

The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactylum*)

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Abstract

A primary goal of molecular ecology is to understand the influence of abiotic factors on the spatial distribution of genetic variation. Features including altitudinal clines, topography and landscape characteristics affect the proportion of suitable habitat, influence dispersal patterns, and ultimately structure genetic differentiation among populations. We studied the effects of altitude and topography on genetic variation of long-toed salamanders (*Ambystoma macrodactylum*), a geographically widespread amphibian species throughout northwestern North America. We focused on 10 low altitude sites (< 1200 m) and 11 high-altitude sites in northwestern Montana and determined multilocus genotypes for 549 individuals using seven microsatellite loci. We tested four hypotheses: (1) gene flow is limited between high- and low-altitude sites; and, (2) gene flow is limited among high-altitude sites due to harsh habitat and extreme topographical relief between sites; (3) low-altitude sites exhibit higher among-site gene flow due to frequent flooding events and low altitudinal relief; and (4) there is a negative correlation between altitude and genetic variation. Overall F_{ST} values were moderate (0.08611; $P < 0.001$). Pairwise F_{ST} estimates between high and low populations and a population graphing method supported the hypothesis that low-altitude and high-altitude sites, taken together, are genetically differentiated from each other. Also as predicted, gene flow is more prominent among low-altitude sites than high-altitude sites; low-altitude sites had a significantly lower F_{ST} (0.03995; $P < 0.001$) than high altitude sites ($F_{ST} = 0.10271$; $P < 0.001$). Use of Bayesian analysis of population structure (BAPS) resulted in delineation of 10 genetic groups, two among low-altitude populations and eight among high-altitude populations. In addition, within high altitude populations, basin-level genetic structuring was apparent. A nonequilibrium algorithm for detecting current migration rates supported these population distinctions. Finally, we also found a significant negative correlation between genetic diversity and altitude. These results are consistent with the hypothesis that topography and altitudinal gradients shape the spatial distribution of genetic variation in a species with a broad geographical range and diverse life history. Our study sheds light on which key factors limit dispersal and ultimately species' distributions.

Keywords: altitude, *Ambystoma macrodactylum*, genetic structure, landscape influence, long-toed salamander, topography

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Introduction

A primary goal of molecular ecology is to understand the influence of abiotic factors, such as, altitude, topography and glacial history on the spatial distribution of genetic variation. Glacial maxima and minima can drastically

affect landscape features, such as topographic relief, consequently having a major influence on species' ranges (Waitt & Thorson 1983; Carstens *et al.* 2005). Features including altitudinal or latitudinal clines and landscape characteristics also affect the proportion of suitable habitat, influence migration patterns and ultimately genetic divergence of populations (Manel *et al.* 2003; Palo *et al.* 2003). In addition, divergent local selection pressures across altitudinal gradients may contribute to local genetic differentiation through local adaptation (Slatkin 1987; Palo *et al.* 2003). Incorporating phylogeographical tools to analyse the effects of landscape characteristics on species' distributions over large spatial scales has provided remarkable insight into the spatial patterns of genetic diversity (Avice 2000). These tools can be ineffective at small spatial scales, however, because sequence diversity may not be rich enough to analyse patterns of colonization and migration and distribution patterns of genetic diversity at local scales. By using highly variable genetic markers and a dense sampling regime across a small, topographically diverse region, it is possible to test the localized effects of altitude and geography on genetic diversity and genetic connectivity across a landscape, yet studies have rarely done so.

Recent advances in population genetics have allowed researchers to study the effect of landscape variables, such as altitude and topography, on the geographical distribution of genetic variation in the emerging field of landscape genetics (Manel *et al.* 2003). For example, altitude was shown to be an important variable that explained differences in flowering time and consequent genetic divergence of three species of snowbed herbs (Hirao & Kudo 2004). Altitudinal relief has also been shown to be significantly negatively correlated with gene flow in both Pacific jumping mice (*Zapus trinotatus*; Vignieri 2005) and tiger salamanders (*Ambystoma tigrinum melanostictum*; Spear *et al.* 2005). Mountain ridges have also been shown to be an important barrier to amphibian dispersal and gene flow (Funk *et al.* 2005).

