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RESEARCH ARTICLE

Population structure and conservation genetics of the Oregon spotted frog, *Rana pretiosa*

Michael S. Blouin · Ivan C. Phillipsen · Kirsten J. Monsen

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Abstract The Oregon spotted frog (*Rana pretiosa*) is one of the most threatened amphibians in the Pacific Northwest. Here we analyzed data from 13 microsatellite loci and 298 bp of mitochondrial DNA in frogs collected from 23 of the remaining *R. pretiosa* populations in order to (1) assess levels of genetic diversity within populations of *R. pretiosa*, (2) identify the major genetic groups in the species, (3) estimate levels of genetic differentiation and gene flow among populations within each major group, and (4) compare the pattern of differentiation among *R. pretiosa* populations with that among populations of *R. cascadae*, a non-endangered congener that also occurs in Oregon and Washington. There is a strong, hierarchical genetic structure in *R. pretiosa*. That structure includes six major genetic groups, one of which is represented by a single remaining population. *R. pretiosa* populations have low genetic diversity (average $H_e = 0.31$) compared to *R. cascadae* (average $H_e = 0.54$) and to other ranid frogs. Genetic subdivision among populations is much higher in *R. pretiosa* than in *R. cascadae*, particularly over the largest geographic distances (hundreds of kilometers). A joint analysis of migration rates among populations and of effective sizes within populations (using MIGRATE) suggests that both species have extremely low migration rates, and that *R. pretiosa* have slightly smaller effective sizes. However, the slight difference in effective sizes between species appears insufficient to explain the large difference in genetic diversity and in large-scale genetic structure. We therefore hypothesize that low connectivity among the more widely-spaced *R. pretiosa* populations (owing to their patchier habitat), is the main cause of their lower genetic diversity and higher among-population differentiation. Conservation recommendations for *R. pretiosa* include maintaining habitat connectivity to facilitate gene flow among populations that are still potentially connected, and either expanding habitat or founding additional 'backup' populations to maintain diversity in the isolated populations. We recommend that special consideration be given to conservation of the Camas Prairie population in Northern Oregon. It is the most geographically isolated population, has the lowest genetic diversity ($H_e = 0.14$) and appears to be the only remaining representative of a major genetic group that is now almost extinct. Finally, because the six major groups within *R. pretiosa* are strongly differentiated, occupy different habitat types, and are geographically separate, they should be recognized as evolutionarily significant units for purposes of conservation planning.

Keywords Ranidae · Evolutionarily significant unit · Pacific Northwest · Amphibian declines · Genetic diversity · Gene flow

M. S. Blouin (✉) · I. C. Phillipsen
Department of Zoology, Oregon State University, 3029 Cordley Hall, Corvallis, OR 97331-2914, USA
e-mail: blouinm@science.oregonstate.edu

K. J. Monsen
Department of Biology and Molecular Biology, Montclair State University, Montclair, NJ 07043, USA

Introduction

Frog species in the genus *Rana* often show low genetic diversity within populations (Garner et al. 2003, 2004; Martínez-Solano et al. 2005; Ficetola et al. 2007) and high genetic differentiation among populations (Monsen and Blouin 2003; Palo et al. 2004a; Funk et al. 2005). This population structuring is usually attributed to behavioral

philopatry and to a limited ability to disperse long distances between aquatic habitats (Seppa and Laurila 1999; Palo et al. 2004b). Dispersal appears to be most strongly restricted in high elevation or other harsh habitats (Monsen and Blouin 2003; Funk et al. 2005). However, some ranid frogs have high genetic diversity within populations (Arens et al. 2007; Crosby et al. 2008) and little genetic differentiation among populations over appreciable distances (Newman and Squire 2001). Therefore, it cannot be assumed that all ranid frogs have genetically impoverished populations that are highly differentiated—these genetic characteristics must be assessed on a case by case basis, particularly in the context of conservation. Characterizing patterns of genetic diversity within populations and genetic differentiation among populations has become an important first step in conservation status assessments for endangered species (Allendorf and Luikart 2007). Towards that end, we here describe the species-wide genetic structure of the Oregon spotted frog (*Rana pretiosa*), one of the most threatened amphibians in the Pacific Northwest (Cushman and Pearl 2007). We also compare this genetic structure to that of a more common and non-threatened congener, *Rana cascadae*, whose range overlaps substantially with that of *R. pretiosa*. We conclude that *R. pretiosa* is subdivided into six major genetic groups, and that *R. pretiosa* populations have unusually low genetic diversity and connectivity for a ranid frog.

R. pretiosa currently occupies only 10–30% of its original range (Hayes 1997; McAllister et al. 1993), which historically spanned northeastern California, western and central Oregon and Washington in the United States, and southern British Columbia in Canada (Stebbins 2003). The species is believed to be now extinct in California and western Oregon, and it persists in only a few scattered locales in Washington, and British Columbia (Fig. 1). *R. pretiosa* is a candidate for federal listing as endangered by the U.S. Fish and Wildlife Service (2005), is listed as "sensitive-critical" by the Oregon Department of Fish and Wildlife and "endangered" in the state of Washington. It is an endangered species in Canada where it is known from only three tiny populations (Seburn and Seburn 2000). Functioning metapopulations (groups of populations connected by gene flow) probably still exist only in the central Cascades region of central Oregon, in the Klamath Basin of southern Oregon (Fig. 1), and in the Chehalis drainage south of Seattle, Washington (populations DC and BC; Fig. 1). With the exception of the Chehalis drainage pair, the few populations remaining in northern Oregon, Washington, and British Columbia are geographically isolated and almost certainly on independent evolutionary trajectories. Therefore, for the purposes of prioritizing conservation efforts in this species (e.g. delineating management units, identifying particularly at-risk or genetically unique populations) it is vital to understand the

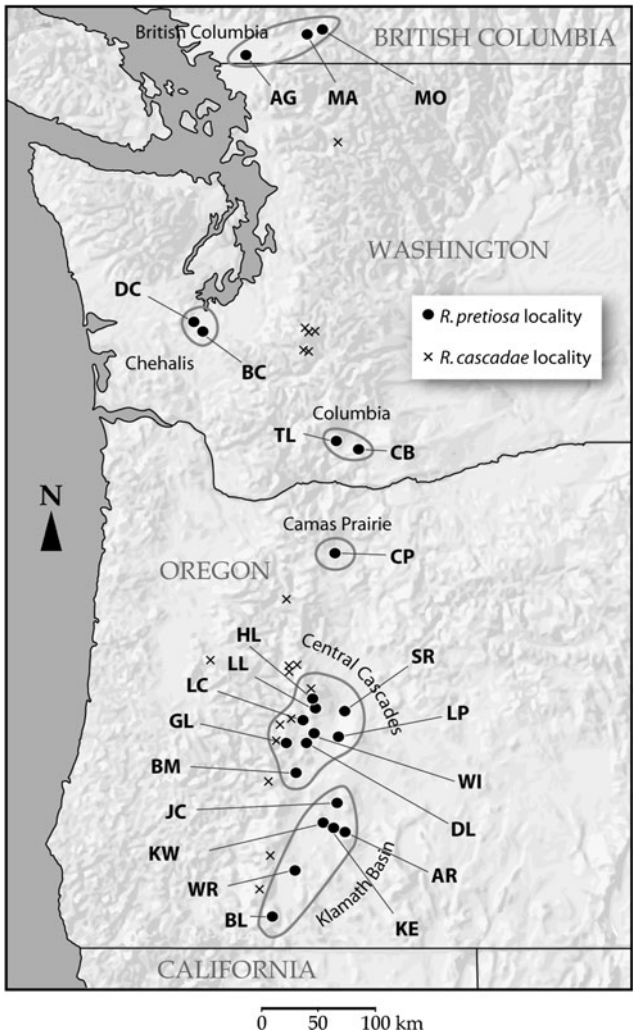

Fig. 1 Map of sampling localities for *R. pretiosa* in the Pacific Northwest. Localities are shown as *black circles* and are labeled with their respective *two-letter codes* (Table 1). The six major genetic groups of populations identified in this study are outlined and labeled. Sampling localities of *R. cascadae* from Monsen and Blouin (2004) are shown as small 'x' symbols

historical genetic relationships among populations, the current patterns of genetic diversity within populations, and the degree of connectivity among populations. This information would be very timely given the species is under review for listing as endangered in the US, and given captive breeding and reintroduction programs are already underway in the US and in Canada (Chelgren et al. 2008; MP Hayes, pers. comm.; P Govindarajulu, pers. comm).

Here we analyzed data from microsatellite and mitochondrial loci collected from most of the remaining *R. pretiosa* populations. The goals of this study were to (1) assess levels of genetic diversity within populations of *R. pretiosa* across its range, (2) identify the major historical genetic groups in the species, (3) estimate levels of genetic

differentiation and gene flow among populations within each of the remaining groups, and (4) compare the pattern of differentiation among *R. pretiosa* populations with that among populations of *R. cascadae*, a more continuously-distributed and non-endangered congener that also occurs in Oregon and Washington.

