed that RMA regression is superior to Ordinary Least Squares regression for this purpose. The computer program IBD (Bohonak 2002) was used to calculate Mantel tests (100 000 randomizations) and the slope, y-intercept, and coefficient of determination (r^2).

No a priori rationale exists for determining the limits of IBD. Thus, various methods of grouping populations were explored. In the Pacific Northwest, glacial history suggests that populations in the north should be recent arrivals. Accordingly, populations were added sequentially from south to north, and regression statistics calculated as each population was added. The grouping with the highest r^2 was defined as the 'optimal' grouping. This 'optimal' grouping was compared with the IBD statistics from three other approaches to grouping populations: (1) all populations

from throughout the range of *T. granulosa* (2) the MDS clusters, and (3) and the 'optimal' solution found for IBD in the mtDNA data set (described below).

Phylogenetic Analysis. Modified Cavalli-Sforza & Edwards (1967) chord distances were used in a Neighbour Joining algorithm to estimate phylogenetic relationships from the allozyme data. The use of chord distances with Neighbour Joining has been shown to be accurate when subjected to simulation and congruence tests (Wiens & Servedio 1998; Wiens 2000). Chord distances and phylogenetic reconstructions were computed using PHYLIP 3.573c (Felsenstein 1993).

MtDNA Cytochrome b Sequence Variation

Population Sampling and Laboratory Techniques: 39 individuals from 23 populations throughout the range of *T. granulosa* were sequenced for a fragment of the mitochondrial cytochrome *b* gene (Fig. 1, Table 1). Three individuals of *Notophthalmus viridescens* were used as an outgroup; there is consistent support for a sister taxon relationship between *Taricha* and *Notophthalmus* (Wake & Özeti 1969; Titus & Larson 1995; Larson *et al*. 2003). The complete data set combines overlapping sequences of two lengths collected at different times with different technologies. For data set #1, 24 individuals (including two *N. viridescens*), average length 363 bp, were amplified using the PCR primers MVZ 15 and Cytb2 (Moritz *et al*. 1992). Sequences were obtained by running single-strand PCR products on gels ('manual sequencing'). See Tan & Wake (1995) for laboratory details. Data set #2 consists of 18 individuals (including one *N. viridescens*), average length 751 bp, amplified using the PCR primers MVZ15 and MVZ16 (Moritz *et al*. 1992). The majority of data set #2 focuses on northern populations, where the shorter sequences of data set #1 yielded a single haplotype. Whole genomic DNA was extracted from ethanol-preserved or frozen tissues (tail tips, liver, intestine) using the 'DNeasy Tissue Kit' (Qiagen, Inc). Amplifications were carried out in a PTC-100 Thermal Cycler (M.J. Research), and included nontemplate controls to monitor contamination. Double stranded PCR products were purified using the QIAquick PCR Purification kit (Qiagen, Inc.). All samples were sequenced in both directions in a 10 μL reaction mixture using dRhodamine and a 377 Automated Sequencer (Applied Biosystems, Inc.). Sequences were visually aligned in SEQUENCHER (Gene Codes Corp.). All sequences are deposited in GenBank under the accession numbers L22714, L22771-L22879, L22880-L22881 (data set #1) and AY627899-AY627912 (data set #2).

Regional Diversity. Patterns of regional diversity within *T. granulosa* were explored using nucleotide diversity (π, the average number of nucleotide differences per site) (Nei 1987), isolation by distance plots, and haplotype networks.

Isolation by distance was examined in the mtDNA data in a manner similar to the allozyme data, by plotting genetic divergence against geographical distance. Saturation curves (described below, under *Phylogenetic Analyses*) suggest that there is little or no mutational saturation within *T. granulosa*, and thus simple percent divergence was used as a measure of genetic distance. Mantel tests and RMA regression statistics were calculated using the program IBD 1.52 (Bohonak 2002). The 'optimal' grouping of populations calculated for the mtDNA data set (defined as the grouping with the highest r^2 value; see *Allozyme Variation: Regional Diversity*, above) was compared to (1) the complete data set (2) the MDS clusters, and (3) the 'optimal' grouping calculated for the allozyme data set. For tests of IBD, the Idaho sample (25) was excluded from regression statistics because of its geographical isolation and because we lack an allozyme sample from Idaho for comparison.

Haplotype networks were generated with TCS 1.13 (Clement *et al*. 2000). Haplotype networks are useful at low levels of divergence, when gene trees may not be bifurcating, and thus the hypothesis of a hierarchical relationship among haplotypes violated. Haplotype networks were generated using (1) all haplotypes, shortened to 375 bp so that sequence length variation between data set #1 and #2 does not impact the results, and (2) the longer sequences from data set #2 from Oregon to Alaska, where only a single haplotype was found with data set #1.

Phylogenetic Analyses. Prior to phylogenetic analysis, mutational saturation in the data set was tested by plotting the absolute number of transitions and transversions at each codon position (1st, 2nd, and 3rd) against maximum likelihood distances (parameters chosen with MODELTEST 3.06 (Posada & Crandall 1998; see below)). Mutational saturation is identified when increasing genetic distances are not reflected by an increase in the absolute number of mutational differences.

Maximum likelihood and Bayesian analyses were conducted. Identical sequences in data set #1 and #2 were identified in MACCLADE 4.0 (Maddison & Maddison 2000) using the 'Search and Merge' option. The resulting combined data set had 19 unique haplotypes, including three outgroup haplotypes. The model that best fits the cytochrome *b* data was determined using a hierarchical likelihood ratio test, as implemented in the program MODELTEST 3.06 (Posada & Crandall 1998). The Transversion Model (TVM) was chosen, and included a parameter for substitution rate heterogeneity among sites ($\Gamma = 0.7376$). Nucleotide frequencies under this model were estimated as: A = 0.2829; C = 0.2862; G = 0.1465; T = 0.2844. All sites were assumed to be variable. This model was used in both maximum likelihood and Bayesian analyses.

Maximum likelihood analyses and nonparametric bootstrapping were carried out with PAUP* 4.0 b10 (Swofford

Table 3 Nei's (1978) – below diagonal) and Rogers' (1972) — above diagonal) genetic distances between population pairs for allozyme variation. Clusters refer to the population grouping pattern in the multidimensional scaling analysis (Fig. 2 and text)