Amphibians are particularly sensitive to effects of altitudinal and topographic variation for several reasons. Amphibians are generally poor dispersers and often highly site philopatric (Rowe *et al.* 2000; Tallmon *et al.* 2000; Funk *et al.* 2005; Kraaijeveld-Smit *et al.* 2005; Spear *et al.* 2005). Low vagility in amphibians is often attributed to dependence on moist habitats or wetland corridors for dispersal (Rothermel & Semlitsch 2002; Tremham & Shaffer 2005) because of desiccation and predation risks associated with terrestrial dispersal (Madison & Farrand 1998; deMaynadier & Hunter 1999; Rothermel & Semlitsch 2002) and slow terrestrial locomotion (Rothermel & Semlitsch 2002). Thus, dispersal of individuals away from their natal sites and consequent range expansion are generally expected to be limited in amphibians.

The long-toed salamander (*Ambystoma macrodactylum*) is abundant and has a wide geographical range throughout the western USA and Canada (Stebbins 1951). Breeding sites for long-toed salamanders are found from low-lying coastal pools near sea level to montane glacial cirques at altitudes of over 2000 m (Anderson 1967; Howard & Wallace 1985). The long-toed salamander exhibits considerable diversity in life-history traits across this altitudinal gradient. At low altitudes, salamanders generally metamorphose within 2–3 months, but at high altitudes, salamanders generally overwinter prior to metamorphosis (Kezer & Farner 1955; Anderson 1967; Howard & Wallace 1985; Maxell *et al.* 2003). Short breeding seasons and harsh habitats at high altitude may contribute to these life-history differences. If differences in life-history trajectory are responses to selection and are not phenotypically plastic in nature, they could increase genetic differentiation between high- and low-altitude populations in addition to the effects of altitude and topography.

The Cabinet Mountains in northwestern Montana contain long-toed salamander breeding sites across an altitudinal gradient (from ~700 m to >2300 m). Frequent flooding events at low altitudes may facilitate moderate to high gene flow because spring is concomitant with the breeding season. While high-altitude populations may have been unsuitable habitat during much of the Pleistocene, receding glaciers carved out depressions, resulting in glacial cirques suitable for colonization by long-toed salamanders from low-altitude refugia. However, due to the generally low vagility of *A. macrodactylum* (Tallmon *et al.* 2000; Rothermel & Semlitsch 2002), colonization of high-altitude sites is probably uncommon, possibly leading to founder effects and decreased genetic diversity in high-altitude sites relative to low-altitude sites. Relative to low-altitude habitats, high-altitude interpond habitats typically have more extreme topographic relief between ponds, longer winters, and significantly more snowfall, all of which likely limit dispersal and consequent gene flow (Funk *et al.* 2005). As a result of either infrequent postglacial colonization and/or restricted gene flow, lower genetic diversity is expected among high-altitude sites than low-altitude sites. This prediction is consistent with a mountain-valley model of amphibian genetic structure that has been proposed and supported by empirical data from Columbia spotted frogs (*Rana luteiventris*) (Funk *et al.* 2005). The mountain-valley model of population structure predicts lower levels of gene flow and genetic diversity among high-altitude sites relative to low-altitude sites due to harsher terrain, topography and climate.

We tested four predictions regarding the population genetic structure of long-toed salamanders in low- vs. high-altitude sites in the Cabinet Mountains using seven microsatellite loci based on the topography of the study area: (i) gene flow will be higher among low-altitude

sites (< 1200 m) than high-altitude sites; (ii) gene flow will be restricted among high-altitude sites; (iii) low-altitude sites, taken together, will be significantly genetically differentiated from high-altitude sites; and, (4) low-altitude sites will have higher genetic diversity than high-altitude sites.

Materials and methods

Study site and sample collection

The Cabinet Mountains of northwest Montana are located within the Cabinet Mountain National Wilderness Area in the Kootenai National Forest. We sampled sites throughout the Bull River Valley region, both along the valley bottom (low altitude; < 1200 m) and throughout intermountain basins (high altitude; > 1200 m). Sixty potential amphibian-breeding sites were surveyed for the presence of long-toed salamanders between April 2003 and July 2005 and 549 tail clips were collected from 21 of these sites (Fig. 1). Potential breeding populations were chosen based on a combination of past amphibian surveys (Maxell *et al.* 2003) and our survey results (A. Giordano, unpublished data).