Methods

Sampling

23 *R. pretiosa* populations were sampled from across the species' range (Fig. 1; Table 1). These represent most of the known populations of the species, which still persists in approximately 33 known sites (Hayes 1997; Cushman and Pearl 2007). Sampling took place in 1999 and 2000, with the exception of the three Canadian populations, one of which was sampled in 1999, and all three of which were sampled in 2007. For the US populations, we took toe-clip samples from adults (samples stored in Drierite dessicant; W.A. Hammond Drierite Co., Xenia, OH). We released all sampled frogs at the site of capture. Sample sizes ranged from 26 to 63 (mean $n = 30$, median = 31; Table 1).

Because of the endangered status of the Canadian populations, we could obtain only samples from egg masses in 2007 ($n = 7, 7$ and 15 egg masses for populations AG, MO and MA, respectively; Table 1). We had previously obtained toe clips from 5 adults and eggs from 8 masses from population AG in 1999. For the egg mass samples we analyzed only one egg per mass in order to avoid including siblings in the population sample. Nonetheless, due to the sampling of eggs and to the small sample sizes for the Canadian populations, we do not include these populations when comparing genetic diversity among populations. Also, we recognize that genetic distances involving these populations may be slightly inflated relative to values one would obtain using randomly-sampled adults.

Microsatellite loci—amplification and genotyping

Each population except the 1999 British Columbia sample was assayed for allelic variation at thirteen microsatellite loci ($n = 26$–44 individuals scored per population, excluding the British Columbia egg samples; Table 1). These loci were developed for *R. pretiosa* (loci with "RP" prefix) or for *R. luteiventris* (loci with "SFC" prefix), which is the sister species of *R. pretiosa* (Green et al. 1997; Hillis and Wilcox 2005; Funk et al. 2008). PCR amplifications (25 µl) were carried out with the components and conditions as described in Monsen and Blouin (2004), but with locus-specific annealing temperatures and fluorescently-labeled forward primers (see Appendix A for primer sequences). One locus (RP385) would not amplify cleanly in the samples from the Klamath Basin (JC, KW, KE, AR, WR, and BL populations), but gave clean, scorable bands from all other populations. Therefore, we removed this locus before estimating genetic structure among the entire set of 23 populations. We used all 13 loci to examine genetic structure within groups of populations other than the Klamath Basin. Another locus (RP15) is a polymorphic dinucleotide $(AT)_n$ repeat locus in the Klamath Basin, but is monomorphic for a single allele in all other populations. Sequencing revealed that most of the repeats were lost to a large deletion in those other populations. Therefore, we excluded this locus as well as RP385 when comparing genetic diversities among all populations.

Microsatellite loci analysis

We tested for deviations from Hardy–Weinberg and genotypic equilibrium within populations using GENEPOP version 3.2a (Raymond and Rousset 1995). To estimate genetic diversity within populations, we calculated average heterozygosity (H_e) and allelic richness (AR). These measures were calculated using data from the 11 loci that were homologous in all populations. Allelic richness for each population was estimated at a common sample size of 15 via rarefaction (hereafter AR_{15}) using the program POPULATIONS (Langella 1999). This sample size reflects the minimum number of individuals in a population that were scored for all 11 loci (i.e. had no missing data). Genetic diversity was not estimated for the three Canadian populations (AG, MO, MA) or for the KW population due to small sample sizes and/or missing data for these populations.

We used information from three methods to identify the hierarchical pattern of genetic structure in this species: (1) a STRUCTURE analysis (Pritchard et al. 2000), (2) a bootstrapped neighbor-joining tree based on Nei's unbiased genetic distance (Nei 1978) between pairs of populations (POPULATIONS software, Langella 1999), and (3) a principle coordinates analysis (PCoA) of genetic distances between individuals (Smouse and Peakall 1999) using GenAlEx (Peakall and Smouse 2006).

Assuming that we would find a hierarchical pattern of genetic structure, we first performed the STRUCTURE analysis using the entire dataset of 685 individuals with K (the hypothesized number of distinct genetic groups) from 1 to 15. We then carried out separate analyses on each major group identified by the first analysis (K from 1 to 10). For each value of K we carried out 20 independent runs under the correlated allele frequencies model allowing admixture. Each run had a total of 1×10^6 iterations with a burn-in of 5×10^4 iterations. For each value of K, we calculated the mean and standard deviation of ln Pr($X|K$) (the estimated likelihood of K) across the 20 runs. We also used the ΔK

Table 1 Locality information for the sampled *R. pretiosa* populations

Locality	Code	Lat.	Long.	Elev. (m)	*n* (mtDNA)	SSCP results	*n* (MS)	H_e	*AR*	AR_{15}	Genetic group
British Columbia, CA											
Aldergrove (1999)	AG	49.072	−122.473	116	13	13 type J[a]	–	–	–	–	British Columbia
Aldergrove (2007)	AG	49.072	−122.473	116	–		7				
Mountain Slough	MO	49.265	−121.691	17	–		7	–	–	–	British Columbia
Maria Slough	MA	49.238	−121.848	18	–		15	–	–	–	British Columbia
Washington, USA											
Dempsey Creek	DC	46.967	−123.000	42	34	34 type A[b]	30	0.47	3.15	3.07	Chehalis Drainage
Beaver Creek	BC	46.883	−122.917	77	26	26 type A[c]	26	0.48	3.38	3.27	Chehalis Drainage
Trout Lake	TL	46.017	−121.533	596	37	37 type C	36	0.17	2.15	1.91	Columbia Drainage
Conboy Wildlife Refuge	CB	45.950	−121.317	555	48	48 type C, 1 type A[d]	39	0.50	4.38	3.98	Columbia Drainage
Oregon, USA											
Camas Prairie	CP	45.137	−121.569	962	25	25 type D	29	0.14	1.69	1.64	Camas Prairie
Hosmer Lake	HL	43.970	−121.773	1518	30	26 type A, 4 type G	31	0.24	1.92	1.87	Central Cascades
Little Cultus Lake	LC	43.803	−121.874	1451	–	–	34	0.25	2.08	2.02	Central Cascades
Little Lava Lake	LL	43.911	−121.757	1445	43	34 type A, 9 type G	37	0.22	1.85	1.78	Central Cascades
Wickiup Reservoir	WI	43.700	−121.771	1325	30	30 type A	32	0.24	1.85	1.85	Central Cascades
Sunriver	SR	43.880	−121.446	1267	63	40 type A, 23 type B	38	0.29	2.31	2.28	Central Cascades
La Pine	LP	43.683	−121.516	1282	–	–	32	0.28	2.54	2.36	Central Cascades
Davis Lake	DL	43.636	−121.857	1346	30	30 type A	31	0.32	2.38	2.38	Central Cascades
Big Marsh	BM	43.392	−121.954	1443	63	63 type A	29	0.22	2.31	2.29	Central Cascades
Gold Lake	GL	43.633	−122.043	1466	32	32 type A	31	0.20	1.85	1.75	Central Cascades
Jack Creek	JC	43.151	−121.537	1497	30	30 type A	30	0.38	2.75	2.54	Klamath Basin
Klamath Marsh East	KE	42.963	−121.586	1381	–	–	34	0.46	3.08	2.92	Klamath Basin
Klamath Marsh West	KW	42.946	−121.748	1379	–	–	27	0.48	3.25	–	Klamath Basin
Aspen Ridge	AR	42.933	−121.483	1387	–	–	31	0.36	2.75	2.61	Klamath Basin
Wood River	WR	42.623	−121.971	1263	25	20 type A, 5 type E	40	0.38	4.08	3.44	Klamath Basin
Buck Lake	BL	42.252	−122.204	1506	15	12 type A, 3 type I	44	0.30	2.92	2.83	Klamath Basin

Sample sizes (*n*) for each locality are listed for SSCP (mtDNA) and for microsatellite loci (MS). Microsatellite genetic diversity measures for each locality are given as expected heterozygosity (H_e), unrarified allelic richness (*AR*), and allelic richness rarified to a common sample size of 15 (AR_{15}). The major genetic group assignment is based on the results of the microsatellite-based STRUCTURE analysis and NJ tree

[a] Two individuals were sequenced for the full mtDNA amplicon (see Online Resource 6) in this population. 1 had an additional substitution in the right hand region (the J* haplotype; see Fig. 7)

[b] Three individuals were sequenced for the full mtDNA amplicon (see Online Resource 6) in this population. All three had an additional substitution in the right hand region (the A* haplotype; see Fig. 7)

[c] Four individuals were sequenced for the full mtDNA amplicon (see Online Resource 6) in this population. All four had an additional substitution in the right hand region (the A* haplotype; see Fig. 7)

[d] Two individuals were sequenced for the full mtDNA amplicon (see Online Resource 6) in this population, one with haplotype C and one with haplotype A (identified via SSCP). The "A" individual had an additional substitution in the right hand region (the A** haplotype; see Fig. 7)

method of Evanno et al. (2005) as an aid in identifying the most likely number of genetic groups at each level of the hierarchy.

To quantify the overall degree of genetic differentiation among populations in a way that is comparable with that in other studies, we estimated F_{ST} (Weir and Cockerham 1984) and Hedrick's (2005) standardized G'_{ST} within each of the major genetic groups that were identified by the STRUCTURE analysis. For each group of populations that we considered to be still connected by gene flow (Klamath Basin group and Central Cascades group), we performed Mantel tests (with 1,000 permutations) between the matrix

of pairwise genetic distances ($F_{ST}/(F_{ST} - 1)$) and the matrix of pairwise geographic distances (ln-transformed Euclidean distance between pairs of populations) using GenAlEx (Peakall and Smouse 2006).

