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PROJECT TITLE:
Population genetic analysis of Columbia spotted frogs (*Rana luteiventris*) in southeastern Oregon

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INTRODUCTION

Columbia spotted frogs (*Rana luteiventris*) are distributed in the Pacific Northwestern United States and Canada, yet in some parts of their range, populations are small and disjunct due to isolation by both natural landscape features (topography) and anthropogenic factors. There is particular concern for populations in the Great Basin distinct population segment (DPS).

We used microsatellite markers to characterize the population genetic structure of Columbia spotted frogs (*Rana luteiventris*) in Southeastern Oregon. Our specific objectives were to: 1. Estimate the level of genetic connectivity (gene flow) among populations of Columbia spotted frogs; 2. Identify the number and spatial distribution of distinct populations of Columbia spotted frogs; 3. Estimate effective population sizes of Columbia spotted frog populations; and 4. Test for population declines of Columbia spotted frogs using genetic bottleneck tests.

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METHODS

Sample collection

Tadpole, juvenile and adult *Rana luteiventris* frogs were sampled over 7 years, between September 2005 and September 2011 (Table 1). Many of the samples from 2009 and 2010 were collected by the USGS ARMI group. The number of samples per collection site ranged from 1 – 33 (Table 1). Population genetic analyses are sensitive to sample size (requires at least 5 individuals) and spatial sampling. To reduce statistical bias in our analyses, we grouped samples from ponds located within 500 m to ensure adequate sample sizes.

Laboratory Methods

Genomic DNA was extracted from either toe clips or buccal swabs using Qiagen DNEasy Kits. We amplified all individuals at 8 microsatellite loci, previously published for *R. luteinventris* and *R. pretiosa* (Monsen and Blouin 2004; Funk et al. 2005). We amplified PCR fragments in two multiplex PCR reactions using QIAGEN multiplex PCR kits. Each 10 µl multiplex PCR reaction contained 5 µl of 2x QIAGEN Multiplex PCR Master Mix (3mM $MgCl_2$), 1 µl of 5X Q-Solution, 0.2 µl of each primer (10 µM), and approximately 10 ng of genomic DNA. Multiplex 1 contained the loci RP17, RP193, SFC134, SFC139 and multiplex 2 contained the loci RP15, RP23, SFC128, RP3. The PCR conditions for both multiplex PCR were as follows: an initial denaturation of 95°C for 15 min, followed by 35 cycles of 95°C for 30 sec, 58°C for 90 sec, 72°C for 60 seconds, followed by a final extension of 60°C for 60 min. The PCR product was prepared for fragment analysis on a capillary sequencer with HIDI formamide and LIZ ladder (500 GeneScan). We scored microsatellite alleles using GeneMarker v 1.91.

Descriptive statistics

The number of alleles per locus, observed and expected heterozygosities and conformation to Hardy-Weinberg Equilibrium (HWE) was examined using Arlequin 3.5 (Excoffier et al. 2005). We evaluated loci for linkage disequilibrium (whether alleles at different loci are non-randomly associated) using GENEPOP version 4.0.10 on the web (Raymond and Rousset 1995; Rousset 2008). We used Micro-checker 2.2.3 (van Oosterhout et al. 2006) to test for the presence of null alleles.

Demographic History

We estimated the effective population sizes (*Ne*) using a Bayesian model implemented in OneSamp (Tallmon et al. 2008). This model requires at least 18 individuals per sampling site (Tallmon et al. 2008). The effective population size (*Ne*) can be thought of as the number of individuals that pass their genes on to the next generation. More specifically, the rate at which genetic diversity is lost in a population due to genetic drift is inversely related to *Ne*. Effective population size is an important parameter to consider in conservation because smaller populations are more likely to suffer from low genetic diversity and are susceptible to genetic drift and the negative consequences of inbreeding.

Demographic history was investigated by evaluating whether sites experienced 'recent' genetic bottlenecks by testing for heterozygosity excess in the program BOTTLENECK 1.2 0.2 (Cornuet and Luikart 1996). More specifically, BOTTLENECK detects bottlenecks that occurred within the last $2N_e$–$4N_e$ generations (Cornuet and Luikart 1996). Thus, for example, if $N_e = 20$ for a given population, then BOTTLENECK could detect a bottleneck that occurred within the last 40 generations. If generation time is 2 years, this translates to 80 years. BOTTLENECK has been shown to be the best approach for detecting recent bottlenecks of relatively low magnitude (Williamson-Natesan 2005). Populations that undergo rapid reduction in size are expected to show an excess of heterozygosity for the observed number of alleles because the loss of rare alleles is faster than the loss of heterozygosity. We used two models, the infinite alleles model (IAM) and the two-parameter model (TPM). Following Funk et al. (2010), we examined the

TPM with *a priori* parameters (variance = 12 and proportion of IAM in the model at 10 %). We tested for significance using the Wilcoxon signed rank test, a test appropriate for datasets containing fewer than 20 polymorphic loci. We also examined the shape of the allele frequency distribution: a skewed distribution indicates a bottleneck whereas a normal distribution is characteristic of stable populations. In general, BOTTLENECK is sensitive to detecting recent bottleneck events.

Population Genetic Structure

Population genetic structure was examined using three complementary approaches: pairwise comparisons between all sites (F_{ST}) within and among three regions (Central Oregon, Blue Mountains, Southeastern Oregon; Table 1), Bayesian model-based assignment tests to identify genetic clusters (STRUCTURE), and analyses of molecular variance (AMOVA) among major groups.

a) Pairwise F_{ST} is the proportion of total genetic variation due to among population differences and ranges from 0 – 1. We calculated F_{ST} for every pair of sites and tested whether it was significantly different from zero using 500 permutations. A non-significant F_{ST} ($F_{ST} = 0$) indicates that those two sites are not significantly divergent. Analyses were conducted in Arlequin 3.11 (Excoffier et al., 2005).

b) We performed an individual-based clustering analyses in STRUCTURE v 2.3.3 (Pritchard et al. 2000) to determine the number of genetic cluster across all sites. This is a Bayesian clustering method that assigns each individual (independent of sampling site) to a particular genetic cluster based on its genotype across all loci. We ran the model with a range of possible genetic clusters (1 – 20). Each model was run 10 times and verified for convergence with log likelihood values. We used an initial burn-in of 100,000 with an additional 3,000,000 iterations. Correlated allele frequencies and admixture were assumed. The most likely number of genetic clusters was determined using the DeltaK method (Evanno et al. 2005) and by calculating the posterior probabilities of each model. We initially conducted analyses on all sampling sites combined. This analysis revealed two major genetic groups, Eastern and Central Oregon Groups (see Table 1). We subsequently conducted STRUCTURE analyses for each of the two genetic clusters (Eastern and Central OR: Table 1) and found that Eastern Oregon sites were further subdivided into Blue Mountains and Southeastern Oregon. Using the parameters above, we then conducted iterative STRUCTURE runs on discrete clusters until K = 1 was supported. Initial STRUCTURE runs showed that two sites grouped with Idaho *R. luteiventris* and one site was highly disjunct from other sites (Parsnip Creek; see Fig. 1). Therefore, we excluded these sites from further STRUCTURE analyses.

c) Genetic structuring between and among the four genetic groups was tested with AMOVA using pairwise F_{ST}, performed in Arlequin v3.11. We tested the level of genetic differentiation within and among each groups with 1023 permutations.