	California cluster								Oregon cluster					Washington to Alaska cluster					
	1	2	3	4	5	6	11	13	15	16	17	18	19	24	27	28	29	30	31
1	—	0.132	0.246	0.221	0.239	0.219	0.276	0.3	0.36	0.38	0.391	0.384	0.422	0.462	0.442	0.436	0.425	0.446	0.435
2	0.009	—	0.215	0.188	0.218	0.211	0.251	0.272	0.34	0.348	0.354	0.348	0.395	0.45	0.42	0.419	0.409	0.428	0.418
3	0.058	0.045	—	0.139	0.18	0.179	0.26	0.266	0.328	0.331	0.353	0.341	0.427	0.453	0.423	0.416	0.406	0.424	0.415
4	0.045	0.033	0.018	—	0.149	0.14	0.2	0.215	0.296	0.303	0.326	0.314	0.403	0.435	0.405	0.398	0.386	0.409	0.396
5	0.054	0.047	0.032	0.021	—	0.117	0.16	0.16	0.298	0.303	0.326	0.314	0.403	0.432	0.399	0.393	0.383	0.402	0.392
6	0.041	0.041	0.029	0.016	0.01	—	0.18	0.209	0.352	0.356	0.374	0.364	0.445	0.468	0.441	0.434	0.423	0.444	0.433
11	0.076	0.065	0.071	0.04	0.024	0.03	—	0.116	0.289	0.287	0.295	0.289	0.381	0.448	0.415	0.412	0.4	0.421	0.41
13	0.092	0.077	0.075	0.047	0.024	0.042	0.011	—	0.281	0.287	0.303	0.295	0.388	0.448	0.412	0.406	0.401	0.41	0.407
15	0.136	0.123	0.114	0.092	0.093	0.13	0.088	0.082	—	0.202	0.201	0.207	0.255	0.386	0.378	0.374	0.362	0.386	0.373
16	0.156	0.131	0.118	0.097	0.098	0.134	0.087	0.087	0.04	—	0.066	0.048	0.234	0.359	0.319	0.317	0.302	0.331	0.315
17	0.166	0.136	0.136	0.114	0.114	0.15	0.092	0.097	0.04	0.003	—	0.03	0.212	0.374	0.34	0.341	0.327	0.354	0.34
18	0.16	0.131	0.127	0.106	0.106	0.142	0.089	0.093	0.043	0.001	0	—	0.223	0.365	0.326	0.326	0.312	0.34	0.325
19	0.196	0.172	0.205	0.181	0.181	0.221	0.16	0.165	0.065	0.055	0.044	0.05	—	0.324	0.32	0.333	0.319	0.346	0.332
24	0.242	0.232	0.234	0.213	0.209	0.247	0.229	0.228	0.159	0.136	0.15	0.142	0.108	—	0.191	0.189	0.184	0.211	0.186
27	0.219	0.199	0.202	0.183	0.177	0.216	0.193	0.189	0.154	0.107	0.122	0.112	0.106	0.031	—	0.079	0.096	0.098	0.08
28	0.212	0.197	0.193	0.175	0.171	0.208	0.19	0.184	0.151	0.106	0.124	0.113	0.117	0.032	0.002	—	0.056	0.058	0.012
29	0.2	0.187	0.184	0.164	0.161	0.197	0.178	0.178	0.14	0.096	0.113	0.103	0.106	0.031	0.006	0.001	—	0.109	0.045
30	0.224	0.207	0.203	0.187	0.18	0.22	0.2	0.188	0.161	0.116	0.135	0.124	0.127	0.042	0.006	0.002	0.011	—	0.07
31	0.21	0.196	0.193	0.174	0.17	0.207	0.189	0.184	0.15	0.105	0.123	0.112	0.116	0.031	0.003	0	0.001	0.003	—

2003), using a heuristic search routine, Tree Bisection and Reconnection (TBR) branch swapping, and a starting tree estimated by neighbour joining.

Bayesian analyses were conducted with MRBAYES 2.01 (Huelsenbeck & Ronquist 2001). The analysis was initiated with random starting trees, and carried out for 5.0×10^5 generations. In all runs, default Metropolis-coupled Markov chain Monte Carlo methods ('heated chains') were used to improve the ability of the Markov chains to find alternate optima. Markov chains were sampled every 100 generations, for a total of 5000 sample points. After excluding 10 000 'burn in' generations (chosen by visualization of a plot of log-likelihood scores against generation), a majority rule consensus tree was constructed. The percentage of times a particular clade is recovered is its posterior probability. For a clade to be significantly supported it should occur in ~95% of the sample points (Lewis 2001). To assure that the posterior probability values had not become 'stuck' on a local optimum, we repeated the analysis five times with different random starting trees. The log-likelihood values and the posterior probabilities of the clades recovered were compared among runs for congruence.

Results

Allozyme variation

Of the 45 loci scored in *Taricha granulosa*, 21 were variable [many of the invariable loci are variable in other species of *Taricha*, or between species; see Kuchta (2002)] (Appendix). Table 3 provides the matrix of Nei's (1978) and Rogers' (1972) genetic distances among population samples.

A scree plot suggests that two axes account for most of the variation in the MDS analysis (data not shown; see Kuchta 2002). Three geographically differentiated clusters of populations were identified (Fig. 2): we label these the 'California', 'Oregon', and 'Washington to Alaska' clusters because they generally follow state boundaries. However, note that the Siskiyou county population (15) in northern CA is grouped within the 'Oregon' cluster, and that there is limited sampling near the Oregon and Washington border, so these labels are not literal. The California cluster loosely mirrors geography, with Santa Cruz (1) at one end and Shasta (13) at the other. The Oregon cluster (pop 15–19) is separated from the California cluster, and, relative to geography, has larger values on the first axis. Population 19 is distinct from 15 to 18. The Washington–Alaska cluster is removed from the Oregon cluster, especially in the second dimension, and strongly deviates from a 'map' of populations that isolation by distance can produce in MDS results. The populations in this cluster also form a tight grouping, in contrast to the large geographical distance encompassed (>900 km).

Several loci show strong shifts in allele frequency among the clusters, and some loci have alleles that are restricted to particular clusters (Appendix). Among California cluster populations, there are four loci with unique, widespread alleles, including ACON2, IDH1, PGD, and PGM. For example, PGM is nearly fixed for allele *b*, except for the

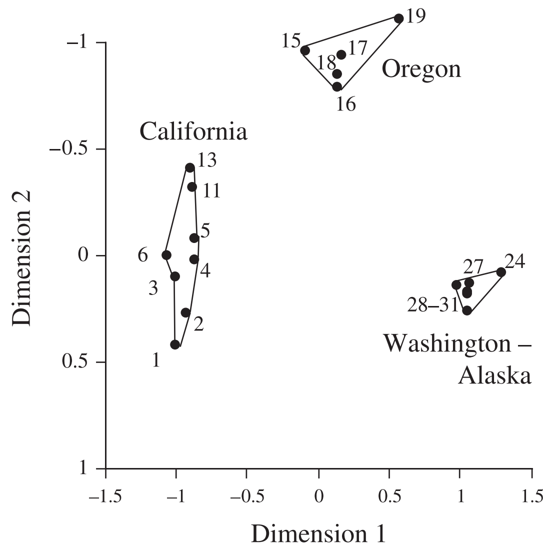

Fig. 2 Results of the multidimensional scaling analysis. Clusters of populations are identified and labelled according their geography (see text for caveats). Population numbers correspond to Table 1, Fig. 1.

a allele at low frequency in population 3. Also in the California cluster, the GDA locus is nearly fixed for the *a* allele (the exceptions are that allele *b* is present at low frequency in population 11, and in the Oregon cluster population 15 is also fixed for the *a* allele). Among Oregon cluster populations, two loci are strongly differentiated. At LDH2, allele *a* is present in all populations, and outside of the Oregon cluster is only found in Washington State (24). At PGD, allele *b* is nearly fixed in the Oregon cluster, and is absent from the Washington–Alaska cluster; however, it is also common in the California region. In the WA–AK cluster, G3PDH, NADH1, and PGD are all nearly fixed for unique alleles, except for low-frequency variants in single populations belonging to other clusters (Appendix).

The average Nei's D_N among populations within clusters is low, ranging from 0.013 among the WA–AK cluster populations to 0.042 among the California cluster populations (Table 3). Nei's genetic distances among clusters (nearest neighbour comparisons) are higher: between the California and Oregon clusters (13–15) $D_N = 0.082$, and between the Oregon and Washington clusters (19–24) $D_N = 0.108$. The largest D_N within the species is 0.247, between populations 6 and 24 (California vs. Washington).