Comparison with *R. cascadae*

R. cascadae is a close congener of *R. pretiosa* (Hillis and Wilcox 2005). The two species have similar ranges that run North–South through Central Oregon and Washington, although *R. cascadae* occur mostly on the wetter Western slopes of the Cascades Mountains, and *R. pretiosa* on the drier Eastern slopes (Corkran and Thoms 2006; Fig. 1). *R. cascadae* populations appear to be relatively healthy and abundant in the Cascades Mountains of Oregon and Washington, and their populations are distributed continuously in this region (Pearl and Adams 2005). We previously showed that *R. cascadae* displays a strong pattern of isolation by distance (IBD) among populations that is apparent at all geographic scales (Monsen and Blouin 2003, 2004). Thus, *R. cascadae* appears to exist as a continuously-distributed network of small populations that are connected by migration that probably approximates a stepping-stone pattern (Kimura and Weiss 1964; Monsen and Blouin 2004). *R. pretiosa* are much more patchily distributed than *R. cascadae*, presumably owing to their more aquatic habit and requirement for permanent bodies of water with both summer feeding zones and thermal refugia for overwintering (Pearl and Hayes 2004). Here we compared patterns of isolation by distance (G'_{ST} vs. geographic distance) in *R. pretiosa* and in *R. cascadae* over the same geographic scale (Fig. 1). The question was whether *R. pretiosa* shows a different pattern of IBD than *R. cascadae*, as one might expect given the patchier distribution of *R. pretiosa* populations. For this comparison we used the 18 *R. cascadae* populations assayed for microsatellite variation in Monsen and Blouin (2004). These *R. cascadae* samples were scored at 6 microsatellite loci, 4 of which were also used in this study on *R. pretiosa*. Sample sizes were slightly smaller in the *R. cascadae* samples (n between 20 and 30 for most of the *R. cascadae* populations vs. between 30 and 40 for most of the *R. pretiosa* populations). We excluded the three Canadian populations of *R. pretiosa* for the purposes of this comparison. We used the t test to test for differences in mean H_e, AR_{15}, and θ (see below) between the two species, after using the Shapiro–Wilks test to confirm that the data did not deviate from normally-distributed.

R. cascadae showed much less genetic differentiation over any given distance than *R. pretiosa* (see results below). To investigate whether this difference results from differences in migration rates or in effective sizes we used MIGRATE 3.0 (Beerli and Felsenstein 2001) to simultaneously estimate interpopulation migration rates ($M = m/\mu$, where m is the migration rate and μ is the mutation rate) and population effective sizes ($\theta = 4N_e\mu$, where N_e = effective size) for clusters of populations of each species on a small scale (pairwise distances less than 40 km). Here we used the Klamath Basin and the Central Cascades clusters of populations for *R. pretiosa*, and three clusters of *R. cascadae* populations in which populations are separated by <25 km. Separate MIGRATE analyses were performed for each cluster within each species. See Online Resource 1 for details of the MIGRATE analyses.

Mitochondrial DNA

We also assayed mtDNA variation in individuals from most of the populations collected in 1999/2000. These included all four Washington populations, 12 out of the 16 Oregon populations, and the 1999 sample from Aldergrove (AG) in Canada (Table 1). We assayed for sequence variation in a 298 bp fragment of mtDNA that includes 257 bp of the 5′ end of the ND1 gene, and 41 bp of the 3′ end of the flanking tRNA-leu (positions 4731 to 5291 in the *Xenopus* complete mtDNA sequence; Roe et al. 1985).

We used single strand conformation polymorphism analysis (SSCP; Orita et al. 1989) to identify new variants. PCR and SSCP protocols followed Monsen and Blouin (2003; primers in Appendix A). We sequenced at least two individuals of every putative unique haplotype in both directions. Homologous sequences from 12 individuals of *R. luteiventris* (the sister species of *R. pretiosa*) were also included in the mtDNA analysis in order to verify that none of our samples were actually *R. luteiventris* (see Online Resource 2). All *R. pretiosa* and *R. luteiventris* haplotypes are accessioned in GenBank (GU056784–GU056800).

Results

Microsatellite loci analysis

Genetic diversity

With the exception of the 2007 sample from AG (one egg from each of 7 egg masses), all populations were in Hardy–Weinberg Equilibrium and showed little genotypic disequilibrium between loci (Online Resource 3). The few pairs of loci showing significant genotypic disequilibrium were not consistent across populations. The average expected heterozygosity (H_e), allelic richness (AR), and allelic richness rarified to $n = 15$ (AR_{15}) of each population are listed in Table 1. Genetic diversity measures for the *R. cascadae* populations are given in Online Resource 4. Levels of genetic variation in populations of *R. pretiosa*

are low for a frog (Ficetola et al. 2007), with mean $H_e = 0.31$ (range 0.14–0.50) versus mean $H_e = 0.54$ in *R. cascadae* (range 0.33–0.74). Similarly, mean $AR_{15} = 2.46$ in *R. pretiosa* (range 1.64–3.98) while mean $AR_{15} = 4.22$ in *R. cascadae* (range 2.17–5.90). In fact, every *R. pretiosa* population has at least one locus that was fixed for a single allele (range 1–7 loci per population fixed for one allele). The differences in H_e and AR_{15} between the two species were statistically significant (*t* test; $P < 0.01$). Note that all but one of the loci used with *R. cascadae* were actually cloned from spotted frog (*R. pretiosa* or *R. luteiventris*) libraries, and that four loci overlap between the two datasets. Thus, the difference in genetic diversity between the two species is unlikely to reflect a bias in choice of loci (if anything, there would be an ascertainment bias towards observing lower diversity in *R. cascadae*). These results suggest that *R. pretiosa* has populations that are very small and/or very isolated, even for a ranid frog.

Major genetic groups

When data from all individuals was analyzed with STRUCTURE, the highest ln Pr(*X*|*K*) was associated with $K = 9$ (Online Resource 5). However, the ln Pr(*X*|*K*) values increased only incrementally above $K = 3$. Applying the ΔK method of Evanno et al. (2005), $K = 3$ had the strongest signature, with $K = 9$ also showing some weak support (Online Resource 5). All individuals were consistently assigned to the same populations across the 20 runs with $K = 3$, and there was very little inferred admixture. Based on this analysis, there appear to be three genetic groups within *R. pretiosa* at the highest hierarchical level of population structure (Fig. 2): (1) a northern group including all populations from Canada south to Camas Prairie (CP) in northern Oregon, (2) the Central Cascades group in central Oregon, and (3) the Klamath Basin group in southern Oregon. These three major groups are particularly obvious in the PCoA plot (Fig. 3).

There is additional substructure within each of the three main groups (Fig. 2). By far the strongest hierarchical substructure occurs within the northern group, in which STRUCTURE revealed three, four or five very distinct groups, depending on what level of the nested hierarchy is being examined (Fig. 2). This nested hierarchy is also clearly evident in the NJ tree (Fig. 4). At $K = 5$ the two Columbia Basin populations (TL and CB) are separated, but we suspect this may reflect, in part, recent genetic drift in the TL population, which has much lower genetic diversity than other Northern populations (Table 1). Therefore, there appear to be four natural subgroups within the Northern clade that also reflect geographic proximities: the British Columbia populations (AG, MO, and MA), the two populations in the Chehalis drainage in WA (DC and BC), the two populations in the Columbia drainage in WA (TL and CB), and the Camas Prairie (CP) population in Oregon. Within this hierarchy the Chehalis and British Columbia groups form the next natural grouping (e.g. see Fig. 3 and the $K = 3$ panel in the Northern group from