RESULTS

Descriptive statistics, Hardy Weinberg Equilibrium, Linkage Equilibrium

Microsatellite genotyping revealed low average number of alleles per locus (mean ± STD = 2.92 ± 1.13; Table 1). The observed heterozygosity across all loci per site ranged from 0.28 – 0.81 (mean ± STD = 0.53 ± 0.02). The number of monomorphic loci per site ranged from 1 – 8 (mean ± STD = 2.8 ± 0.29), consistent with an overall reduction in allelic richness and diversity across all sites. Several sites were not in HWE ($p > 0.05$; Table 1). The *P* value for all loci combined is provided for each population. Pairwise comparison of all loci (combined across sites) showed no evidence of linkage.

Demographic History

The effective population sizes, estimated for 10 sites with sufficient samples sizes (> 17 individuals), are very small, ranging from 6.8 – 30.21 (average ± STD = 19.21 ± 1.78; Table 1).

Bottleneck analyses using IAM, TPM and allele distribution shape provide evidence that 5 sites have recently experienced a severe loss in allelic diversity (Table 2). We found strong support (2 or more significant tests) for a bottleneck for two of these sites (Page Springs and Little Fish Creek), indicating a decline in population size, but only moderate model support for a bottleneck (IAM or allele shape only) for the other 3 sites.

Population Genetic Structure

a) Pairwise F_{ST} comparisons were high overall, indicating substantial reductions in genetic connectivity among most sites. For sites within the Central cluster, pairwise F_{ST} values ranged from 0 – 0.247 (average = 0.082), with 28 of 36 pairwise comparisons showing significant genetic differentiation (Table 3). Pairwise F_{ST} values for all sites in the Eastern group ranged from 0 – 0.655 (average = 0.287), with 566 of 600 (95%) pairwise comparisons showing significant genetic differentiation (Table 4). For the Blue Mountain sites, pairwise F_{ST} values ranged from 0 – 0.648 (average = 0.226), and for the Southeastern Oregon group, pairwise F_{ST} values were similarly high, ranging from 0 – 0.628 (average = 0.33; Table 4). Only two sites were contained in the 'Idaho' cluster, and these showed no evidence of genetic differentiation from each other ($p = 0.176$).

As expected, pairwise comparisons of sites within clusters showed lower levels of genetic divergence than comparison among clusters. That is, pairwise comparisons between clusters showed very high average F_{ST} values: 0.24 (Eastern v Central), 0.33 (Blue v Southeastern OR), 0.63 (Central v Idaho), and 0.73 (Eastern v Idaho). Two isolated sites, Long and Dry, showed high average pairwise F_{ST} to both the Blue and Southeastern clusters. The average pairwise F_{ST} of Long to all Blue sites was 0.49, and 0.51 between Long and all Southeast sites. For Dry, the average pairwise F_{ST} to all Blue sites was 0.41, and 0.50 to all Southeast sites. These high values underscore their isolation and low affinity with either group. Due to their high genetic divergence from either major group, the placement of Long with the Blue genetic group, and Dry with the Southeastern Oregon group, remains uncertain and tentatively based on geographic location (Figs. 3-4).

b) STRUCTURE. The clustering method implemented in STRUCTURE confirmed that Columbia spotted frogs in Oregon are partitioned into five main genetic clusters (Fig. 1). Two sites, Coburn Creek and Castro Spring Reservoir are more related to Idaho sites in

the Owyhee Mountains than to other Oregon sites, and therefore were excluded from subsequent STRUCTURE analyses. Similarly, Parsnip Creek was highly isolated and distinct and therefore also excluded from subsequent STRUCTURE analyses.

The Central cluster includes Silver Creek, Nicoll Creek, Claw Creek, Camp Creek, and North Fork Crooked River (Fig. 2; Table 1). The most likely number of genetic clusters for this group ranged from 3-6, with 3 clusters identified using the DeltaK method (Evanno et al. 2005) and 6 demes using posterior probabilities of each model. Visual inspections of assignment plots corroborate the presence of three primary clusters. Most individuals from the Silver Creek sites were assigned primarily to cluster Central 'a', with the exception of individuals from SICR_CR45 and 4150SICR that were assigned to an alternate cluster, Central 'b' (Table 1). All other sites had a high proportion of individuals assigned to cluster Central 'b'. There is a third cluster (in low proportion) that occurs in almost all sites, except for Crook Cr, which only contains individuals assigned to Central 'b'.

The Eastern cluster is further sub-divided into the Blue Mountains (Blue) and Southeastern Oregon (SEO; Fig. 1; Table 1). The most likely number of genetic groups ranged from 2 – 13: 2 demes were identified using the ΔK maximum value (Blue and SEO), and 13 demes using posterior probabilities of each model. Visual inspection of the assignment plots verified that Blue and SEO are distinct groups, and the additional clusters (up to 13) further partitioned genetic clusters with respect to each main group. We therefore conducted subsequent STRUCTURE analyses for each of these two main groups. STRUCTURE analyses of the Blue Mountain sites revealed 7 clusters (Table 1; Fig. 1). We found limited admixture among most clusters with two exceptions: Casper Mining and Bendire 2 both contained individuals assigned to 2-3 distinct clusters (Table 1; Fig. 3). The interpretation is that some individuals sampled at Casper (based on combined genotype) are more similar to individuals assigned to cluster 'a', while others are more similar to cluster 'b' individuals. For the Southeastern cluster, STRUCTURE analyses revealed 4 clusters, with admixture observed in Mud Creek only (Table 1; Fig. 4).

c) AMOVA analyses provided strong support for five major genetic clusters of Oregon populations of *R. luteiventris*: 1) Parsnip Creek, 2) 'Idaho' (Coburn and Castro), 3) Central Oregon 4) Blue Mountain and 5) Southeastern Oregon (Table 1). These analyses showed that 25.15% of genetic variation could be explained by genetic cluster with only 13.73% of the variation partitioned among sites within each cluster (Table 5).

DISCUSSION

Rana luteinventris in Oregon occur in small populations that are highly differentiated from most other sites. We used several approaches to understand the genetic relationship among sites and identify the number of distinct genetic clusters, estimate the effective population sizes, and estimate the probability that sites have experienced recent (and/or historical) bottleneck events.

The level of genetic connectivity (gene flow) among populations and the number and spatial distribution of distinct populations

Rana luteiventris sites within Oregon consist broadly of five genetic clusters that are highly differentiation from each other (Figure 1a; Table 5), with substantial differentiation among sites within each cluster (Tables 3-4). The high level of genetic differentiation both among genetic groups as well as within some of the groups, provides substantial evidence that *R. luteiventris* sites comprise several isolated and distinct genetic groups. F_{ST} values range from zero – 1, with zero indicating ongoing gene flow (no differentiation) and 1 indicating no gene flow. For amphibians, it is generally agreed that a F_{ST} value greater than 0.3 provides evidence of very restricted gene flow. This study revealed many instances of pairwise F_{ST} values greater than 0.3. For example, Dry Creek is highly isolated from all other Southeastern OR sites (pairwise F_{ST} values ranged from 0.38 - 0.54 (Table 4). Similarly, the pairwise F_{ST} values between Long Creek and other Blue Mountain populations was greater than 0.60, underscoring the isolation of both Long and Dry sites to other *R. luteiventris* sites.