Isolation by Distance. The relationship between geographical and genetic distance for all populations sampled is complex, forming two discrete clusters of points, as if two regressions ought to be made (Fig. 3a; Mantel test $P < 0.0001$; $r^2 = 0.42$). Populations from central coastal California (1) to Washington State (24) possess a linear pattern of IBD (Fig. 3b; $P < 0.0001$) with a higher coefficient of determination ($r^2 = 0.79$) than for the complete data set. This is the optimal IBD result in that it gives the highest r^2 value of any contiguous geographical sample of populations. Note that the Washington sample (24) fits the isolation by distance model quite well (Fig. 3b). North of Washington (27–31), there is no relationship between geographical and genetic distance from southern British Columbia to Alaska (Fig. 3b; $r^2 = 0.05$; $P = 0.43$). When the populations are grouped to match the MDS clusters (Fig. 3c), the fit to the data is not as good as the optimal IBD grouping ($r^2 = 0.19–0.39$). IBD is significant in the California cluster ($P = 0.004$) and the Washington–Alaska cluster ($P = 0.03$) but is not significant in the Oregon cluster ($P = 0.16$), although the sample size in this cluster is small (four populations). When the allozyme data is put into the same population groupings as the optimal mtDNA IBD pattern (see *mtDNA variation: Regional Diversity*) (Fig. 3d), the fit to the data is good but lower than the optimal allozyme IBD pattern ($r^2 = 0.71$ vs. 0.79; $P < 0.0001$). IBD is identified between populations from Washington to Alaska ($r^2 = 0.39$; $P = 0.03$).

Phylogenetic Results. Fig. 4 shows the results of an unrooted neighbour joining analysis of Cavalli-Sforza & Edwards (1967) chord distances. Populations from central to northern California (1–13) form a well-supported monophyletic clade (bootstrap = 97%); nested within this clade, populations from Santa Cruz and San Mateo Counties, CA form a monophyletic clade (bs = 85%). Populations from Washington to Alaska also form a well-supported clade (bs = 96%), as do populations from British Columbia to Alaska (bs = 71%). On the whole, there is a general trend for populations to be ordered in a manner reflective of geographical proximity.

MtDNA variation

Regional Diversity. Sequence divergence between the ingroup and outgroups is high — average uncorrected divergence between *T. granulosa* and *T. rivularis* is 11.1% (maximum likelihood corrected divergence = 19.3%), and the average uncorrected divergence between *T. granulosa* and *Notophthalmus viridescens* is 16.0% (ML corrected = 37.2%). Within *T. granulosa*, in contrast, sequence divergence is relatively low, with a maximum divergence of 2.8% (3.1% ML corrected). Many geographically adjacent populations differ by only a single mutational step (Fig. 5a). Despite the low divergence, there is strong geographical structure among haplotypes in central California (1–4), with each population possessing its own unique haplotypes. In contrast, in northern California, one haplotype was found in Mendocino (6), Humboldt (7), Trinity (8), and Shasta (10) Counties (Fig. 5b); satellite haplotypes were found in Tehema (14) and Shasta (9, 12) Counties. In the north, one

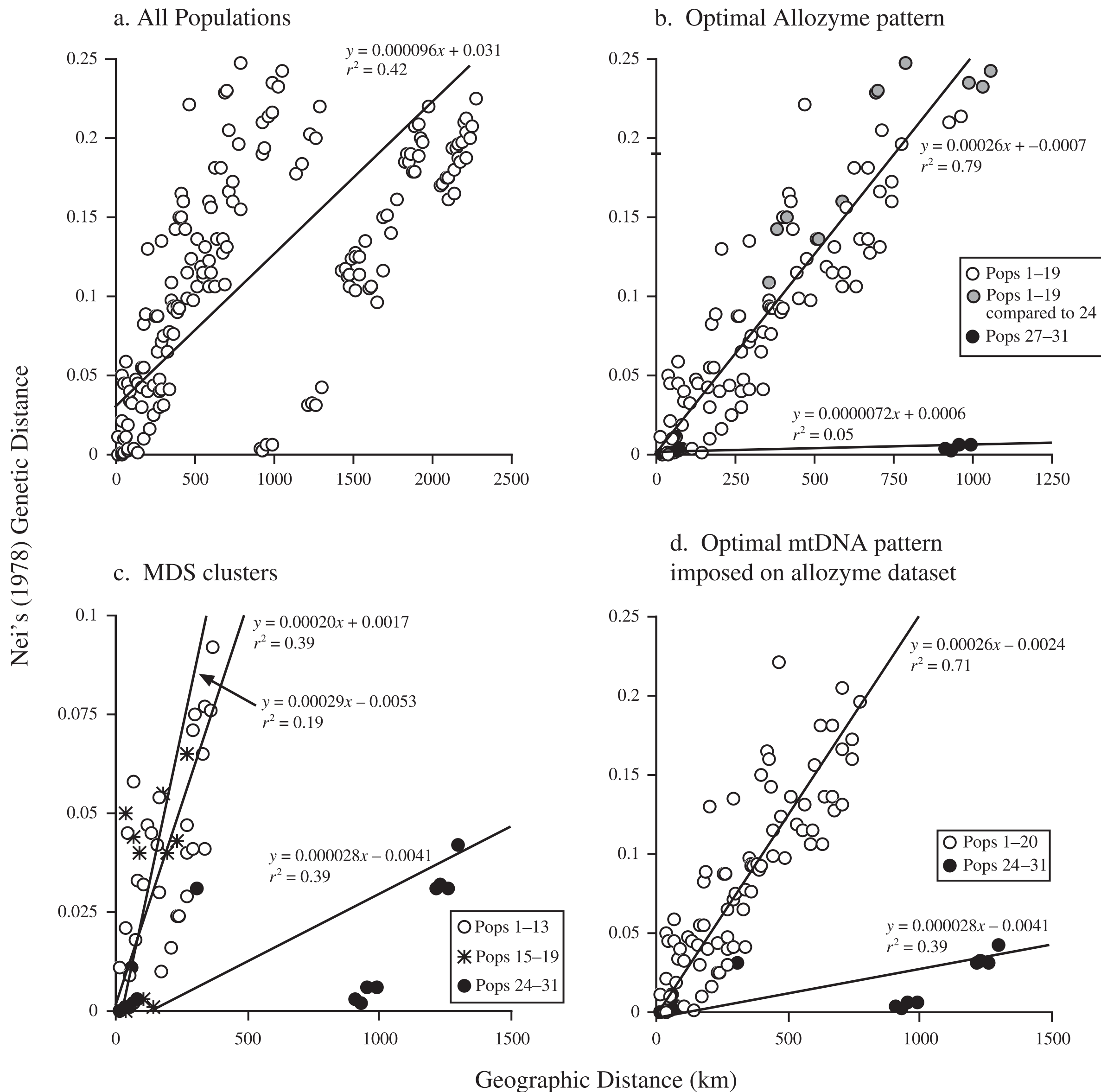

Fig. 3 (a) Relationship between geographical distance (km) and Nei's (1978) genetic distance (D_N) among all populations. (b) The 'optimal' IBD pattern determined for the allozyme data (defined as the regression with the highest r^2 value; see text). Regressions are calculated separately for populations 1–24 and 27–31. (c) Tests of IBD for clusters identified in the MDS analysis (Fig. 2). (d) The 'optimal' IBD pattern for the mtDNA data (see text; Fig. 6), applied to the allozyme data.

haplotype is broadly distributed from northern CA (15) to Alaska (32); satellite haplotypes were found in Latah County, ID (25) and Pacific County, WA (21).