Equal numbers of adult and larval salamanders were captured among sampling sites during the breeding season (March–August) using dip-nets and minnow traps. Individuals were sampled haphazardly from throughout

each breeding site to maximize the chance of collecting unrelated individuals. Nearly 90% of all captures were larvae, and there were no differences in percentage of larvae vs. adults among sites (or among high- vs. low-altitude sites). When dip-netting, individuals were either encountered visually or captured in a random sweep of pond vegetation. Minnow traps were used for overnight capture of adults. Tail clips (~0.5–1.0 cm) were taken from individuals from each pond and stored in 70% ethanol at –20 °C until DNA extraction. Sample sizes ranged from $n = 10$ to $n = 31$ (Appendix I).

DNA isolation and microsatellite amplification

DNA was extracted from 96 of the 549 individuals using a QIAGEN DNeasy plate extraction kit. DNA from all other individuals was extracted using a standard phenol–chloroform protocol (Sambrook *et al.* 1989).

Seven polymorphic microsatellite loci were used: Aje D03, Aje D94, Aje D162 and Aje D422 were originally isolated in *Ambystoma jeffersonianum* (Julian *et al.* 2003a). Ama C40, Ama D95 and Ama D184 were originally isolated in *Ambystoma maculatum* (Julian *et al.* 2003b). Each locus contains a tetranucleotide repeat, and all but AmaC40 showed high levels of allelic variation (Table 1).

All loci were amplified using either standard or touch-down polymerase chain reaction (PCR) protocol performed on either a Bio-Rad I-cycler or a MJ PTC-100 (Appendix II).

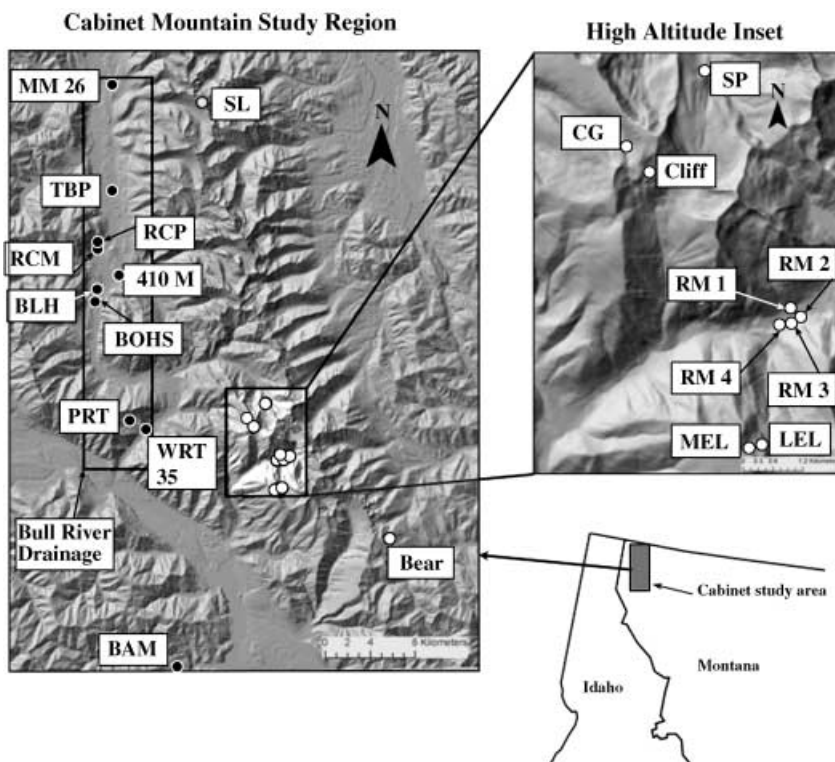


Fig. 1 Shaded relief map of the northwest Montana study area. High-altitude sites are indicated by white circles, low-altitude sites are indicated by black circles. An outline map of the Idaho panhandle and western Montana show the general region of the study area. A high-altitude inset shows the detail of the locations of the nine high-altitude sites.