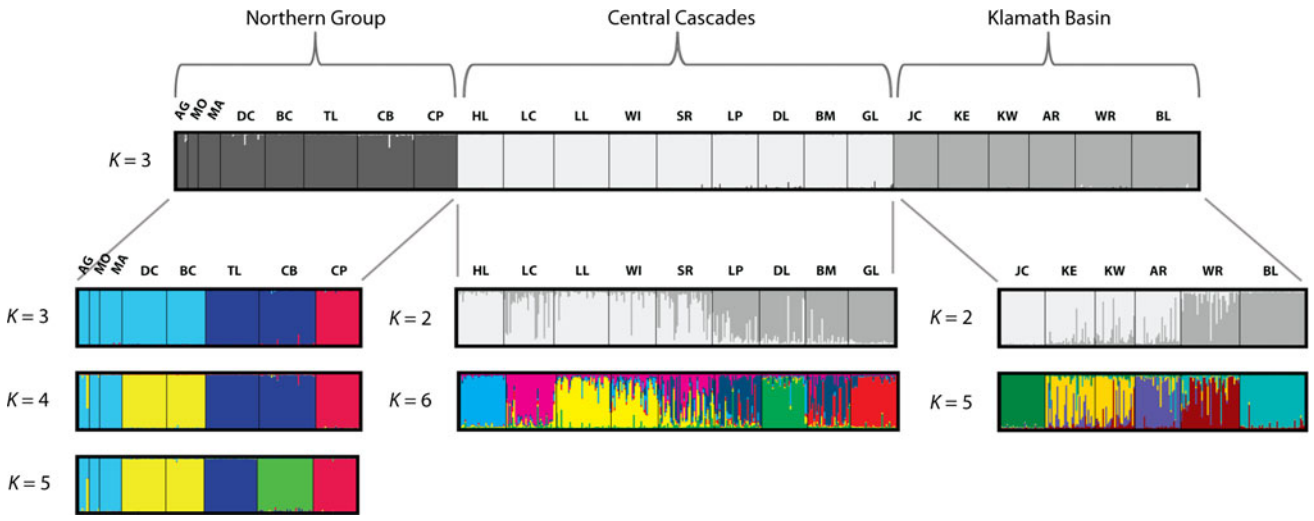

Fig. 2 Plots of population assignment of *R. pretiosa* individuals based on the STRUCTURE analysis. Each sampling locality is designated by its *two-letter code* (Table 1). Results for the analysis using the entire dataset ($n = 685$ frogs) is shown in the top bar. When *K* was set to 3, all individuals were unambiguously assigned to 3 genetic groups: a Northern group (populations in British Columbia and WA plus the Camas Prairie population), a Central Cascades group, and a Klamath Basin group. Subsequent analyses were run on each of these three major groups. A strong hierarchical structure is evident in the Northern group, which gives strong support for $K = 3$, 4 or 5 populations (*left side panels*). Much weaker structure is evident in the Cascades Lakes group and in the Klamath Basin group

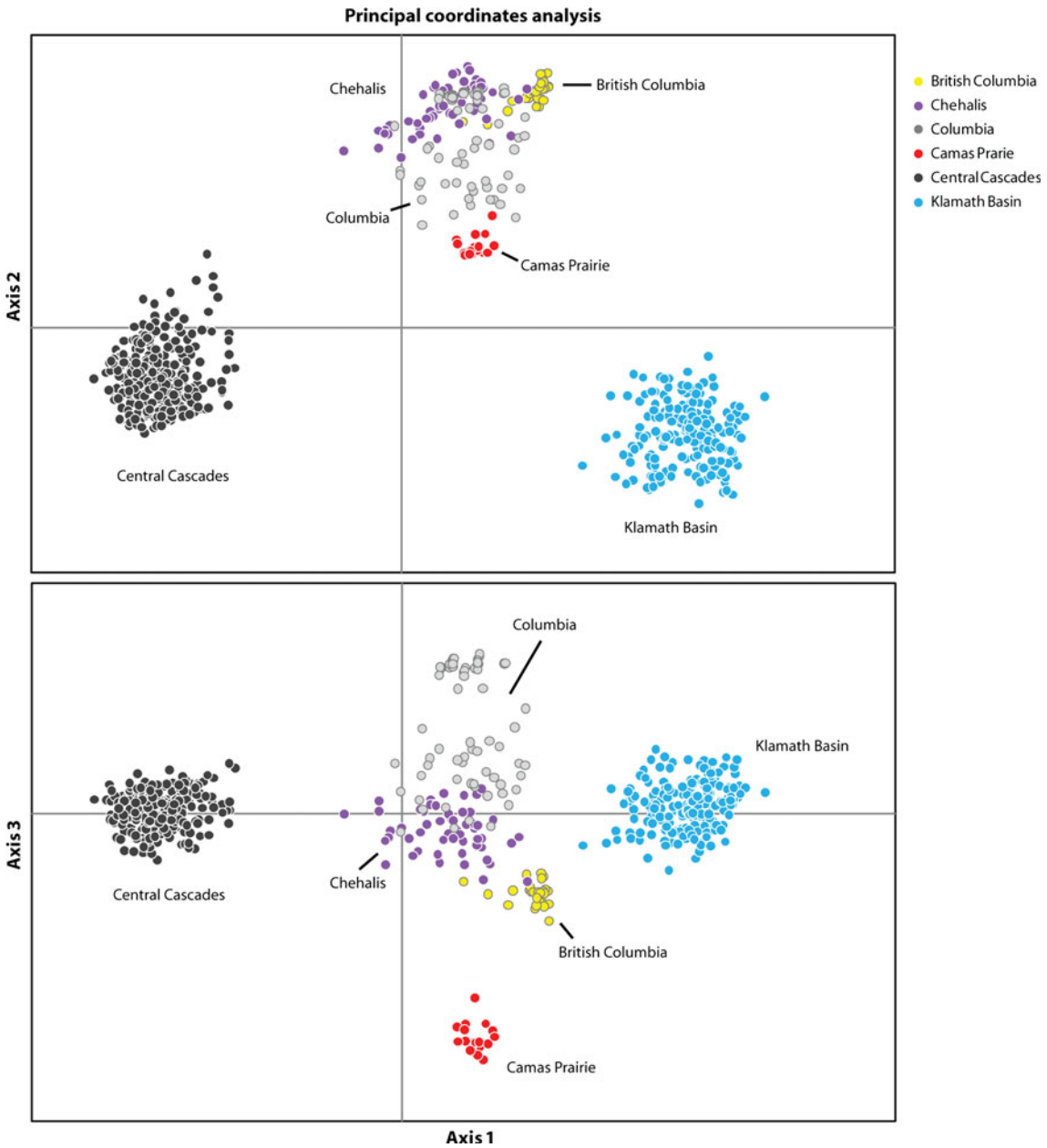

Fig. 3 Results of principal coordinates analysis on genetic distances between all *R. pretiosa* individuals. Top Coordinate 1 vs. 2. Bottom Coordinate 1 vs. 3. Individuals in each of the major genetic groups identified in the STRUCTURE analysis and NJ tree form distinct clusters when plotted for the first three principal coordinates

Fig. 2), which is again consistent with their geographic proximity to each other.

STRUCTURE revealed further substructure within the Central Cascades and within the Klamath Basin groups. Here there was support for $K = 2$ or 6 in the Central Cascades and for $K = 2$ or 5 in the Klamath Basin, with the larger numbers mostly identifying individual populations (Fig. 2). However, the structuring within these two major groups is minor relative to that observed further North (also evident in the NJ tree, Fig. 4). Thus, we recommend that the Central Cascades group and the Klamath Basin group each be considered a single group.

The distinctness of the CP population (e.g. Fig. 2) within the Northern group results in part because it carries several alleles in high frequency that are absent or rare in other populations. However, it is also the least genetically diverse population in the study ($H_e = 0.14$, $AR = 1.64$). Thus, recent genetic drift may have inflated genetic distances

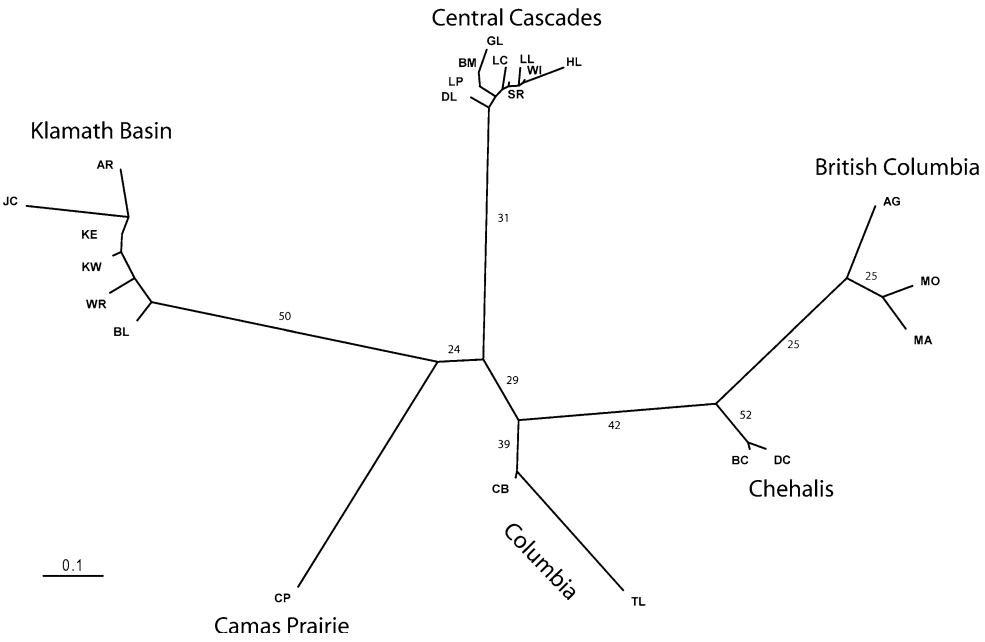

Fig. 4 Bootstrapped neighbor-joining tree based on pairwise genetic distances among *R. pretiosa* populations. The distance measure is Nei's unbiased genetic distance (Nei 1978). The six major genetic groups are labeled. Bootstrap values are shown on branches

between it and the other groups. Recent drift may also explain the long branch connecting CB to TL (Columbia drainage) in the NJ tree, as TL has very low diversity relative to the other Washington populations (Table 1). The three Canadian populations also appear to be well differentiated from each other in the NJ tree. However, the genetic distances among these populations are almost certainly inflated by the small sample sizes and the fact that we sampled egg masses (gametes from one season's breeders), rather than frogs from the entire population's age structure.