Both the STRUCTURE and AMOVA analyses support a break between Southeastern OR and Blue Mountains (Table 1). This is fairly consistent with results from mitochondrial DNA-based phylogenetic analyses that revealed a Great Basin DPS occurring at a similar juncture (Funk et al. 2008). An important difference is the grouping of Kingsbury Gulch with the Blue Mountains using microsatellite data presented in this report. The spatial distribution of genetic differentiation among sites within each Oregon cluster is shown in Figures 2- 4.

The Central Oregon cluster contains some highly isolated sites (Nicoll, NF Crook, Camp). The Silver Creek sites contain two distinct clusters, with genetic connectivity among some, but not all sites (Table 3). Our analyses suggest that the Central Oregon sites contain two genetic demes. However, despite that the pairwise F_{ST} values were different from zero (indicating some restricted gene flow), they are relatively low (pairwise F_{ST} values < 0.1). These two lines of evidence combined suggest that there is some genetic structure among those sites, but that the genetic differentiation is low to moderate (as opposed to high genetic differentiation that would suggest genetic isolation).

In Eastern Oregon, we identified two sub-groups, consistent with a previous study that showed that Great Basin sites (from Southeastern Oregon) are distinct from Blue Mountain sites (Funk et al. 2008). There are a few sites of particular interest, and therefore highlighted on Fig. 1b with respect to their position to Highway 20 and with their assignment to genetic groups. The sites that are north of Highway 20 are: King G, King Hib, Calf and Cotton, while the sites just south of Highway 20 are Long and Dry. Our analyses support the grouping of King G, King Hib, Calf and Cotton with the Blue Mountain Group. However, Long and Dry are highly genetically distinct from both each other and to the other sites in either the Blue Mountains or Southeastern OR, and could therefore be considered to be isolated and independent groups. However, we tentatively placed Long with the Blue Mountain Group and Dry with the Southeastern OR group based on the habitat in which they both occur.

Sites within the Blue Mountains are partitioned into 7 groups, with limited connectivity among those groups (Fig. 3). We found a case where one site (Casper) contains individuals assigned to two different genetic clusters. At Casper, three individuals clustered with individuals sampled from L. Green, Slab, Burnt, Pine, and Calf, while

three others were assigned to a distinct cluster that was observed only in one other individual (from L Green). We made certain that individuals at Casper were sampled at the same time and from pools in close proximity to rule out temporal and spatial sampling bias. One explanation is that individuals from Casper form two distinct breeding populations; however, a larger sample size, combined with analyses of mitochondrial and nuclear sequence data is required to robustly test this hypothesis.

The Southeastern Oregon group has 3 highly structured groups (Little Fish 8 and 1, Dry, and Bridge) that are genetically distinct, whereas 2 sites show varying levels of genetic mixing (Page, Mud). Page is comprised of individuals from a single genetic cluster, while Mud contains individuals assigned more closely to either Page individuals or Bridge individuals, suggesting admixture among those sites.

The high level of genetic differentiation both among the five clusters, as well as within each cluster, provides substantial evidence that *R. luteiventris* populations are isolated and distinct genetic groups. This is evidenced by high levels of genetic differentiation within and between each cluster (Tables 3,4).

We are applying landscape genetic approaches to rigorously test the role of landscape and geological features in shaping the gene flow among these sites. Specifically, we are applying gravity models (Murphy et al. 2010) to test the relative importance of topography, slope position, wetland distribution, stream network, and temperature and moisture on connectivity among these sites. The strength of gravity models is the incorporation of 'at-site' and 'between-site' landscape variables in predicting the factors that play a role in population connectivity. These analyses are time-intensive and are not completed at this time, but will be included in forthcoming publications in scientific journals.

Estimates of effective population size and tests for population declines using genetic bottleneck tests.

Overall, *R. luteiventris* sites contain very small effective population sizes and exhibit low hetrozygosity and low allelic richness in most sites (Table 1). Effective population size is an important measure in conservation because small populations lose allelic diversity faster and are at higher risk of extinction than larger populations due to the stochastic process of genetic drift. Measuring effective population size allows us to estimate how vulnerable a population is to the effects of genetic drift and is a measure of both the current size of the population as well as the historical size. Often, *Ne* is significantly smaller than the total number of individuals observed in a population (termed the 'census size', *Nc*). Several factors make *Ne* smaller than *Nc*, including fluctuations in population size over time, unequal sex ratios, and high variance in reproductive success. A meta-analysis of *Ne* in many species of plants and animals found that the *Ne*:*Nc* ratio averaged approximately 0.10 (Frankham 1995).

A prior comparative study of *Ne* within and among four pacific northwestern ranid species revealed low estimates of genetic diversity and small effective populations sizes (overall *Ne* < 50; Phillipsen et al. 2011). Estimates of *Ne* for the 19 populations of *R. luteiventris* in our analyses ranged from 6.81 – 30.20 (average = 18.693), which was within the range of *Ne* for *R. luteinventris* revealed in the Phillipsen et al (2011) study.

The sites with exceptionally low estimates of *Ne* (Table 1) are of particular concern as these sites are more vulnerable to the negative effects of loss of genetic variation (e.g. inbreeding depression).

We found evidence of bottlenecks in two groups (Southeastern Oregon group and Parsnip Creek). In Southeastern OR, there was strong evidence of a population bottleneck at two sites (Page Springs and Little Fish; Table 2), and only moderate evidence for Mud, Bridge, and Parsnip (Table 2). The consequences of population bottlenecks on frog populations include reduced population viability and persistence and reduced genetic variation (which results in lower fitness). These results, combined with strong evidence of small effective population sizes and low genetic diversity, place *R. luteiventris* sites from Southeastern Oregon and Parsnip Creek as a conservation concern.

Management implications

The Columbia spotted frog populations in Oregon are small (small effective population sizes), exhibit low genetic variation, and are highly differentiated from most other sites. The threats to small and isolated populations include susceptibility to reductions in genetic diversity, inbreeding effects, and demographic stochasticity, all of which can increase local extinction probabilities. These sites have a small probability of survival due to the overall low levels of migration rates among sites. The distinct units identified by our analyses include a Central cluster, the Blue Mountains, Southeastern Oregon and Parsnip Creek. Of particular concern is the Southeastern Oregon group that also shows evidence of recent population contraction (Table 3). Parsnip Creek is geographically isolated, has an extraordinarily small *Ne* (Table 1), and also shows some evidence of a recent population bottleneck (Table 2).

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Table 1. Sampling sites and microsatellite marker characteristics for *Rana luteinventris* in Oregon. Sites are contained within four major clusters based on genetic differentiation (see Results). The sub-cluster (s-cl) of each main genetic cluster indicated by lower case letters. Some sites contain more than one sub-cluster, indicated by multiple letters. For each site, the site abbreviation and sample size (N), the average number of alleles per locus (No.All), number of monomorphic loci (M), and observed heterozygosity (Ho) and expected heterozygosity (He) given Hardy Weinburg Equilibrium (HWE). *P*-value for tests of HWE provided, with significant (P < 0.05) indicating departure from HWE. Effective population size (*Ne*) for sites containing at least 18 individuals.