Among the longer sequences in data set #2, haplotypes between Oregon (20) and Alaska (32) differ by a maximum of five bp (Fig. 5b). Unlike central California populations, haplotypes in the north tend to be widely distributed. For example, one haplotype was found in populations 21, 22, 23, 26, and 32 (from Benton Co., OR to Juneau Co., AK); another haplotype (differing from the first by 3 bp) was found in populations 20, 22, and 23 (Benton Co., OR, to Pierce Co., WA). Finally, two individuals from a population in Idaho (25) share an identical, unique haplotype. It is located at a tip of the haplotype networks (Fig. 5a,b), and is one base pair different from a haplotype that is broadly distributed in Oregon and Washington.

IBD patterns in the mtDNA data are similar to the allozyme data. When all the populations are considered, there are two separate clusters of points (Fig. 6a). Significant IBD is present (Mantel test $P = 0.003$), however, the coefficient of determination is low ($r^2 = 0.13$). The optimal IBD pattern (defined as the regression with the highest r^2) includes all

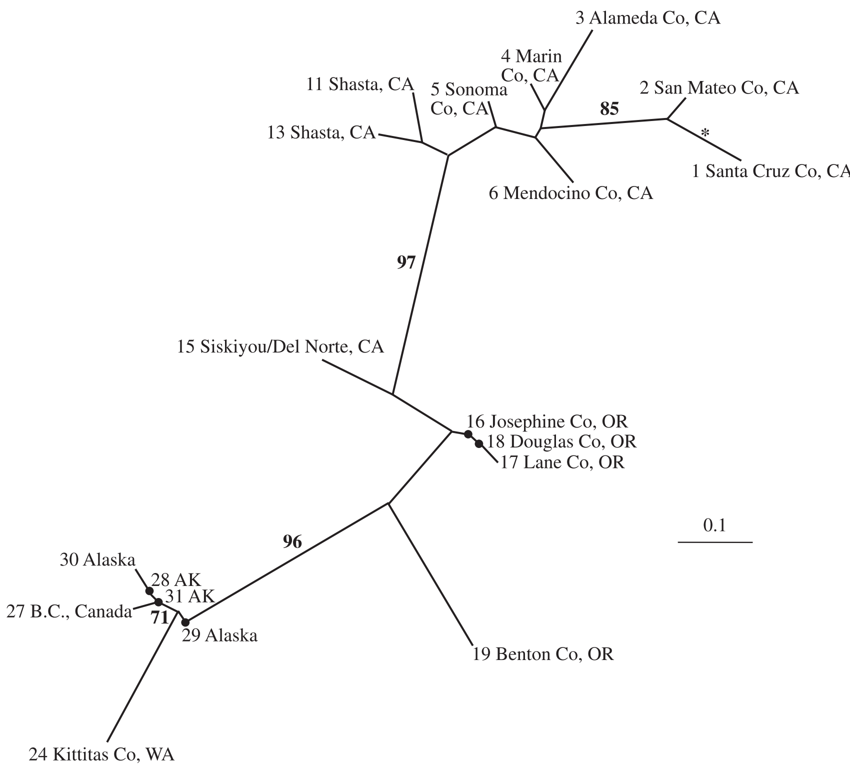

Fig. 4 Unrooted neighbour joining tree, constructed using chord distances (Cavalli-Sforza & Edwards 1967). Bootstrap support values are in bold along internodes (values < 70% not shown). The asterisk shows the root identified by the Bayesian/ML analysis of the mtDNA data (Fig. 7).

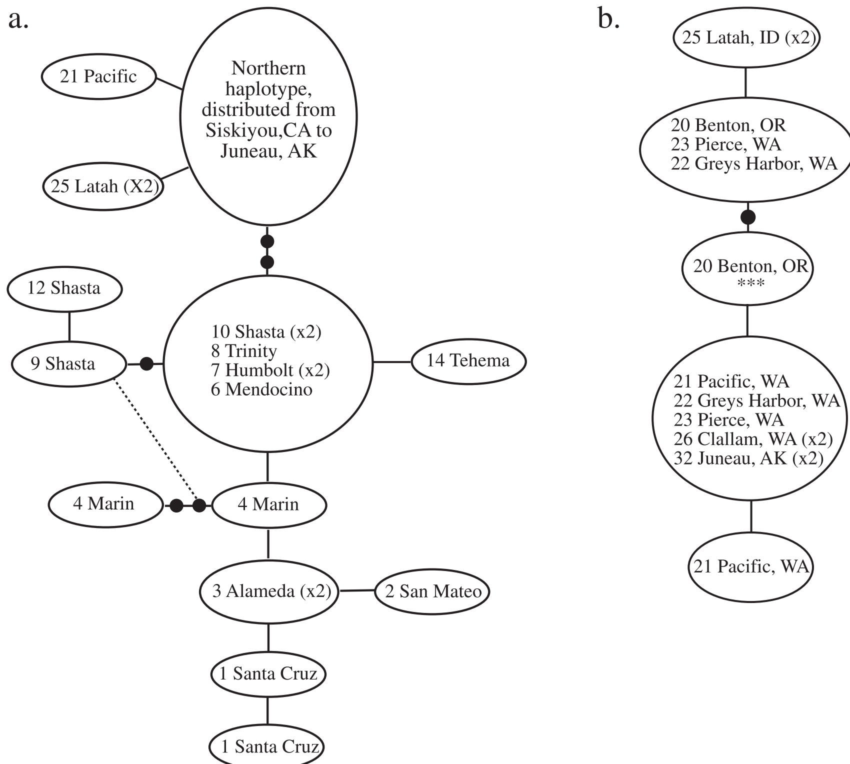

Fig. 5 Haplotype networks. (a) All populations, with longer haplotypes from Data set #2 shorted to match Data set #1 (275 bp). (b) Longer haplotypes (up to 778 bp) from Data set #2 for haplotypes found from Oregon to Alaska, where the shorter sequences in (a) show very little diversity (the 'northern haplotype' in Fig. 5a). The asterisks indicate where the network connects to haplotypes from California.

the populations from Santa Cruz Co., CA (1) to Benton Co., OR (20) ($r^2 = 0.57$; $P < 0.0001$) (Fig. 6b). No IBD is present in populations from Washington to Alaska (21–33, excluding the Idaho population, 25) ($P = 0.84$; $r^2 = 0.08$) (Fig. 6b). Comparisons between populations from Washington–Alaska with the Idaho population (25) also fail to show a pattern of isolation by distance (Fig. 6b). When the MDS pattern is imposed on the mtDNA data (Fig. 6c), there