Genetic differentiation among populations within major groups

There is substantial allele frequency variation among populations on a local scale (Appendix B). Considering each of the three major groups at the highest hierarchical level, global F_{ST} is 0.23 among the Central Cascades populations, 0.21 among the Klamath Basin populations, and 0.53 among the northern populations (or $F_{ST} = 0.45$ if we exclude the CP population). The corresponding G'_{ST} values for these groups were 0.31, 0.34, and 0.77, respectively.

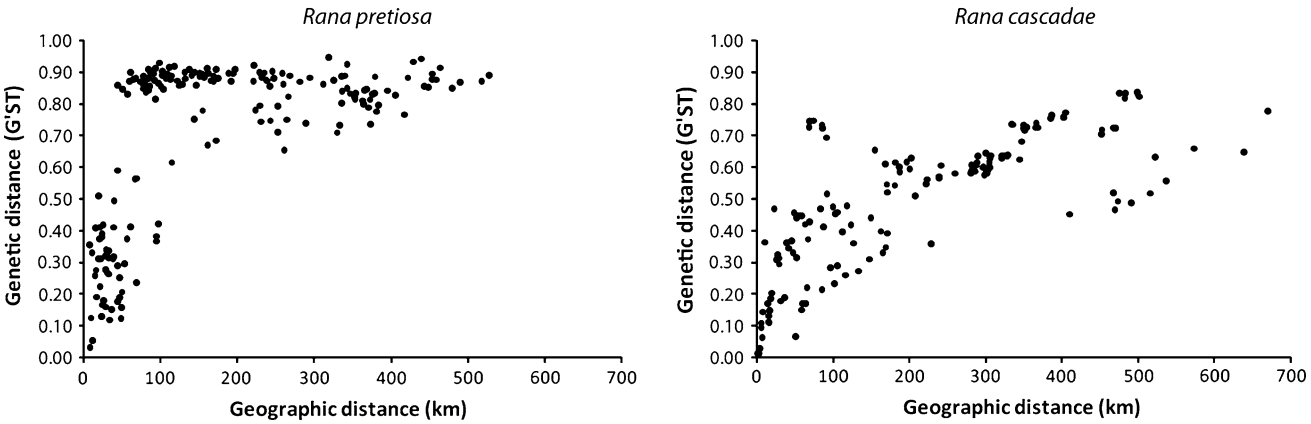

Fig. 5 Plots of pairwise genetic versus geographic distance for *R. pretiosa* (*left*) and *R. cascadae* (*right*). Genetic distances between population pairs are measured as G'_{ST} (Hedrick 2005). *R. pretiosa* shows very high differentiation between populations at all but the shortest geographic distances, suggesting that gene flow is very limited between most populations. *R. cascadae*, on the other hand, more closely fits an isolation-by-distance pattern at both small and large geographic scales. Populations of *R. cascadae* are probably interconnected in a stepping-stone pattern of gene flow, whereas *R. pretiosa* populations are mostly isolated

Pairwise tests for differences in global F_{ST} between the three groups were highly significant for comparisons between the Northern group and each of the other two groups ($P < 0.01$; based on 5,000 permutations performed in FSTAT), but not between the Central Cascades and Klamath Basin groups ($P = 0.742$; FSTAT permutation test). Mantel tests showed a significant correlation between genetic ($F_{ST}/(F_{ST} - 1)$) and geographic distances for populations in the Klamath Basin ($r = 0.703$, $P = 0.008$), but not for populations in the Central Cascades ($r = 0.102$, $P = 0.293$). This latter result is in sharp contrast to the pattern in *R. cascadae*, in which there is strong IBD over similar geographic scales (Monsen and Blouin 2004). Plots of G'_{ST} versus geographic distance for each species over their entire ranges also show very different patterns of IBD (Fig. 5; similar pattern obtained using F_{ST}, not shown). *R. cascadae* shows a more continuous pattern of IBD over all geographic scales. Although *R. pretiosa* populations are not obviously less connected at the smallest geographic scales (e.g. <80 km), at larger scales they show the signature of complete isolation (Hutchison and Templeton 1999).

Comparison of gene flow and effective sizes between R. pretiosa and R. cascadae

The MIGRATE analysis revealed two interesting patterns. First, *R. pretiosa* populations have slightly lower long-term effective sizes (*t* test, $P < 0.01$). Average $\theta = 0.87$ in *R. pretiosa*, versus average $\theta = 1.04$ in *R. cascadae*, a 1.2-fold difference (Fig. 6). These values of θ would correspond to long-term effective sizes of 435 and 525, given a typical vertebrate microsatellite mutation rate of $\mu \approx 5 \times 10^{-4}$ (Goldstein and Schlötterer 1999). However, this difference in θ is not sufficient to explain the difference between species in observed genetic diversity (assuming the two species have similar mutation rates). A 2.6-fold difference in effective sizes would be required to explain the difference in average heterozygosities of 0.31 and 0.54, assuming mutation-drift equilibrium in closed populations and an infinite alleles model of mutation (Nei 1987, p. 375; e.g. N_e's of 112.3 and 587.0 if $\mu \approx 5 \times 10^{-4}$). An approximately 3.4-fold difference in effective sizes would be required under a stepwise mutation model (Nei 1987, p. 379; e.g. N_e's of 68.8 and 232.8). That the MIGRATE results are reasonable is suggested by a comparative study of short-term N_e estimated via the linkage disequilibrium and temporal methods in populations of four species of ranid frog (Phillipsen, *unpub. data*). In that dataset *R. pretiosa* effective sizes were again only slightly smaller than those of *R. cascadae*. Thus, the higher heterozygosities in *R. cascadae* populations probably result more from higher gene flow than from larger effective sizes.

The second interesting result from the MIGRATE analysis is that for populations of both species separated by greater than about 10 km, the estimated migration rates are remarkably low—on the order of the mutation rate (Fig. 6). Gene flow appears to be much higher between populations separated by less than ~10 km (see also Monsen and Blouin 2004), so gene flow over larger distances probably occurs by a stepping stone process. But most *R. pretiosa* populations are farther apart than 10 km, and they are much less continuously distributed over the species-wide landscape than are populations of *R. cascadae*. So again, we hypothesize that low connectivity owing to more widely spaced populations is the key factor causing *R. pretiosa* to have less diverse populations and greater genetic structure than *R. cascadae*.

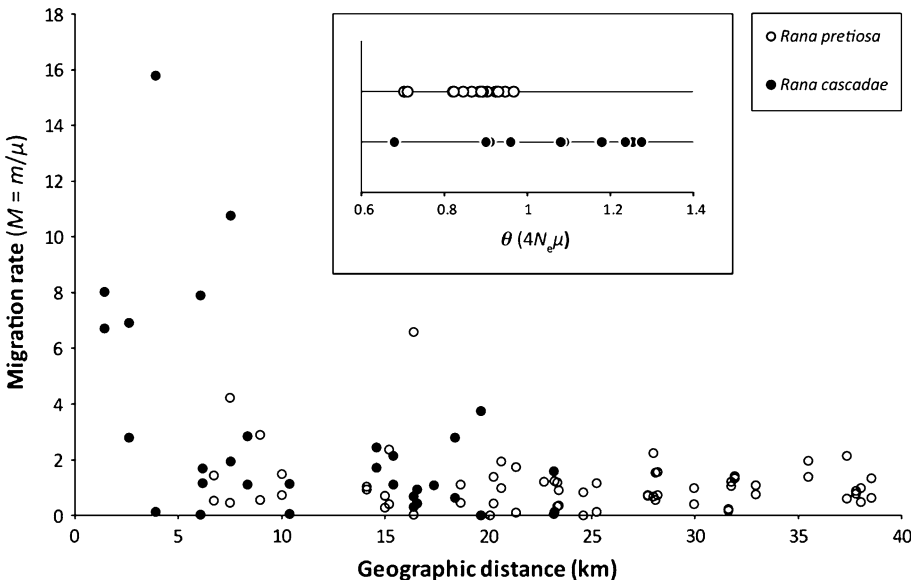

Fig. 6 MIGRATE results: one-way migration rates ($M = m/\mu$) plotted against pairwise geographic distances on a small geographic scale for *R. cascadae* populations (*filled circles*) and for *R. pretiosa* populations (*open circles*). Notice that migration rates are similar for the common pairwise geographic range over which the two species were both sampled (~5 to 25 km), and that migration rate is extremely small in both species beyond about 10 km pairwise distance (i.e. $m \approx \mu$). *Inset panel* shows estimated θ ($4N_e\ \mu$) for each population. Average. $\theta = 0.87$ in *R. pretiosa* and 1.05 in *R. cascadae*