Genetic Cluster	s-cl	Site Name	Site Abb.	Ho	He	HWE	No.All	M	Sample Date	N	*Ne* mean (range)
Central	b	Camp Creek	Camp	0.42	0.64	0.008	3.63	1	Jun 2007	7	
Central	b	North Fork Crooked River	NF Crook	0.54	0.55	0.660	2.38	3	Jun 2007	4	
Central	b	Claw Creek & Claw Creek Alt 1-10	Claw	0.47	0.53	0.001	2.75	2	Sep 2009 Jul 2010	11	
Central	b	Nicoll Creek	Nicoll	0.65	0.64	0.000	4.63	2	Jul, Oct 2009	27	24.68 (19.8132.60)
Central	b	Silver Creek below CR 45	SICR_CR45	0.56	0.61	0.006	5.25	1	Jul 2010	22	28.61 (21.89-45.94)
Central	a	Silver Creek	SICR	0.62	0.61	0.000	4.25	1	Aug 2008	19	20.49 (16.57- 28.15)
Central	a	Silver Creek_4150	SICR_4150	0.57	0.65	0.094	3.38	1	Oct 2009	7	
Central	a	Silver Creek RNA_10.09	SICR_RNA_09	0.46	0.50	0.000	4.50	1	Oct 2009	29	25.98 (20.13- 39.55)
Central	a	Silver Creek RNA	SICR_RNA	0.52	0.57	0.000	5.13	1	Aug 2009	29	25.98 (20.13-39.55)
Eastern_Blue	a	Little Greenhorn	L Green	0.60	0.61	0.869	4.50	1	Sep 05,06	19	18.46 (14.81 -26.59)
Eastern_Blue	a	N Fork Burnt	NF Burnt	0.78	0.70	1.000	3.50	3	Aug 2006	8	
Eastern_Blue	a	Pine Cr Pond	Pine Cr	0.65	0.56	0.990	2.50	2	Aug 2006	10	
Eastern_Blue	a	Slab Creek	Slab	0.44	0.50	0.233	2.86	2	Sep 2005	8	

Eastern_Blue	a,b	Casper Mining Claim	Casper	0.39	0.69	0.000	2.88	1	2011	6	
Eastern_Blue	e	Cottonwood S. Outlet	Cotton	0.41	0.48	0.322	1.63	4	Jul 2008	9	10.96 (7.53-16.8)
Eastern_Blue	e	Mormon Basin	Mormon	0.54	0.52	0.974	2.00	4	2011	12	
Eastern_Blue	e,c,f	Bendire 2	Bendire	0.38	0.62	0.626	1.50	5	Jul 2008	2	
Eastern_Blue	d	Summit Prairie	Summit	0.80	0.67	1.000	1.88	5	Jul 2008	2	
Eastern_Blue	d	Pinecreek 1	Pinecreek1	0.47	0.73	0.304	2.38	3	Jul 2008	3	
Eastern_Blue	d	Hibernaculum (Kingsbury)	King Hib	0.52	0.48	0.024	3.63	1	Aug 2006, 2008	33	24.51 (19.50-36.06)
Eastern_Blue	d	Kingsbury Gulch	King G	.	.	.	.	8	Aug 2006	1	
Eastern_Blue	c	Calf Creek	Calf	0.47	0.52	0.012	3.38	1	Jun 2010	17	13.43 (10.73-18.84
Eastern_Blue	f	Bear Creek	Bear	0.42	0.41	0.002	3.50	1	Jun, Jul 2010	29	20.56 (15.08-29.70)
Eastern_Blue	f	Little Malheur River, sites 6-8	LMR 6-8	0.30	0.34	0.058	3.38	2	Jun, Jul 2010	28	30.21 (21.97-50.46)
Eastern_Blue	f	Little Malheur River, sites 9-11	LMR 9-11	0.58	0.45	0.763	1.50	6	Jul 2010	3	
Eastern_Blue	g	Long Creek	Long	0.40	0.57	0.256	1.57	2	na	11	
Eastern_SEO	h	Dry Creek	Dry	0.41	0.36	0.000	2.13	3	Aug 2006	25	7.04 (5.07-10.63)
Eastern_SEO	i	Bridge Creek, site 15	Bridge 15	0.76	0.69	0.036	3.75	4	Aug 2010	23	14.97 (12.19-21.89)
Eastern_ SEO	i	Bridge Creek, site 27-30	Bridge 2730	0.69	0.69	0.850	2.57	4	Aug 2010	4	
Eastern_ SEO	j	Page Springs	Page Sp	0.67	0.63	0.006	3.25	3	Aug 2008	19	9.22 (7.20-12.49)
Eastern_ SEO	i,j	Mud Cr	Mud	0.81	0.73	0.439	4.50	3	Aug 2006	21	19.46 (15.91-25.80)

Eastern_ SEO	k	Little Fish Creek Alt 8	L Fish 8	0.61	0.64	1.000	1.88	2	na	3	
Eastern_ SEO	k	Little Fish Creek [Alt 1-03, 1-04-15, 1-16-18, 1-19-25, 18,19]	L Fish 1	0.62	0.59	0.377	2.13	3	Aug 2010	22	9.86 (7.69-13.34)
Disjunct	a	Parsnip Creek	Parsnip	0.46	0.41	0.167	2.00	4	Aug 2006,10	21	6.81 (4.81-10.23)
Idaho	a	Castro Springs Reservoir 1 and 6	Castro	0.28	0.28	0.012	1.75	5	Jul 2010	20	14.30 (8.88-25.24)
Idaho	3	Coburn Creek	Coburn	0.30	0.26	0.881	1.38	6	Jul 2010	11	

Table 2. Test of genetic bottleneck for populations with sufficiently high sample sizes (> 17 individuals). The *P*-values from the Wilcoxon-Rank sum tests (1000 replications) of heterozygosity excess under the IAM and TPM model, implemented in BOTTLENECK. Test of allele frequency distribution shape in BOTTLENECK indicates whether sites are stable or shifted (evidence of a bottleneck). We interpret these results by indicating whether there is support (no, some, or strong) for a recent bottleneck.

Site	IAM	TPM	Distribution	Bottleneck
Central Cluster				
Nicoll Creek	0.43	0.43	Stable	no
Silver Creek RNA_10.09	1.00	0.07	Stable	no
Silver Creek	0.02	0.57	Stable	no
Silver Creek below CR 45	0.37	0.81	Stable	no
Blue Cluster				
Little Greenhorn	0.37	1.00	Stable	no
Hibernaculum (Kingsbury)	0.37	0.57	Stable	no
Little Malheur River, sites 6-8	0.37	0.07	Stable	no
Bear Creek	1.00	0.05	Stable	no
Southeastern Cluster				
Dry Creek	1.00	1.00	Stable	no
Bridge Creek, site 15	0.03	0.18	Stable	some
Mud Cr	0.03	0.03	Stable	some
Page Springs	0.03	0.62	Shifted	strong
Little Fish Creek	0.03	0.03	Shifted	strong
Parsnip Creek	0.09	0.62	Shifted	some
Idaho Cluster				
Castro Springs Reservoir 1 and 6	1.00	0.18	Stable	no

Table 3. Pairwise genetic differentiation among *Rana luteiventris* in Central Oregon (see Table 1). F_{ST} estimated in Arelequin along bottom diagonal. Pairs that are significantly different in bold. The *p* value for each comparison shown on upper diagonal, corrected for multiple comparisons.