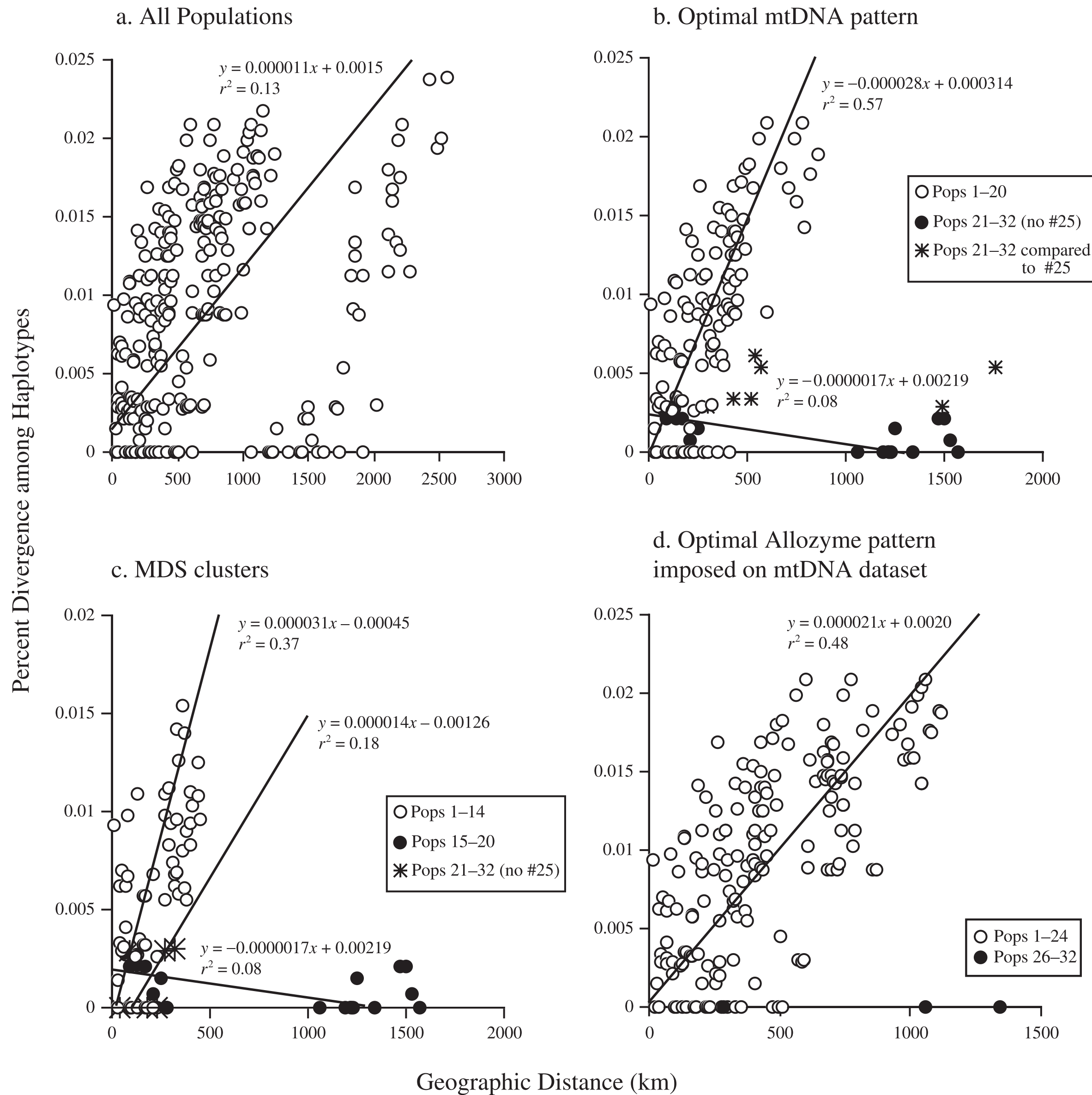

Fig. 6 (a) Relationship between geographical and mtDNA percent sequence divergence for all populations sampled. (b) The 'optimal' IBD pattern determined for the mtDNA data (defined as the regression with the highest r^2 value; see text). (c) Tests of IBD for the three clusters identified in the MDS analysis (Fig. 2). (d) The 'optimal' IBD pattern for the allozyme data (see text; Fig. 3), applied to the mtDNA data.

is significant IBD in the California cluster ($P = 0.002$; $r^2 = 0.37$). However, both the Oregon cluster and the WA–AK cluster fail to show significant IBD ($P > 0.05$; $r^2 = 0.18$ and 0.08, respectively). When the optimal IBD pattern for the allozyme data is applied to the mtDNA (Fig. 6d), there is significant IBD detected ($P < 0.0001$), but the coefficient of determination is lower than the optimal mtDNA pattern ($r^2 = 0.48$ vs. 0.57).

Nucleotide diversity (Nei 1987) was calculated for the California populations and the Oregon to Alaska populations separately (Table 4). For the California to Oregon region (corresponding to the optimal mtDNA IBD model; see above), $\pi = 0.0090$, and for the Washington to Alaska region, $\pi = 0.0026$. These values are significantly different ($t = -5.78$; $P < 0.001$), and show that northern populations have reduced nucleotide diversity relative to southern populations.

Phylogenetics. Within *T. granulosa*, plots of transversions and first and second position transitions against a maximum likelihood estimate of genetic distance do not suggest mutational saturation; however, between ingroup and outgroup sequences a plot of third position transitions

Table 4 Regional diversity estimates based on mtDNA cytochrome *b* sequences

Region	Number of populations	Number of individuals	Number of haplotypes	Number of usable base pairs*	Number of polymorphic sites	Nucleotide diversity† π (SD)
California to Oregon	18	23	18	338	13	0.009 (0.005)
Washington to Alaska	11	16	11	349	2	0.001 (0.001)

*Sites with > 5% missing data were excluded.
†Necleotide diversity (π) is, for the region, the probability that two randomly sampled homologous nucleotides are different (Nei 1987).

does show mutational saturation (data not shown; Kuchta 2002). Accordingly, phylogenetic methods incorporating a model of evolution were employed to improve the odds of correctly rooting the tree. Five separate Bayesian runs with separate random starting trees converged on similar log likelihood values after about 3000 generations, and topology, branch length values, and substitution parameter estimates were also stable and similar. Thus the posterior probability values did not become stuck on local optima in separate runs. To be conservative, the first 10 000 generations were excluded as 'burn in' from the first run. This left 4900 trees, from which a majority rule consensus tree was constructed.

T. granulosa is recovered as monophyletic in both Bayesian and ML analyses (Bayesian posterior probability = 100%; bootstrap value = 88%), but phylogenetic support for structure within *T. granulosa* is limited (Fig. 7). The overall pattern is that central California populations (e.g. Santa Cruz County) are basal to more northern populations in a stepwise manner. Populations from Marin County, CA northward form a weakly supported clade (pp = 91; bs = 60), and, a clade including all the populations between Benton County, OR and Alaska is well supported (*P* = 100%; bs = 98). Within this northern clade are two clades (pp = 100 & 98%; bs = 86 & 66%); however, haplotypes from both clades co-occur in populations 22 and 23. Finally, samples from Latah Co. ID are sister to a haplotype recovered from Oregon and Washington populations (*P* = 100%; bs = 86%).

Discussion

Isolation by distance

Isolation by distance, the accumulation of genetic divergence among populations under geographically restricted dispersal (Wright 1943; Slatkin 1993), characterizes populations in the southern part of the range of *Taricha granulosa*. This is shown by both mtDNA and allozyme data sets, although they differ in their identification of the geographical scope of IBD. In the allozyme data, IBD characterizes populations from central California north to Washington State (population 24) (Fig. 3b), whereas the mtDNA data set identifies IBD to northern Oregon (20) (Fig. 6b). To some extent, this may reflect a sampling discrepancy: in Washington state, one population was sampled for allozymes, whereas five were sampled for mtDNA sequence variation (Table 1; Fig. 1). Nevertheless, for the allozyme data, comparisons between Washington State (24) and more southerly populations (1–19) are consistent with the IBD model (Fig. 3b). In contrast, for the mtDNA data, comparison between Washington State populations (21–24, 26) and more southerly populations all show less genetic divergence than expected given the geographical distances involved (Fig. 8) — that is, all comparisons involving the Washington State sample fall to the right of the regression line identifying IBD among populations from California through Oregon (1–20). Thus, it appears the discrepancy in IBD patterns among the data sets is real, albeit denser sampling for allozymes in Washington and northern Oregon could make this incongruence geographically smaller than it currently appears.