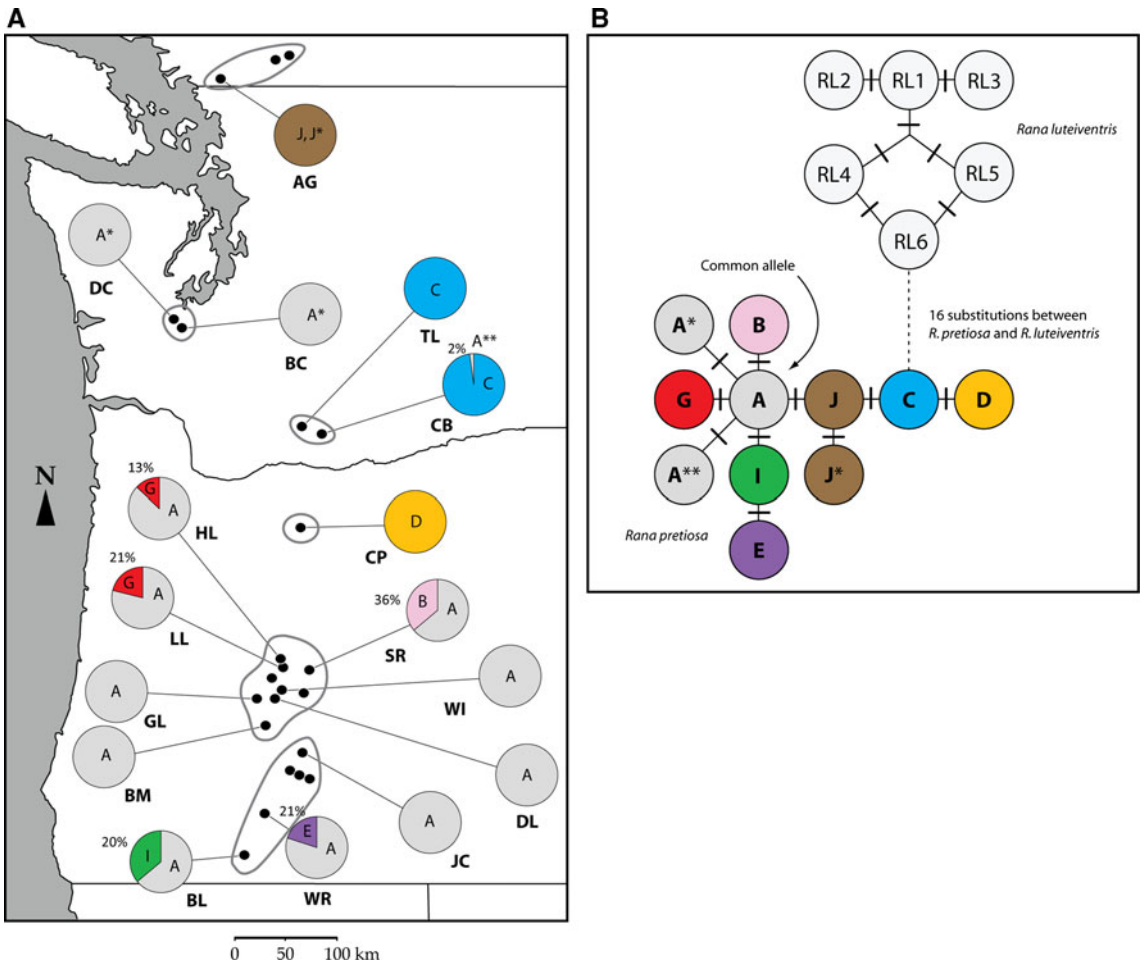

Fig. 7 **A** Relative frequencies of mtDNA alleles identified by SSCP and sequencing (sample sizes and allele counts in Table 1). Note the high frequencies of the private alleles. Asterisked alleles were identified only by sequencing (e.g. "A*" and "A**" appear to be "A" alleles by SSCP; see Online Resource 6). All 7 sequenced alleles from DC and BC were "A*", so we assume that all the "A" alleles were "A*", but cannot be sure. The relative frequency of "J" and "J*" in AG was not determined. **B** Parsimony network of all mtDNA alleles identified by sequencing in this study. *Color codes* match the alleles in panel A

Mitochondrial DNA analysis

All *R. pretiosa* sequences were more closely related to each other than to the sequences from *R. luteiventris* (Fig. 7; see also Funk et al. 2008). We found 8 unique *R. pretiosa* haplotypes via SSCP. One haplotype, "A", was found in Washington, the central Cascades and Klamath Basin. To explore whether this was really a single, wide-spread allele, we sequenced an additional 263 bp of *ND1* 3′ of the SSCP fragment in representative "A" individuals from throughout Washington and Oregon and in representatives of each of the other 7 haplotypes (see Online Resource 6 for more information). We found three new haplotypes (indicated by asterisks in Fig. 7A). In particular, the "A" allele in Washington does indeed appear to be distinct from the "A" allele in Oregon (Fig. 7B; Online Resource 6).

We observed a single haplotype in most populations, and at most two haplotypes, despite large sample sizes assayed per population (n of up to 63 individuals in some samples, with most sample sizes > 30; Table 1). Also, several private alleles (i.e. found in only a single population) were found in the Central Cascades and Klamath Basin metapopulations, and these were at very high frequencies (20–36%; Fig. 7A). These private haplotypes all appear to be single-step mutations derived from the ancestral "A" haplotype (Fig. 7B). Haplotypes that originate in a population and drift to high frequency without spreading to nearby populations indicate that these populations are connected by very small numbers of migrants per generation (Slatkin and Barton 1989). This result reinforces the microsatellite-based conclusion that gene flow among populations has been very low, even on a small geographic scale.

Discussion

Major genetic groups

Synthesizing the results of the STRUCTURE analyses, the neighbor-joining tree, and the PCoA, we conclude that there are six major population groups within *R. pretiosa*. Four of these groups—British Columbia, Chehalis drainage, Columbia drainage, and Camas Prairie—are related and form the larger "northern" group. Within this Northern hierarchy, the Chehalis and British Columbia populations form the next natural grouping, which seems reasonable given their proximity and use of similar lowland marsh habitat. The uniqueness of the Camas Prairie population (e.g. Fig. 2) is supported not only by its very different allele frequencies, but also by its unique mtDNA haplotype and by the three microsatellite alleles (one at each of three loci) that were found only in the Camas Prairie population. This population thus appears to be the sole remaining representative of a distinct genetic group that once existed in northern Oregon.

Genetic diversity within populations

It is not unusual for an amphibian to exhibit low within-population genetic diversity, relative to other vertebrates (Sjögren 1991; Rowe et al. 1999; Spear et al. 2005; Allentoft et al. 2009). However, our comparison between *R. cascadae* and *R. pretiosa* populations highlights that *R. pretiosa* populations have low diversity even for a ranid frog (e.g. average H_e was 0.31 for *R. pretiosa* in our study, whereas most microsatellite-based studies of ranid frogs have reported values higher than 0.40; Newman and Squire 2001; Zeisset and Beebee 2003; Brede and Beebee 2004; Hoffman and Blouin 2004; Funk et al. 2005). Whether low diversity results mostly from isolation or from small population sizes, or both, can be difficult to disentangle. In this case, however, the results from MIGRATE show that individual populations of *R. pretiosa* have only slightly smaller estimates of long-term effective size than populations of *R. cascadae*. Furthermore, gene flow is extremely small beyond about 10 km, a distance much smaller than that which separates most *R. pretiosa* populations. Therefore, we hypothesize that lower connectivity is an important cause of the lower genetic diversity within *R. pretiosa* populations. In other words, because *R. cascadae* are much more continuously distributed in their habitat than are *R. pretiosa*, unsampled *R. cascadae* populations between our sampled sites probably keep those sites linked by a constant trickle of stepping stone migration that maintains genetic diversity. Such intervening stepping stone populations do not exist for *R. pretiosa*.

There is a range in diversities among the extant *R. pretiosa* populations (Table 1). It is interesting to note that, despite their greater isolation, three of the Washington populations (DC, BC, and CB) have higher genetic diversity than populations from the Central Cascades or Klamath Basin groups. One explanation is that the Washington populations exist at much lower elevations, and so may maintain larger population sizes than the montane populations (indeed, the CB population has yearly egg mass counts in the thousands; M. Hayes, pers. comm.). A similar difference in diversity between high and low elevation populations was also observed in *R. luteiventris* (Funk et al. 2005).

Gene flow and genetic differentiation among populations

Genetic subdivision is very high in *R. pretiosa* relative to that in *R. cascadae*. We observed five very distinct, historical groups within *R. pretiosa* over the same geographic range from Oregon to Washington in which *R. cascadae* shows a classic pattern of isolation by distance (Monsen and Blouin 2003). We see the same pattern of higher differentiation in *R. pretiosa* on a smaller scale. For example, we obtained low estimates of gene flow, and no pattern of IBD within the Central Cascades metapopulation, even though these populations still inhabit relatively pristine habitat in national forests. *R. cascadae*, in contrast, shows strong IBD at this geographic scale (Monsen and Blouin 2004). The high frequency of mtDNA private alleles in the two *R. pretiosa* metapopulations also suggests a history of historical (rather than recent) isolation among individual populations. Thus, something about the biology of *R. pretiosa* predisposes it to substantial fragmentation, even in relatively undisturbed habitat. Again, we hypothesize that low connectivity, rather than small effective sizes, is the primary explanation. *R. pretiosa*'s highly aquatic habit seems like the obvious cause of this low connectivity. For example, the distinctness of the Klamath Basin group from populations to the North is particularly striking when one considers that the southernmost Central Cascades population (BM) and the northernmost Klamath Basin population (JC) are only 43 km apart. A low-gradient divide between the Deschutes River and Klamath River watersheds is all that separates these two populations topographically. Note also that all the major groups identified in this study correspond to distinct watersheds. Thus, connectivity between *R. pretiosa* populations probably depends more on the local distribution and connectivity of streams, rivers, and lakes, than on distance *per se* (Hayes 1997; Cushman and Pearl 2007).