Table 1 Results of five independent runs of a nonequilibrium, Bayesian assessment of migration proportions by population (BAYESASS; Wilson & Ranalla 2003). Bolded terms along the diagonal represent proportion of nonmigrants within a population; values in rows represent migrant genes received from other sites; values in columns represent migrant genes donated to other populations. * represents values present in four out of five simulations; dashes separate values for sites where the algorithm ascertained one value three times and one value twice

	Low sites											High sites								
	BAM	BLH	BOHS	MM 26	PRT	RCM	RCP	TBP	CG	SP	Cliff	RM1	RM2	RM3	RM4	SL	BEAR	MEL	LEL	
BAM	0.679																			
BLH		0.679	0.215	0.0/0.066																
BOHS			0.984				0.0/0.026													
MM 26			0.240/0.0	0.703/0.983																
PRT			0.072	0.06/0.0	0.679		0.23/0.0									0.042	0.112			
RCM			0.253			0.679	0.0/0.01													
RCP			0.0/0.198				0.974/0.678		0.0/0.03											
TBP			0.098	0.0/0.011			0.239/0.0	0.678										0.0/0.046		
CG									0.984											
SP										0.985*										
Cliff											0.987*									
RM1												0.983*								
RM2													0.979							
RM3													0.201	0.684*				0.012/0.0		
RM4													0.258		0.678					
SL																0.985				
Bear																				
MEL																			0.679	
LEL																			0.235	
																			0.984	

PCR products were multiplexed according to allele size and diluted at a concentration of 1:200 fluorescently labelled PCR product:water. Genotypes at each locus were obtained using an ABI 3730 (Applied Biosystems, Inc.) automated capillary and visualized and scored using GENEMAPPER version 3.7 software (Applied Biosystems).

Microsatellite data analysis

We used FSTAT 2.9 to test whether loci were in Hardy–Weinberg equilibrium (HWE) and linkage equilibrium, as well as to calculate expected heterozygosities, and allelic richness for each locus (Goudet *et al.* 1996). Fisher's exact tests for linkage equilibria were calculated using GENEPOP 3.3 (Raymond & Rousset 1995). FSTAT 2.9 was also used to calculate estimators of Wright's F -statistics (Wright 1931): Θ , F and f , respectively (Weir & Cockerham 1984).

To test the hypotheses that gene flow is higher among low-altitude sites than among high-altitude sites, we estimated pairwise F -statistics between all pairs of low-altitude and high-altitude sites, respectively. We also used BAYESASS (version 1.3; Wilson & Rannala 2003) to estimate current migration rates among populations. BAYESASS 1.3 relies on multilocus genotypes and a Markov chain Monte Carlo (MCMC) algorithm to estimate proportions of non-migrants and the source of migrants for each sampling site (Wilson & Rannala 2003). As BAYESASS is a nonequilibrium Bayesian method that does not require data sets to conform to HWE proportions, we therefore included all loci in the analysis. However, the software is limited to analysis of 19 sites (Wilson & Rannala 2003), so we excluded sites Wpt 35 and 410 M, the two sites with the lowest sample sizes ($n = 10$ each). Wilson & Rannala (2003) suggest that more accurate results are obtained from runs when the number of proposed changes for the variables m (migration rate), P (allele frequencies), and F (inbreeding coefficient) are between 40% and 60% of the total chain length. We performed five independent replicate runs of the algorithm for 9×10^6 iterations with 3×10^6 iterations discarded as burn-in. Delta values of $m = 0.30$, $P = 0.15$, and $F = 0.15$ yielded an average number of changes in the accepted range.

We used the program BAPS (Corander *et al.* 2003; Corander & Marttinen 2006) to test the hypothesis that gene flow was restricted among high-altitude sites by determining whether high-altitude sites formed a higher number of genetically distinct populations than low-altitude sites. Specifically, we used BAPS to detect clusters of genetically similar populations and to estimate individual coefficients of ancestry (q) with regard to the detected clusters. BAPS uses a stochastic optimization algorithm for analysing Bayesian models of population structure, which greatly improves the speed of the analysis compared to traditional MCMC-based algorithms (Corander & Marttinen 2006).

Furthermore, comparison tests have shown that BAPS has comparable statistical power to STRUCTURE, and increased power over small geographical distances (Corander & Marttinen 2006; Latch *et al.* 2006). When testing for population clusters, we ran 10 replicates for every level of k (k is the maximum number of clusters) up to $k = 20$. When estimating individual ancestry coefficients via admixture analysis, we utilized only clusters that had at least 10 individuals present within them. In addition, we used the recommended number of reference individuals (200) and repeated the admixture analysis 50 times per individual.