Postglacial range expansion

Because rapid range expansions, especially those involving long-range dispersal during the colonization process, are thought to involve repeated founder events, reduced population structure and genetic variation is expected in formerly glaciated regions (Hewitt 1996, 1999; Ibrahim *et al*. 1996; Hewitt & Ibrahim 2001). Both the allozyme data and the mtDNA data show such evidence of rapid range expansion. In the allozyme data, expansion from southern British Columbia to SE Alaska is indicated: no significant pattern of IBD was found (Fig. 3b), and Nei's D_N is < 0.01 over > 900 km. One complication with this interpretation is that, of the five northern populations sampled for allozymes, four are from islands. Thus, an alternative explanation for the reduced diversity in the north is that allelic diversity was lost during island colonization. This may be partly

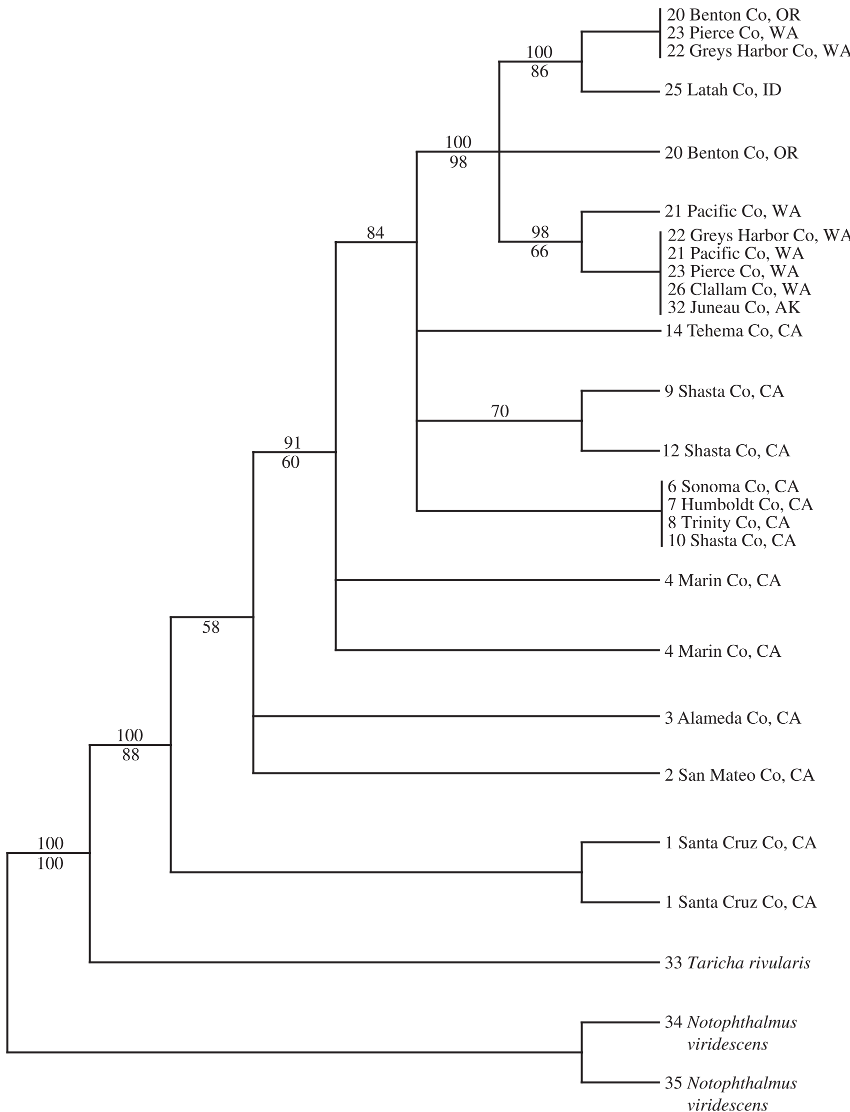

Fig. 7 Majority-rule consensus of 4900 Bayesian trees; ML gives a similar but less resolved reconstruction. Posterior probabilities are shown above branches, and indicate Bayesian support for the node; ML bootstrap values are provided below branches (values < 50% not shown).

responsible for the observed lack of variation; however, population 31 is not on an island, yet has an allelic profile nearly identical to the nearby island populations (28–30), as well as to the Vancouver Island sample (27) (Appendix). In addition, if islands were colonized from a more genetically diverse mainland source, one would expect founder effects and genetic drift to result in genetically distinctive populations on each island. Instead, populations 28–31 are nearly identical: the average genetic distance between mainland population 31 and populations 28–30 (Alaska) is $D_N = 0.0013$, and between 31 and 27 (Vancouver Island) $D_N = 0.003$.

The mtDNA expansion signature, in contrast to the allozyme data, has its southern limit in Washington (24): from Washington to SE Alaska no significant pattern of IBD was found (Fig. 6b). The topology of the mtDNA haplotype network is also congruent with range expansion. Among the shorter sequences (data set #1 and #2 combined; see *Materials and Methods*), one haplotype is distributed from Siskiyou Co., in northern CA, to Juneau Co., AK (Fig. 5a). This pattern is unlike this distribution of haplotypes in central California populations, in which each population possesses unique haplotypes (Fig. 5a). Longer sequences (data set #2) from Oregon to Alaska do show some haplotype diversity. However, it primarily stems from two geographically widespread haplotypes that co-occur in Oregon and Washington (Fig. 5b). Finally, the average number of nucleotide differences per site (π) is significantly less between the Washington to Alaska region and the California to Oregon region, which is also consistent with a northern range expansion from a southern refugium.

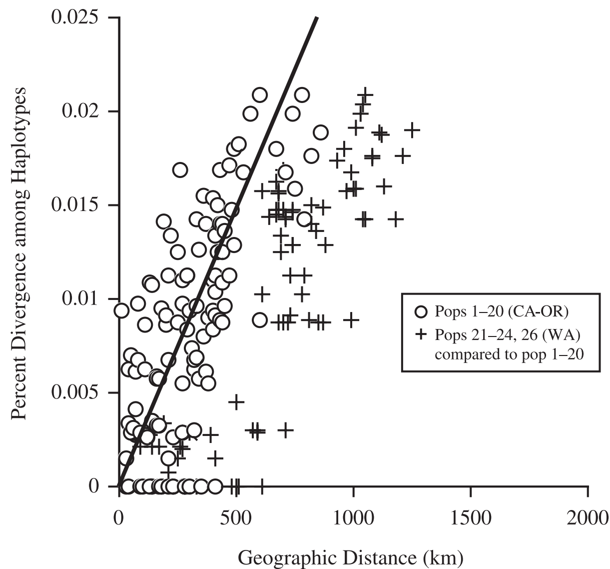

Fig. 8 Relationship between geographical distance (km) and mtDNA percent sequence divergence. This is similar to Fig. 6b, but comparisons between Washington (21–25, 26) and populations to the south (1–20) are highlighted (plus symbols vs. open circles). The RMA regression goes through populations 1–20 ($r^2 = 0.57$). Note that all the comparisons with Washington populations (21–23, 26) fall to the right of the regression, indicating less genetic change than expected given the distances involved.

Biogeography and the discordance between data sets

As discussed above, the boundary between the region characterized by IBD and the region characterized by range expansion is geographically discordant between the allozyme and mtDNA data sets. Different transmission biology and resolution may be the cause. MtDNA has a low-effective population size (N_e) relative to nuclear loci because it is haploid and maternally transmitted; the rule of thumb is the N_e of mtDNA is one quarter that of nuclear loci (Moore 1994). As a result, single populations typically maintain only a small number of closely related haplotypes (Avise 2000). It follows that when an expansion originates from a single source population, low levels of diversity are expected to typify the recently colonized region. If the expansion involves leptokurtic (long range) dispersal, which imposes diversity-reducing population bottlenecks at the leading edge of the expansion, even lower diversity may result (Ibrahim *et al*. 1996; Hewitt 2000).