Conservation Implications

Our data suggest that the six major genetic groups within *R. pretiosa* have been reproductively isolated for many generations, certainly pre-dating European influences. These groups also occupy different habitats (Pearl and Hayes 2004). For example, the Northernmost populations are found in lowland marshes, and the Central Cascades populations are found in high elevation lakes. These six groups thus seem like obvious candidates for designation as evolutionarily significant units (ESUs; Crandall et al. 2000) or distinct population segments (DPSs; Federal Register 1996) for the purposes of conservation management, Thus it would be prudent to keep track of the group membership of any frogs used in captive breeding programs, reintroductions, or translocations. Knowing the collection locality of a frog will in most cases be enough to determine its group membership. However, if uncertainties arise, the molecular markers used in this study could be used to unambiguously assign individuals to their respective groups.

We observed a wide range of genetic diversities among *R. pretiosa* populations. Whether low diversity *per se* is a threat to any of these populations has not been demonstrated. Indeed, many of these populations persist despite what appear to be remarkably low levels of microsatellite DNA (and presumably genome-wide) diversity. Nevertheless, a general correlation between population fitness and genetic marker diversity has been demonstrated in a number of species (Reed and Frankham 2003), and studies suggest that this correlation is true for amphibians as well (Lesbarrères et al. 2005; Rowe et al. 1999; Andersen et al. 2004). So given the isolation of many *R. pretiosa* populations, it would seem reasonable to focus some management effort on minimizing future genetic drift and inbreeding by expanding the sizes of key populations. This is probably best accomplished by improving, or expanding the available wetland habitats at each site. We recommend against supplementation of extant populations with captive reared individuals, as this strategy can actually harm wild populations (Araki et al. 2007, 2009; MciGinnity et al. 2009). One could also found additional populations in suitable habitat near their source populations. This strategy was used to successfully found a new *R. pretiosa* population in artificially-constructed ponds in the central Cascades, using a handful of founders (20 adults and 9 egg masses; Chelgren et al. 2008). Despite the obvious founder effect and subsequent inbreeding that must have taken place, this population appears to be thriving.

Low connectivity among populations, in addition to naturally small population sizes, appears to be an important cause of low diversity within populations and high differentiation among populations in *R. pretiosa*. Thus, preserving habitat connectivity among populations to maintain natural patterns of gene flow should be a high priority in the management of this species. This recommendation is most relevant to populations in the Central Cascades and Klamath Basin, where some gene flow among populations is likely to still occur. Research to identify what specific habitat or landscape features enhance or discourage movement among populations is critically needed.

Finally, special consideration should be given to the Camas Prairie population. This population appears to be the only remaining representative of a major genetic group that is now almost extinct. CP also has the lowest genetic diversity and is the most geographically isolated population of *R. pretiosa*. Egg mass surveys and mark-recapture of adults in 2001 and 2002 revealed about 30 breeding females per year and an estimated adult size of <100 (Blouin, unpub. data). Thus, measures to expand the size of this population or to found additional populations nearby should be considered.

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Appendix

See Tables 2, 3

Table 2 PCR primers for mtDNA and microsatellite loci

Locus		Primers	T_a (°C)	No. of alleles
ND1 (mtDNA)			50	
	F: MB77	TGGCAGAGCTTGGTTATGCAAAAGA		
	R: MB129	GGAAATGGGGTTCATATGATTATG		
	R: MB130	GAAATGGTAAAAGAAGAGAGGGT		
Microsatellite loci				
RP3			45	12
	F: MB165	CCTGAGAGCCATCCAATAAGTGCCA		

Table 2 continued

Locus		Primers	T_a (°C)	No. of alleles
	R: MB166	GAAAGCAAAACTGGGAAAGTACATA		
RP15			50	5
	F: MB146	CTTGATACAGTGTGCAAGAGGC		
	R: MB147	ATACTCGTGATAGGGAGTTT		
RP17			50	4
	F: MB149	GTCTCTACTTCCATCCAAC		
	R: MB171	GTGTAGACAAACAAATGAAAGTCAG		
RP22c			55	18
	F: MB150	ACCCCACCAGCAGAATACAATGA		
	R: MB198	AGACCAGAGCCAGAGCAACC		
RP23			45	11
	F: MB152	ACATAGATACAATAGATAGATAGAC		
	R: MB153	CACAGGAATGTAAAATCTGGCTTTC		
RP26			58	10
	F: MB154	TAGATAGTAACTCCATACAGTGACA		
	R: MB155	TAGTCTCCTTACAAAATATAACTGC		
RP193			50	9
	F: MB224	CCATTTTCTCTCTGATGTGTGT		
	R: MB225	TGAAGCAGATCACTGGCAAAGC		
RP385			50	10
	F: MB232	ATTGAAACTTGCGGCTCTCT		
	R: MB233	GGCATGTGTCCACAATGTAA		
RP415			45	9
	F: MB234	AAGTTTCATTAAAGCAGATT		
	R: MB235	GGTATATCTTAGGGTTACCT		
RP461			45	7
	F: MB236	AAGGGTTTGAGTACAACTAC		
	R: MB237	GTAACACATGAACTGTCACG		
SFC104			58	6
	F: MB256	CCCTGTTAAACCACTGGCTAT		
	R: MB257	GTCATTTCAGAAGTCCGCTTTCAG		
SFC120			56	26
	F: MB262	AACCCTGGTAGTATGACCAAC		
	R: MB263	GTGGAACTCCAGTTATGATCC		
SFC134			57	21
	F: MB276	TGGGAAAAGACTCTGTGGT		
	R: MB277	AGGAAATGTGTGGAAGCAT		

Table 3 Matrix of pairwise genetic differentiation between *R. pretiosa* populations

	AG	MO	MA	DC	BC	TL	CB	CP	HL	LC	LL	WI	SR	LP	DL	BM	GL	JC	KE	KW	AR	WR	BL
AG	–	**0.33**	**0.42**	0.32	**0.30**	**0.69**	0.43	**0.73**	0.71	0.66	0.71	0.69	0.64	**0.62**	**0.60**	**0.70**	0.71	**0.58**	0.49	**0.49**	0.59	0.54	**0.61**
MO	**0.45**	–	0.33	0.40	**0.39**	0.73	0.49	0.78	0.76	0.72	0.76	0.75	0.70	0.69	0.66	0.76	0.77	0.63	0.53	0.53	0.63	0.58	0.64
MA	**0.55**	0.40	–	0.49	0.48	0.74	0.52	0.78	0.77	0.73	0.77	0.76	0.71	0.71	0.69	0.76	0.77	0.66	0.57	0.58	0.65	0.61	0.66
DC	0.56	0.64	0.75	–	0.03	0.53	0.38	0.62	0.56	0.53	0.57	0.55	0.51	0.50	0.48	0.55	0.56	0.55	0.48	0.47	0.55	0.51	0.57
BC	**0.56**	**0.63**	0.74	0.06	–	0.51	0.37	0.60	0.56	0.53	0.57	0.56	0.52	0.51	0.48	0.55	0.56	0.54	0.46	0.45	0.53	0.49	0.55
TL	**0.90**	0.90	0.91	0.78	0.75	–	0.35	0.77	0.72	0.69	0.72	0.71	0.67	0.66	0.62	0.70	0.72	0.69	0.61	0.62	0.69	0.64	0.67
CB	0.75	0.77	0.79	0.68	0.67	0.51	–	0.57	0.53	0.49	0.54	0.53	0.48	0.46	0.41	0.49	0.51	0.49	0.39	0.37	0.47	0.42	0.48
CP	0.92	0.92	0.92	0.88	0.87	0.93	0.81	–	0.74	0.70	0.75	0.75	0.70	0.70	0.69	0.75	0.76	0.68	0.59	0.62	0.68	0.64	0.67
HL	0.94	0.95	0.95	0.83	0.84	0.90	0.78	0.90	–	0.32	0.28	0.21	0.25	0.31	0.24	0.45	0.47	0.64	0.58	0.60	0.65	0.62	0.66
LC	0.92	0.94	0.94	0.81	0.82	0.88	0.74	0.89	0.41	–	0.21	0.20	0.11	0.19	0.23	0.19	0.30	0.62	0.56	0.58	0.64	0.61	0.64
LL	0.94	0.95	0.95	0.83	0.85	0.90	0.79	0.91	0.36	0.28	–	0.13	0.14	0.24	0.25	0.33	0.40	0.64	0.59	0.61	0.66	0.63	0.67
WI	0.93	0.95	0.95	0.83	0.84	0.90	0.79	0.91	0.27	0.26	0.17	–	0.09	0.17	0.24	0.25	0.30	0.61	0.56	0.58	0.64	0.61	0.64
SR	0.91	0.93	0.93	0.80	0.82	0.87	0.74	0.89	0.32	0.16	0.18	0.12	–	0.10	0.21	0.18	0.29	0.59	0.53	0.54	0.61	0.58	0.62
LP	**0.90**	0.93	0.93	0.80	0.82	0.86	0.72	0.90	0.42	0.26	0.31	0.22	0.13	–	0.20	0.09	0.14	0.59	0.53	0.54	0.61	0.58	0.62
DL	**0.90**	0.91	0.92	0.78	0.79	0.82	0.65	0.89	0.32	0.31	0.34	0.33	0.29	0.28	–	0.25	0.31	0.59	0.53	0.54	0.61	0.58	0.62
BM	**0.93**	0.95	0.95	0.83	0.84	0.88	0.73	0.91	0.56	0.25	0.41	0.31	0.24	0.12	0.34	–	0.13	0.60	0.54	0.56	0.62	0.59	0.64
GL	0.94	0.95	0.95	0.83	0.85	0.89	0.75	0.91	0.59	0.39	0.50	0.38	0.38	0.17	0.41	0.16	–	0.63	0.56	0.59	0.65	0.62	0.67
JC	0.95	0.96	0.96	0.94	0.93	0.95	0.86	0.92	0.91	0.89	0.90	0.88	0.88	0.88	0.90	0.86	0.88	–	0.18	0.21	0.26	0.34	0.40
KE	0.88	0.88	0.90	0.88	0.85	0.89	0.73	0.86	0.89	0.87	0.88	0.85	0.85	0.84	0.87	0.83	0.84	0.31	–	**0.02**	0.07	0.12	0.23
KW	**0.89**	0.88	0.90	0.88	0.86	0.89	0.71	0.87	0.89	0.87	0.89	0.87	0.86	0.85	0.87	0.85	0.86	0.38	**0.03**	–	0.11	0.09	0.23
AR	0.93	0.92	0.94	0.91	0.90	0.93	0.80	0.90	0.92	0.90	0.92	0.90	0.89	0.88	0.91	0.88	0.90	0.42	0.13	0.19	–	0.19	0.28
WR	0.88	0.87	0.89	0.87	0.85	0.88	0.73	0.87	0.90	0.88	0.90	0.87	0.86	0.86	0.89	0.86	0.88	0.57	0.21	0.17	0.30	–	0.12
BL	**0.90**	0.89	0.90	0.89	0.87	0.88	0.76	0.87	0.90	0.88	0.90	0.87	0.87	0.88	0.88	0.86	0.89	0.62	0.38	0.37	0.42	0.19	–