	Nicoll	SICR_ 4150	SICR_ RNA	SICR_ RNA_09	Claw	SICR	SICR_ CR45	Camp	NF Crook
Nicoll	*	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SICR_ 4150	0.02	*	0.30	0.10	0.17	0.15	0.34	0.02	0.00
SICR_ RNA	**0.05**	0.01	*	0.71	0.00	0.20	0.00	0.00	0.00
SICR_ RNA_09	**0.07**	0.03	0.00	*	0.00	0.01	0.00	0.00	0.00
Claw	**0.06**	0.03	**0.08**	**0.09**	*	0.00	0.00	0.00	0.00
SICR	**0.03**	0.01	0.01	0.03	**0.05**	*	0.00	0.00	0.00
SICR_ CR45	**0.06**	0.01	**0.05**	**0.06**	**0.06**	**0.04**	*	0.02	0.00
Camp	**0.09**	**0.07**	**0.09**	**0.13**	**0.13**	**0.09**	**0.05**	*	0.00
NF Crook	**0.13**	**0.13**	**0.14**	**0.18**	**0.16**	**0.12**	**0.12**	**0.12**	*

Table 4. Pairwise genetic differentiation among *Rana luteiventris* in Eastern Oregon (see Table 1). Sites from the Blue Mountains shaded in light grey (sites 10 – 26) and sites from Southeastern Oregon shaded in dark grey (sites 27 – 33). F_{ST} estimated in Arelequin along bottom diagonal. Pairs that are significantly different in bold. The *p*-value for each comparison shown on upper diagonal, corrected for multiple comparisons.

		10	11	12	13	14	15	16
10	L Green	*	0.02	0.00	0.00	0.01	0.99	0.00
11	Slab	**0.08**	*	0.00	0.00	0.01	0.99	0.00
12	NF Burnt	**0.15**	**0.08**	*	0.00	0.01	0.99	0.00
13	Pine Cr	**0.22**	**0.19**	**0.14**	*	0.02	0.99	0.00
14	Calf	**0.24**	**0.13**	**0.12**	**0.14**	*	0.99	0.00
15	Pinecreek1	**0.27**	0.16	0.16	0.21	0.19	*	0.21
16	King G	**0.14**	**0.13**	**0.13**	**0.14**	**0.10**	0.01	*
17	King Hib	**0.24**	**0.19**	**0.11**	**0.22**	0.09	0.32	**0.25**
18	Casper	**0.21**	**0.09**	**0.11**	**0.16**	0.16	0.27	**0.13**
19	Mormon	**0.36**	**0.20**	**0.22**	**0.33**	**0.19**	0.31	**0.18**
20	Summit	**0.24**	**0.11**	**0.08**	**0.15**	-0.05	-0.07	0.02
21	LMR 6-8	**0.31**	**0.19**	**0.25**	**0.31**	**0.29**	0.28	**0.14**
22	LMR 9-11	**0.34**	**0.20**	**0.27**	**0.34**	0.33	0.31	**0.15**
23	Bear	**0.20**	**0.09**	**0.19**	**0.24**	**0.26**	**0.28**	**0.12**
24	Bendire	**0.30**	**0.15**	**0.16**	0.13	0.12	0.34	**0.13**
25	Cotton	**0.39**	**0.22**	**0.30**	**0.36**	**0.40**	**0.45**	**0.22**
26	Long	**0.55**	**0.36**	**0.42**	**0.47**	**0.58**	0.65	**0.37**
27	Dry	**0.45**	**0.32**	**0.45**	**0.45**	**0.52**	**0.60**	**0.33**
28	Bridge 2730	**0.13**	**0.18**	**0.24**	**0.30**	**0.28**	0.36	**0.29**
29	Bridge 15	**0.11**	**0.21**	**0.27**	**0.31**	**0.29**	**0.36**	**0.31**
30	Mud	**0.07**	**0.11**	**0.16**	**0.19**	**0.17**	0.14	**0.18**
31	Page Sp	**0.14**	**0.20**	**0.21**	**0.21**	**0.21**	0.21	**0.19**
32	L Fish 8	**0.20**	**0.09**	**0.15**	**0.27**	0.25	0.27	**0.19**
33	L Fish 1	**0.19**	**0.16**	**0.14**	**0.27**	**0.19**	0.29	**0.22**

Table 4 (continued)

		17	18	19	20	21	22	23	24	25	26
10	L Green	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00
11	Slab	0.00	0.03	0.00	0.02	0.00	0.00	0.00	0.05	0.00	0.00
12	NF Burnt	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.02	0.00	0.00
13	Pine Cr	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.09	0.00	0.00
14	Calf	0.16	0.08	0.01	0.82	0.00	0.09	0.00	0.50	0.00	0.00
15	Pinecreek1	0.21	0.14	0.07	0.64	0.07	0.23	0.00	0.68	0.00	0.08
16	King G	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.03	0.00	0.00
17	King Hib	*	0.01	0.00	0.06	0.00	0.02	0.00	0.08	0.00	0.00
18	Casper	**0.23**	*	0.00	0.15	0.00	0.02	0.00	0.17	0.00	0.00
19	Mormon	**0.38**	**0.27**	*	0.01	0.00	0.00	0.00	0.01	0.00	0.00
20	Summit	0.17	0.13	**0.17**	*	0.02	0.09	0.00	0.34	0.00	0.01
21	LMR 6-8	**0.42**	**0.23**	**0.24**	**0.16**	*	0.71	0.00	0.04	0.00	0.00
22	LMR 9-11	**0.45**	**0.29**	**0.32**	0.16	-0.03	*	0.00	0.11	0.00	0.00
23	Bear	**0.37**	**0.18**	**0.23**	**0.21**	**0.09**	**0.16**	*	0.03	0.00	0.00
24	Bendire	0.30	0.18	**0.24**	0.17	**0.25**	0.38	**0.17**	*	0.02	0.01
25	Cotton	**0.52**	**0.31**	**0.26**	**0.30**	**0.15**	**0.23**	**0.17**	**0.34**	*	0.00
26	Long	**0.62**	**0.53**	**0.50**	**0.56**	**0.43**	**0.59**	**0.35**	**0.60**	**0.53**	*
27	Dry	**0.61**	**0.40**	**0.45**	**0.50**	**0.33**	**0.46**	**0.23**	**0.48**	**0.29**	**0.66**
28	Bridge 2730	**0.23**	**0.27**	**0.49**	0.26	**0.44**	**0.43**	**0.38**	0.43	**0.51**	**0.63**
29	Bridge 15	**0.24**	**0.27**	**0.46**	**0.29**	**0.43**	**0.41**	**0.37**	**0.42**	**0.46**	**0.55**
30	Mud	**0.16**	**0.13**	**0.31**	**0.13**	**0.30**	**0.25**	**0.24**	**0.23**	**0.30**	**0.39**
31	Page Sp	**0.24**	**0.23**	**0.34**	**0.17**	**0.34**	**0.30**	**0.31**	**0.30**	**0.37**	**0.41**
32	L Fish 8	**0.30**	**0.24**	**0.34**	0.22	**0.30**	**0.37**	**0.16**	**0.27**	**0.43**	**0.43**
33	L Fish 1	**0.16**	**0.23**	**0.32**	**0.20**	**0.33**	**0.36**	**0.26**	**0.30**	**0.41**	**0.39**

Table 4 (continued)