The resolution of allozyme variation differs from mtDNA haplotype variation. Allozymes are diploid and encoded by nuclear loci, but have lower resolution because only amino acids substitutions can be detected. Divergence among populations here is summarized by Nei's (1978) D_N. As with mtDNA, an expansion involving leptokurtic dispersal is expected to result in low levels of divergence among populations in a recently colonized region. On the other hand, allozymes may not necessarily suffer a reduction in intrapopulational measures of diversity (e.g. heterozygosity, polymorphism) during an expansion if that expansion does not involve leptokurtic dispersal, even if there is a single source population.

With this in mind, we hypothesize that *T. granulosa* expanded north out of present-day northern California prior to the last glaciation, perhaps with the Klamath/Siskiyou region functioning as a refuge (Soltis *et al*. 1997). Prior to (and during) the last glacial maximum, the Pacific coast of North America remained forested (Barnosky *et al*. 1987; Whitlock 1992; Hewitt & Ibrahim 2001), and it would have been possible for populations of *T. granulosa* to expand northward into coastal Washington State. Such an expansion may have been relatively slow, and/or gene flow among populations may have restored genetic variation lost by expanding populations. The result is that populations from California to Washington show IBD for allozymic variation (Fig. 3b). Nonetheless, mtDNA haplotype diversity north of northern CA is limited, is not sorted geographically, and remains as a relict of past expansion (Fig. 5b). IBD in the mtDNA data sets extends to Benton County, OR, but does not include Washington State (Figs 6b and 8). A subsequent recent, rapid expansion of *T. granulosa* north into Alaska is suggested by the lack of diversity in both the mtDNA and allozyme data sets (Figs 3, 5a and 6). The Cordilleran ice sheet began to recede about 14 500–14 000 years ago, and *T. granulosa* may have expanded northward toward Alaska as early as 10 000 years ago, when the ice rapidly retreated, exposing large expanses of open land (Josenhans *et al*. 1995; Mann & Hamilton 1995). It is not clear, however, that *T. granulosa* could have inhabited the initial, early successional habitats, and thus expansion northward likely occurred even more recently.

Our results parallel many other phylogeographical studies conducted on Pacific NW taxa. For example, Soltis *et al*. (1997) found a north–south break in chloroplast DNA in six plant taxa located in central Oregon (also see Avise 2000; Brunsfeld *et al*. 2001). In all cases, there was only a single northern haplotype, while southern populations had multiple haplotypes. Interestingly, rDNA and allozyme studies of these taxa failed to identify a north–south division, unlike the current study. However, other studies have shown northern populations to exhibit reduced allozymic variation relative to southern populations (for reviews, see Soltis *et al*. 1997; Brunsfeld *et al*. 2001). Given the common pattern of geographical discordance between organellar and nuclear genomes among a diversity of organisms, it seems that the discordance is the result of how these genomes are impacted by range shifts, and is not the result of introgression or selective sweeps of organellar genomes. The smaller N_e of organellar genomes increases the probability of loss of allelic diversity at the leading edge of

an expansion, because genetic drift is a stronger force with low N_e. Also, if there is male-biased dispersal, this may further reduce the chance that mtDNA diversity will persist through an expansion event because this also lowers N_e.

Like *T. granulosa,* the salamander *Ensatina eschscholtzii* also shows reduced diversity in the Pacific NW relative to California. For mtDNA sequences, a single clade extends from northern California to British Columbia, whereas several highly divergent clades are found in northern California (Moritz *et al*. 1992; Kuchta, Parks, Schneider, and Wake, unpublished data). Allozyme diversity is also greater in California than in northern populations (Wake & Yanev 1986). In contrast to *E. eschscholtzii* and *T. granulosa*, many other salamanders show large genetic breaks between Pacific Northwest and more southern populations. For example, *Rhyacotriton kezeri* and *R. variegates* (Good & Wake 1992), *Dicamptodon tenebrosus* and *D. ensatus* (Good 1989), and *Aneides vagrans* and *A. ferreus* (Jackman 1998) all show deep divergence in allozymes among sister taxa in the region between central Oregon and northern California.

Status of disjunct populations in Idaho

The presence of *T. granulosa* populations in the northern Rocky Mountains of Idaho is of biogeographical interest. Nussbaum & Brodie (1971) analysed the body proportions of 50 specimens from an Idaho population, and compared their data with Riemer (1958) and additional populations from Oregon and Washington. They found that the Idaho populations were most similar to populations from the southern part of the range of *T. granulosa* (e.g. Santa Cruz County, CA) in head shape and pigment distribution, and concluded that the Idaho populations were introduced from central coastal California. The mtDNA data presented here reject this hypothesis, because the haplotype from Idaho is unique, and, more importantly, is one base pair different from another haplotype broadly distributed throughout Washington and Oregon. Taken together, the genetic and morphometric data suggest the Idaho populations could be native. Alternatively, it is conceivable that the Idaho populations were introduced from Oregon or Washington, and the morphometric differences documented by Nussbaum & Brodie (1971) a result of phenotypic plasticity, sampling artifacts, or a rapid response to natural selection. If the populations in Idaho are native, they are of conservation concern (Nussbaum *et al*. 1983).

Lending credence to the possibility that the Idaho populations could be native is the fact that over 100 species of plants and animals are disjunctly distributed in the mesic forests of the northern Rockies in Idaho and Montana, and the Cascades and Coast Ranges of western Washington, Oregon, and northern California ('Mesic Forest Disjunct Distribution'; Brunsfeld *et al*. 2001). Amphibian species with this distribution include *Ascaphus truei/montanus* (Ascaphidae) (Nielson *et al*. 2001), *Dicamptodon ensatus/tenebrosus/atterimus* (Dicamptodontidae) (Good 1989), *Plethodon vandykei/idahoensis* (Plethodontidae) (Howard *et al*. 1993), and *Rana pretiosa/luteiventris* (Ranidae) (Green *et al*. 1996). In all these instances, sister taxa are found between the northern Rocky Mountains and the mesic forests of Washington and Oregon. However, this is not the situation in other taxa, such as the plants *Tiarella trifoliata* (Saxifragaceae) (Soltis *et al*. 1992), *Alnus rubra* (Betulaceae) (Soltis *et al*. 1997), and *Polystichum munitum* (Dryopteridaceae) (Soltis *et al*. 1997), and the lizard *Elgaria coerulea* (Anguidae) (Good 1985). Brunsfeld *et al*. (2001) reviewed the comparative phylogeography of western North America, and proposed two contrasting biogeographical scenarios to explain these distributional patterns. The first is the 'Ancient Vicariance' hypothesis, which posits that Rocky mountain populations have been demographically separated from coastal mountain populations for a long time (perhaps millions of years; see Brunsfeld *et al*. 2001), as is the case with the amphibian taxa cited above. The alternative hypothesis is the 'Inland Dispersal' hypothesis, and is the result of recent colonization of the Rocky Mountains by Coast Range populations, probably either via the Okanogan Highlands in northern Washington or the Central Oregon Highlands. Most plant taxa so far studied are best explained by some version of this second hypothesis, as is the lizard *E. coerulea*. If the Idaho populations of *T. granulosa* are native, the genetic data is also most consistent with the inland dispersal hypothesis. Because the haplotype in Idaho is most similar to a haplotype found in central Oregon, the Central Oregon Highlands are the most likely dispersal corridor.

In summary, *T. granulosa* is characterized by isolation by distance in the southern part of its range, but has reduced genetic diversity in the north because of the effects of recent range expansion. This result is congruent with other phylogeographical studies of Pacific Northwest taxa, especially plants, yet differs from most other salamander species thus far studied. Finally, we showed that Rocky Mountain populations (in Idaho) are most closely related to populations in Washington and Oregon, and we suggest they could be native. More work is required, however, to deduce the history of these populations with confidence.