Two-letter population codes are as given in Table 1. Pairwise F_{ST} are above the diagonal and G'_{ST} are below the diagonal. Non-significant values are in *bold*

References

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden, MA

Allentoft M, Siegismund H, Briggs L, Andersen L (2009) Microsatellite analysis of the natterjack toad (*Bufo calamita*) in Denmark: populations are islands in a fragmented landscape. Conserv Genet 10:15–28

Andersen L, Fog K, Damgaard C (2004) Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). Proc Biol Sci 271:1293–1302

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318:100–103

Araki H, Cooper B, Blouin MS (2009) Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendents in the wild. Biol Lett 5:621–624

Arens P, van der Sluis T, van't Westende W, Vosman B, Vos C, Smulders M (2007) Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in The Netherlands. Landsc Ecol 22:1489–1500

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Brede E, Beebee T (2004) Contrasting population structures in two sympatric anurans: implications for species conservation. Heredity 92:110–117

Chelgren ND, Pearl CA, Adams MJ, Bowerman J (2008) Demography and movement in a relocated population of Oregon spotted frogs (*Rana pretiosa*): influence of season and gender. Copeia 2008:742–751

Corkran C, Thoms C (2006) Amphibians of Oregon, Washington, and British Columbia. Lone Pine Publishing, Auburn, WA

Crandall K, Bininda-Emonds OR, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends Ecol Evol 15:290–295

Crosby M, Licht L, Fu J (2008) The effect of habitat fragmentation on fine scale population structure of wood frogs (*Rana sylvatica*). Conserv Genet 10:1707–1718

Cushman K, Pearl C (2007) A conservation assessment for the Oregon spotted frog (*Rana pretiosa*). Report to the USDA Forest Service; USDI Bureau of Land Management

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Federal Register (1996) Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Fed Regist 61:4722–4725

Ficetola GF, Garner TWJ, De Bernardi F (2007) Genetic diversity, but not hatching success, is jointly affected by postglacial colonization and isolation in the threatened frog, *Rana latastei*. Mol Ecol 16:1787–1797

Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. Mol Ecol 14:483–496

Funk WC, Pearl CA, Draheim HM, Adams MJ, Mullins TD, Haig SM (2008) Range-wide phylogeographic analysis of the spotted frog complex (*Rana luteiventris* and *Rana pretiosa*) in northwestern North America. Mol Phylogenet Evol 49:198–210

Garner TWJ, Angelone S, Pearman PB (2003) Genetic depletion in Swiss populations of *Rana latastei*: conservation implications. Biol Conserv 114:371–376

Garner TWJ, Pearman PB, Angelone S (2004) Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. Mol Ecol 13:1047–1053

Goldstein DB, Schlötterer C (1999) Microsatellites: evolution and applications. Oxford University Press, New York

Green DM, Kaiser H, Sharbel TF, Kearsley J, McAllister KR (1997) Cryptic species of spotted frogs, *Rana pretiosa* complex, in western North America. Copeia 1997:1–8

Hayes MP (1997) Status of the Oregon spotted frog (*Rana pretiosa sensu stricto*) in the Deschutes Basin and selected other systems in Oregon and northeastern California with a range wide synopsis of the species' status. Final report prepared for The Nature Conservancy under contract to US Fish and Wildlife Service, Portland, Oregon

Hedrick P (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Hillis DM, Wilcox TP (2005) Phylogeny of the new world true frogs (*Rana*). Mol Phylogenet Evol 34:299–314

Hoffman E, Blouin MS (2004) Historical data refute recent range contraction as cause of low genetic diversity in isolated frog populations. Mol Ecol 13:271–276

Hutchison D, Templeton A (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561–576

Langella O (1999) Populations 1.2.30: a population genetic software. CNRS UPR9034. http://www.pge.cnrs-gif.fr/bioinfo/populations/index.php

Lesbarrères D, Primmer CR, Laurila A, Merila J (2005) Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. Mol Ecol 14:311–323

Martínez-Solano I, Rey I, García-París M (2005) The impact of historical and recent factors on genetic variability in a mountain frog: the case of *Rana iberica* (Anura: Ranidae). Anim Conserv 8:431–441

McAllister KR, Leonard WP, Storm RM (1993) Spotted frog (*Rana pretiosa*) surveys in the Puget Trough of Washington, 1989–1991. Northwest Nat 74:10–15

MciGinnity P, Jennings E, DeEyto E, Allott N, Samuelsson P, Rogan G, Whelan K, Cross T (2009) Impact of naturally spawning captive-bred Atlantic salmon on wild populations: depressed recruitment and increased risk of climate-mediated extinction. Proc R Soc B 27:3601–3610

Monsen KJ, Blouin MS (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. Mol Ecol 12:3275–3286

Monsen KJ, Blouin MS (2004) Extreme isolation by distance in a montane ranid frog *Rana cascadae*. Conserv Genet 5:827–835

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). Mol Ecol 10:1087–1100

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 86:2766–2770

Palo JU, Lesbarreres D, Schmeller DS, Primmer CR, Merila J (2004a) High degree of population subdivision in a widespread amphibian. Mol Ecol 13:2631–2644

Palo JU, Schmeller DS, Laurila A, Primmer CR, Kuzmin SL, Merila J (2004b) Microsatellite marker data suggest sex-biased dispersal in the common frog *Rana temporaria*. Mol Ecol 13:2865–2869

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pearl CA, Adams MJ (2005) *Rana cascadae* Slater 1939: cascade frog. In: Lannoo MJ (ed) Amphibian declines: the conservation status of United States' species. University of California Press, Berkeley, pp 538–540

Pearl CA, Hayes MP (2004) Habitat associations of the Oregon spotted frog (*Rana pretiosa*): a literature review. Final report. Washington Department of Fish and Wildlife, Olympia, Washington, USA

Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Reed DH, Frankham R (2003) The correlation between population fitness and genetic diversity. Conserv Biol 17:230–237

Roe BA, Ma DP, Wilson RK, Wong JF (1985) The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. J Biol Chem 260:9759–9774

Rowe G, Beebee TJC, Burke T (1999) Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. Anim Conserv 2:85–92

Seburn D, Seburn C (2000) Conservation priorities for the amphibians and reptiles of Canada. Prepared for World Wildlife Fund Canada and the Canadian Amphibian and Reptile Conservation Network

Seppa P, Laurila A (1999) Genetic structure of island populations of the anurans *Rana temporaria* and *Bufo bufo*. Heredity 82: 309–317

Sjögren P (1991) Genetic variation in relation to demography of peripheral pool frog populations (*Rana lessonae*). Evol Ecol 5:248–271

Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43: 1349–1368

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–573

Spear S, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). Mol Ecol 14:2553–2564

Stebbins R (2003) A field guide to western reptiles and amphibians. Houghton Mifflin, New York

U.S. Fish and Wildlife Service (2005) Species assessment and listing priority assessment form. Western Washington Fish and Wildlife Office, Lacey, Washington. http://ecos.fws.gov/docs/candforms_pdf/r1/D02A_V01.pdf

Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Zeisset I, Beebee TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. Mol Ecol 12:639–646