		27	28	29	30	31	32	33
10	L Green	0.00	0.04	0.00	0.00	0.00	0.02	0.00
11	Slab	0.00	0.00	0.00	0.00	0.00	0.01	0.00
12	NF Burnt	0.00	0.00	0.00	0.00	0.00	0.01	0.00
13	Pine Cr	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	Calf	0.00	0.02	0.00	0.00	0.00	0.09	0.00
15	Pinecreek1	0.03	0.17	0.04	0.05	0.05	0.26	0.05
16	King G	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	King Hib	0.00	0.02	0.00	0.00	0.00	0.04	0.00
18	Casper	0.00	0.00	0.00	0.00	0.00	0.04	0.00
19	Mormon	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	Summit	0.00	0.06	0.00	0.01	0.01	0.09	0.01
21	LMR 6-8	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	LMR 9-11	0.00	0.02	0.00	0.00	0.00	0.10	0.00
23	Bear	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	Bendire	0.00	0.05	0.00	0.00	0.00	0.13	0.00
25	Cotton	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	Long	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	Dry	*	0.00	0.00	0.00	0.00	0.00	0.00
28	Bridge 2730	**0.54**	*	0.67	0.11	0.00	0.03	0.00
29	Bridge 15	**0.48**	-0.03	*	0.00	0.00	0.00	0.00
30	Mud	**0.38**	0.02	**0.05**	*	0.00	0.00	0.00
31	Page Sp	**0.50**	**0.18**	**0.20**	**0.09**	*	0.00	0.00
32	L Fish 8	**0.54**	**0.33**	**0.33**	**0.19**	**0.27**	*	0.35
33	L Fish 1	**0.50**	**0.27**	**0.27**	**0.19**	**0.24**	0.00	*

Table 5. Analysis of Molecular Variance (AMOVA) for *Rana luteiventris* in Oregon. Sites sampled from five genetic clusters identified by STRUCTURE analysis. See Table 1 and Figure 1a for site and cluster information. * = $p < 0.001$.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of Variation
Among clusters	4	490.198	0.59174	25.15*
Among sites within clusters	31	312.338	0.32308	13.73*
Within sites	992	1426.785	1.43829	61.12*

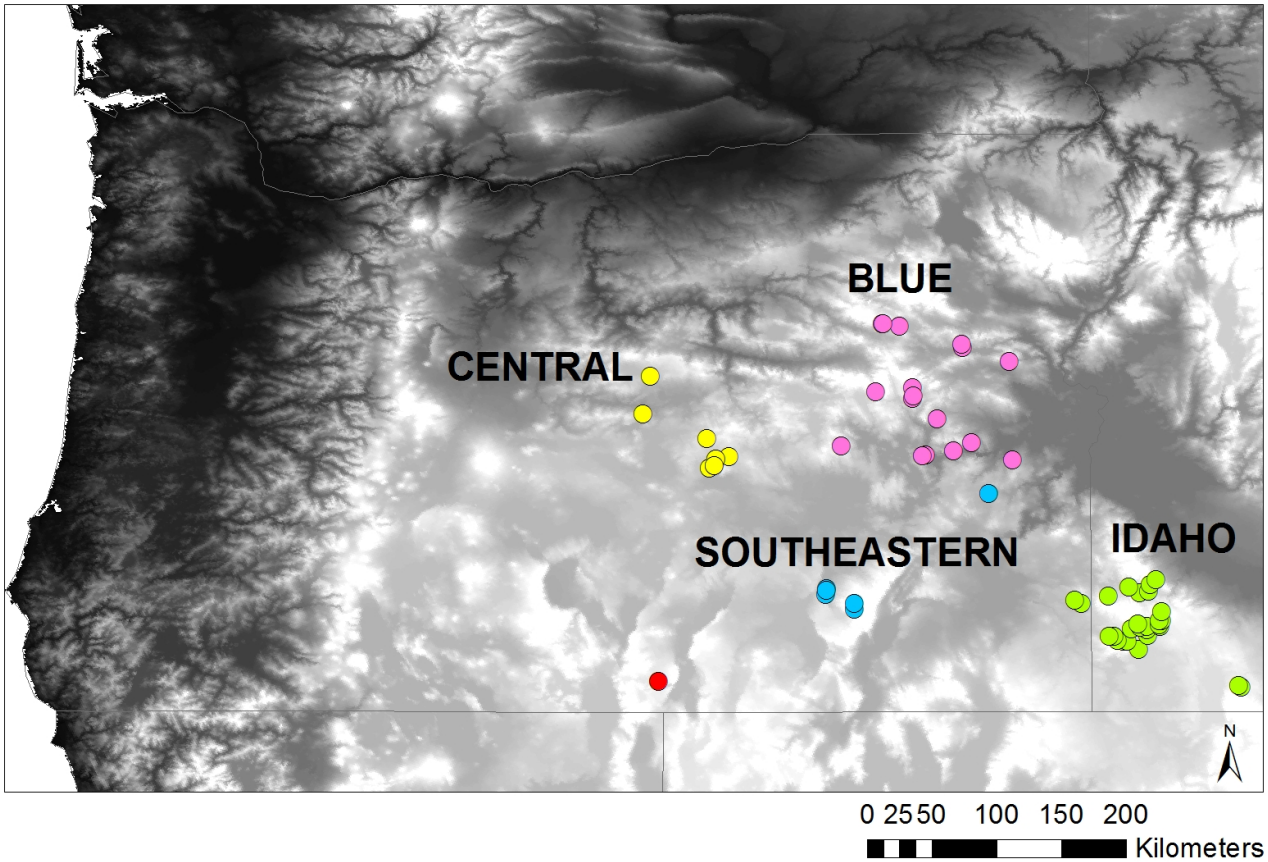

Figure 1a. Sampling sites for *Rana luteiventris* in Eastern Oregon. Genetic analyses based on multilocus genotyping supports five distinct genetic groups: Central Oregon (yellow), Blue Mountains (pink), Southeastern Oregon (blue), Idaho (green), and Parsnip Creek (red). Topographic relief shown from white (high elevation) to dark (low elevation). Drawn from ArcGIS v 9.3.1.