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Appendix

Allozyme allele frequencies in populations of *Taricha granulosa*. Clusters refer to the results of the multidimensional scaling analysis (Fig. 2).

	T. granulosa — California Cluster								*T. granulosa* — Oregon Cluster					*T. granulosa* — Washington to Alaska Cluster					
Pop. #	1	2	3	4	5	6	11	13	15	16	17	18	19	24	27	29	30	31	32
(n)	3	6	10	10	9	2	10	9	3	8	6	10	3	2	3	4	3	5	3
AAT1																			
c	1.000	1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.875	1.000
d	—	—	—	—	—	—	0.200	—	—	—	—	—	—	—	—	—	—	0.125	—
AAT2																			
a	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.500	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ACON1																			
a	—	—	0.050	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
c	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACON2																			
a	1.000	1.000	1.000	1.000	0.111	0.750	0.150	—	—	—	—	—	—	—	—	—	—	—	—
b	—	—	—	—	0.556	—	0.600	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	—	—	—	—	0.333	0.250	0.250	—	—	—	—	—	—	—	—	—	—	—	—
ADA1																			
a	0.833	0.583	1.000	1.000	1.000	1.000	0.150	0.556	0.500	0.375	—	0.100	—	1.000	0.833	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	0.700	0.444	0.167	—	—	—	—	—	—	—	—	—	—
c	0.167	0.417	—	—	—	—	0.150	—	0.333	0.625	1.000	0.900	1.000	—	0.167	—	—	—	—
ADA2																			
b	—	0.083	—	—	0.056	—	—	—	—	—	—	—	—	—	0.500	—	—	—	—
c	—	—	—	—	—	—	—	—	—	—	—	—	0.667	0.500	—	—	—	—	—
d	1.000	0.917	1.000	1.000	0.944	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.333	0.500	0.500	1.000	1.000	1.000	1.000
ADH2																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ALDO																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	1.000	—	—	—	—	—
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CA1																			
a	—	—	—	—	—	—	—	—	—	—	—	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	—	—	—	—	—	—
c	1.000	1.000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
EST2																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Appendix *Continued*

	T. granulosa — California Cluster								*T. granulosa* — Oregon Cluster					*T. granulosa* — Washington to Alaska Cluster					
Pop. #	1 3	2 6	3 10	4 10	5 9	6 2	11 10	13 9	15 3	16 8	17 6	18 10	19 3	24 2	27 3	29 4	30 3	31 5	32 3
ESTD																			
a	1.000	1.000	0.700	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	0.300	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G3PHD																			
a	—	—	—	—	—	—	—	—	—	—	—	—	—	1.000	1.000	1.000	0.833	1.000	1.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	—	—	0.167	—	—
GDA																			
a	1.000	1.000	1.000	1.000	1.000	1.000	0.900	1.000	1.000	—	—	—	—	—	—	—	—	—	—
b	—	—	—	—	—	—	0.100	—	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IDH1																			
a	0.167	0.250	0.100	—	—	—	—	—	—	—	0.083	0.050	—	—	—	—	—	—	—
b	0.333	0.500	0.900	0.650	0.389	—	—	0.056	1.000	1.000	0.917	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.500	0.250	—	0.350	0.611	1.000	1.000	0.944	—	—	—	—	—	—	—	—	—	—	—
LA1																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LA2																			
a	1.000	1.000	0.450	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	0.550	0.100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LDH1																			
a	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.333	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LDH2																			
a	—	—	—	—	—	—	—	—	1.000	0.125	0.333	0.200	1.000	0.500	—	—	—	—	—
b	0.333	0.667	1.000	1.000	1.000	1.000	1.000	1.000	—	0.875	0.667	0.800	—	0.500	1.000	1.000	1.000	1.000	1.000
c	0.667	0.333	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LGG																			
b	—	0.167	—	0.200	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
c	1.000	0.583	1.000	0.800	1.000	1.000	0.900	0.889	1.000	1.000	0.917	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000
d	—	0.250	—	—	—	—	0.100	0.111	—	—	0.083	0.100	—	—	—	—	—	—	—
MDH1																			
a	0.500	0.500	0.850	0.400	0.500	0.750	0.400	0.555	—	0.063	0.083	0.100	—	—	—	—	—	—	—
c	—	0.167	0.050	—	0.278	—	—	0.167	—	—	—	—	—	—	—	—	—	—	—
d	0.500	0.333	0.100	0.600	0.222	0.250	0.600	0.278	1.000	0.938	0.917	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Appendix *Continued*

	T. granulosa — California Cluster								*T. granulosa* — Oregon Cluster					*T. granulosa* — Washington to Alaska Cluster					
Pop.#	1	2	3	4	5	6	11	13	15	16	17	18	19	24	27	29	30	31	32
	3	6	10	10	9	2	10	9	3	8	6	10	3	2	3	4	3	5	3
NADH1																			
a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
d	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	—	—	—	—	—	—
e	—	—	—	—	—	—	—	—	—	—	—	—	0.333	1.000	1.000	1.000	1.000	1.000	1.000
NADH2																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ODH																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PAP																			
b	1.000	0.917	0.900	1.000	0.889	1.000	1.000	0.667	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.667	1.000	0.300	0.750
c	—	0.083	0.100	—	0.111	—	—	0.333	—	—	—	—	—	—	0.333	0.333	—	0.700	0.250
PGD																			
a	—	—	—	—	—	—	—	—	—	—	—	0.100	—	1.000	1.000	1.000	1.000	1.000	1.000
b	—	0.167	0.100	0.250	0.111	—	0.550	0.889	1.000	1.000	1.000	0.900	1.000	—	—	—	—	—	—
d	1.000	0.833	0.900	0.450	0.889	1.000	0.450	0.111	—	—	—	—	—	—	—	—	—	—	—
e	—	—	—	0.300	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PGM																			
a	—	—	0.200	—	—	—	—	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000	—	—	—	—	—	—	—	—	—	—	—
SOD																			
a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
XDH																			
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
% poly morphic loci	13.3	17.8	17.8	11.1	13.3	4.4	15.6	13.3	2.2	6.7	8.9	13.3	4.4	4.4	6.7	2.2	2.2	4.4	2.2
Observed Hetero-zygosity (SD)	0.030 (0.014)	0.070 (0.031)	0.033 (0.015)	0.029 (0.013)	0.022 (0.018)	0.022 (0.016)	0.044 (0.023)	0.012 (0.010)	0.022 (0.022)	0.008 (0.006)	0.011 (0.006)	0.020 (0.009)	0.000 (0.000)	0.000 (0.000)	0.044 (0.027)	0.015 (0.015)	0.007 (0.007)	0.019 (0.014)	0.011 (0.011)
Expected Hetero-ygosity (SD)	0.074 (0.029)	0.080 (0.029)	0.050 (0.018)	0.049 (0.022)	0.051 (0.023)	0.022 (0.016)	0.063 (0.024)	0.048 (0.021)	0.016 (0.016)	0.019 (0.012)	0.022 (0.012)	0.027 (0.011)	0.024 (0.017)	0.030 (0.021)	0.033 (0.019)	0.012 (0.012)	0.007 (0.007)	0.016 (0.012)	0.010 (0.010)

Invariable Loci: ADH1, AK, ALDH, CA2, CK, EST1, G6PD, GAPDH, GDH, GLUD, HADH, IDH2, ME, MDH2, MPI, SORD.