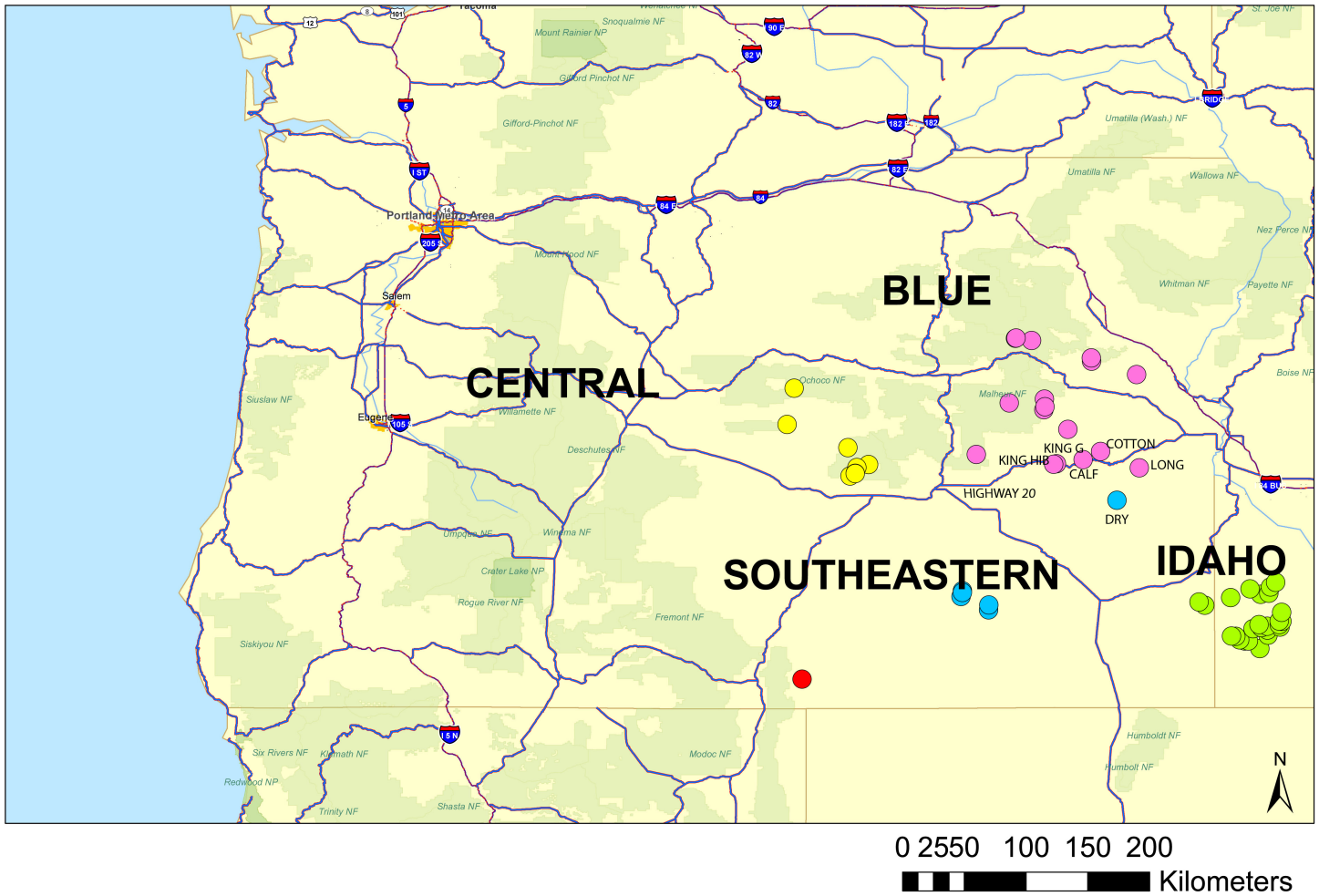

Figure 1b. Sampling sites for *Rana luteiventris* in Eastern Oregon with major highways shown. Specifically, the site localities of some of the southern Blue Mountain sites and Dry Creek from the Southeastern group, relative to Highway 20. Genetic analyses based on multilocus genotyping supports five distinct genetic groups: Central Oregon (yellow), Blue Mountains (pink), Southeastern Oregon (blue), Idaho (green), and Parsnip Creek (red). Drawn from ArcGIS v 9.3.1.

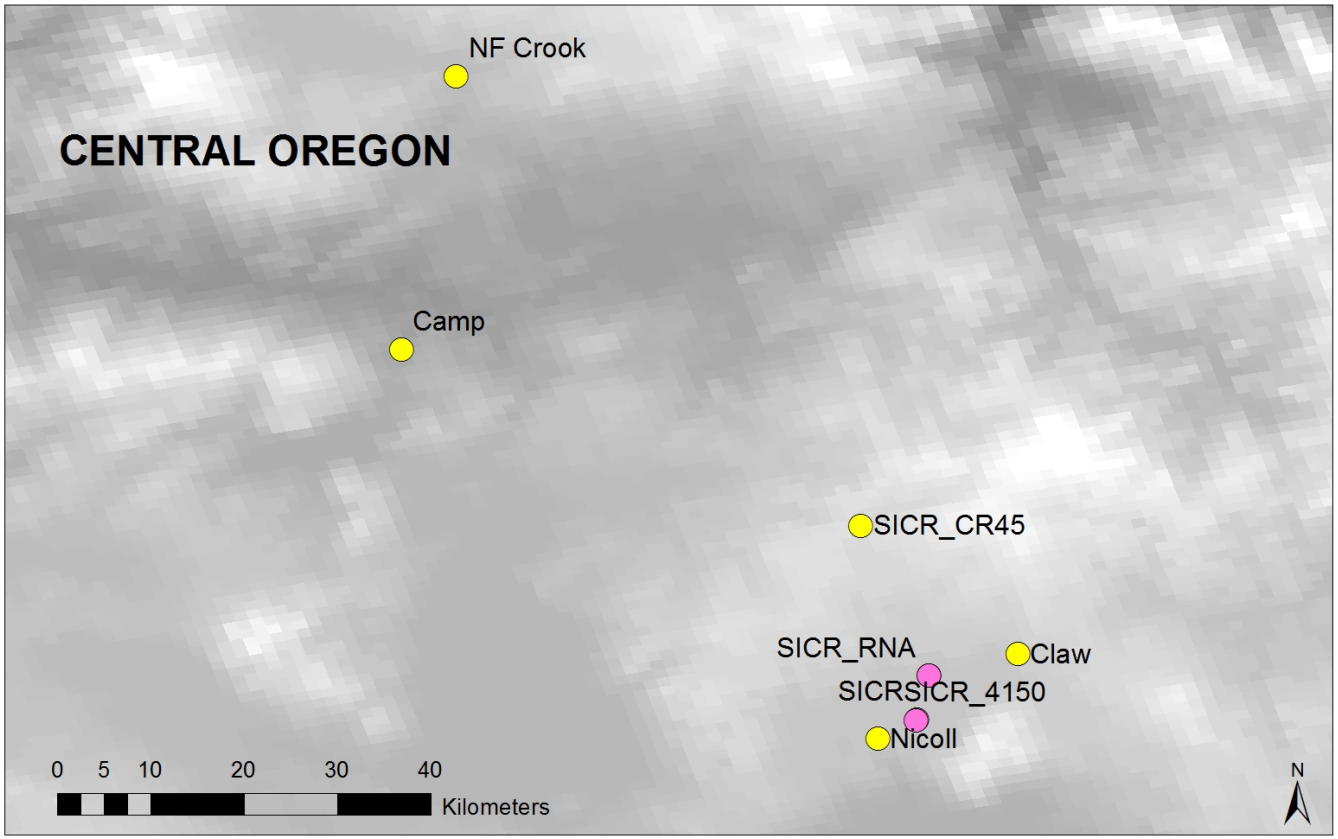

Figure 2. Population assignment to one of two clusters in the Central Oregon group, based on STRUCTURE analyses. Genetic clusters shown as distinct colors.

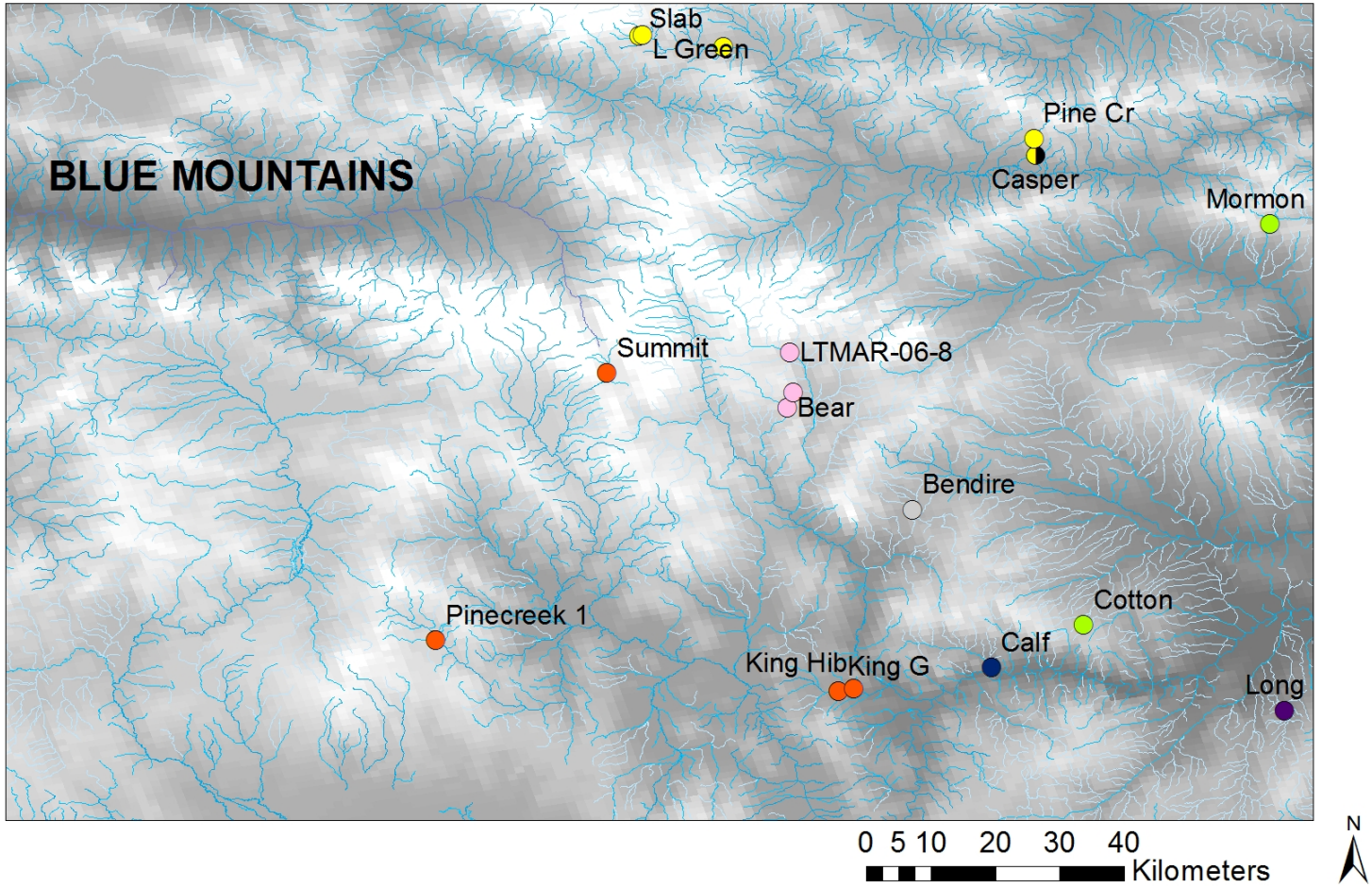

Figure 3. Population assignment to one of seven clusters in the Blue Mountains group, based on STRUCTURE analyses. Genetic clusters shown as distinct colors. The occurrence of two distinct clusters at Casper (yellow and black) shown as dichromatic circle. Bendire contains admixed individuals from 3 clusters, not shown here, but see Table 1.

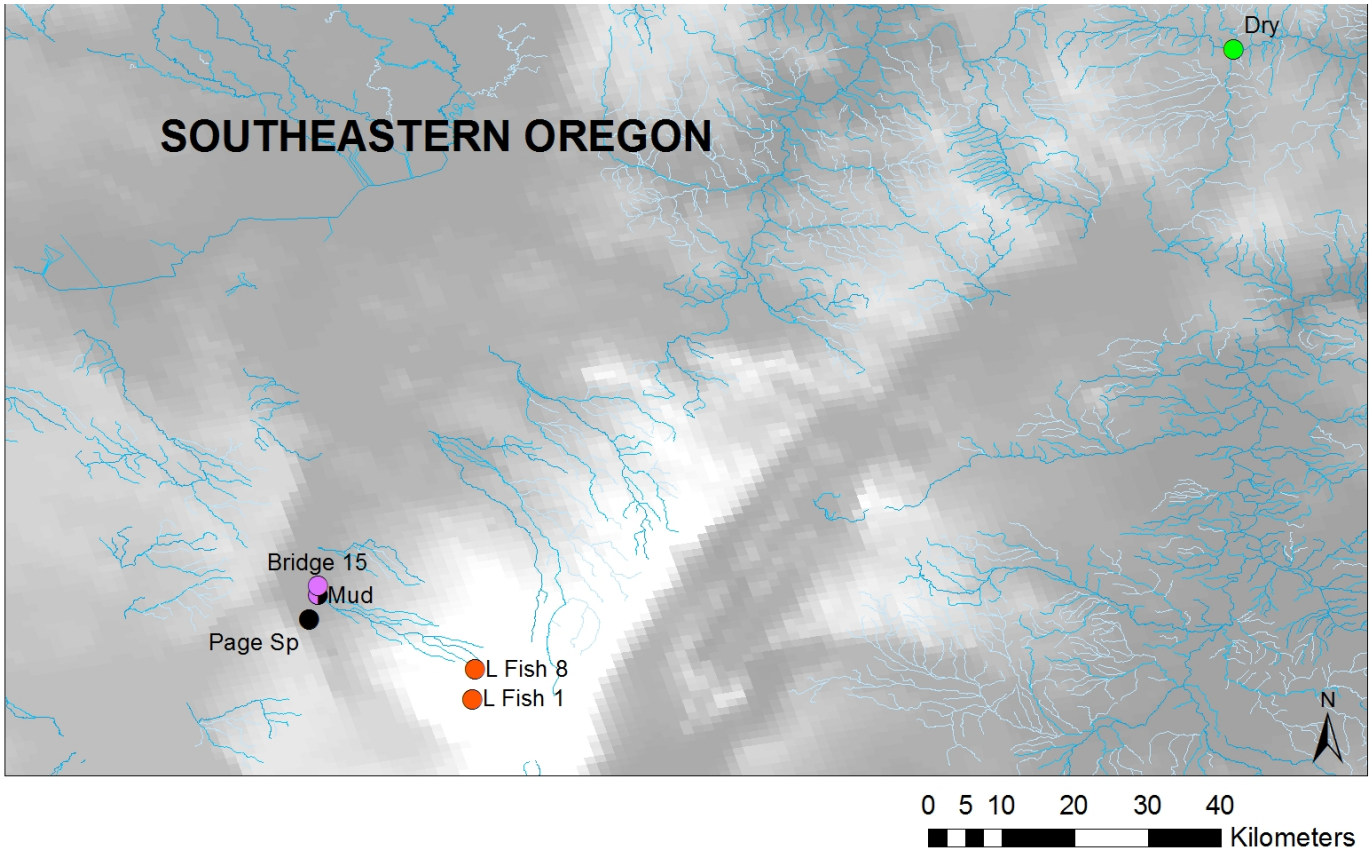

Figure 4. Population assignment to one of four clusters in the Southeastern group, based on STRUCTURE analyses. Genetic clusters shown as distinct colors. The occurrence of two distinct clusters for Mud (pink and black) shown as dichromatic circle.