To determine whether high-altitude and low-altitude sites represent distinct genetic clusters, a multivariate population graphing approach (Dyer & Nason 2004) was also performed. Each sampling site in a population graph is represented by a node, the diameter of which is determined by the level of within site heterozygosity (Dyer & Nason 2004). Lines (i.e. edges) connecting nodes indicate that they are not significantly genetically differentiated from one another and the length of each line is proportional to the among-site genetic variation and visualized by POPGRAPH (<http://dyerlab.bio.vcu.edu/wiki/index.php/Software>). A saturated population graph contains $n(n-1)/2$ edges ($n =$ the total number of nodes), meaning no significant differentiation among any sampling sites. Genetic structuring is tested with a chi-squared significance test to determine if there are significantly fewer edges than predicted for a saturated graph. A binomial test for the significance of among- and within-group relationships is performed by assessing deficiencies of edges between nodes relative to the saturated graph (Dyer & Nason 2004). We used binomial tests to determine whether there was significantly restricted gene flow: (i) between high- and low-altitude groups of sites; and (ii) among high-altitude sites and among low-altitude sites.

To test the hypothesis that genetic diversity should be lower in high-altitude sites vs. low-altitude sites, we performed regressions of both allelic richness and expected heterozygosities by altitude of sampling sites using SYSTAT 11 software (SPSS).

Results

Departures from HWE were found in 13 of 21 sites for locus Aje D162, six of 21 sites for Aje D94, 2 populations for Ama D184 and one of 21 sites for Ama D95, all after Bonferroni corrections for multiple comparisons. All other loci were in HWE. These departures could signify a problem with null alleles at these loci; however, it is also possible that departures at Aje D162 and Aje D94 are due to sampling error (i.e. low sample size relative to allelic number), as we detected more than 30 alleles at each of these two loci (Table 1). Fisher's exact tests detected significant linkage disequilibrium between Aje D03 and

Aje D422 as well as between Aje D162 and Ama D184. Permutation tests performed in *FSTAT* detected significant deviations from genotypic equilibrium in 5 out of 21 total comparisons after Bonferroni correction for multiple comparisons. Expected heterozygosities ranged from 0.498 to 0.701 within populations (Appendix I). Allelic richness was determined to be between 3.565 and 5.780 alleles per locus (Appendix I).

Twenty-two of 45 of pairwise comparisons (49%) between low-altitude sites did not have F_{ST} values significantly different from zero (Table 2). Nine of the 10 low-altitude sites are located along the Bull River drainage or along streams that flow directly in to the drainage (Fig. 1). The tenth site, BAM, is in a separate drainage (Fig. 1); all pairwise F_{ST} s including BAM and other low-elevation sites are significant (Table 2). In contrast, 44 of 55 (92.8%) pairwise comparisons between high-altitude sites had F_{ST} values that were significant from zero (Table 2). The proportion of significant high-altitude comparisons (44/55) is statistically larger than the proportion of significant low-altitude comparisons as detected using a one-tailed Fisher's Exact Test ($P = 0.0022$). Thus, nearly all pairwise high-altitude combinations showed significant genetic subdivision. Comparisons between high- and low-altitude sites also showed significant subdivision, as 90% (99 of 110) had significant F_{ST} values (Table 2). Of these 11 comparisons that were nonsignificant, eight are between a high-altitude site and the two sites with low sample sizes: 410 M and Wpt35.

The proportion of migrants accepted and donated by each site was estimated using *BAYESASS* (Table 1). Most low-altitude populations are either exchanging or receiving migrants at a high rate, while high-altitude populations exchange migrants only within basins. *BAYESASS* also detects directional migration within basins. For example, migration between RM2, RM3 and RM4 is directional with RM2 as the apparent source of migrants (Table 1). Similarly, MEL appears to be the recipient of migrants from LEL (Table 1). BOHS is identified as a source population for all of the low-altitude Bull River sites, with RCP also providing a large proportion of migrants to other populations (Table 1).

Analyses using *BAPS* identified 10 genetic clusters overall ($P = 0.0035$; Fig. 2a, b). There were two genetic clusters among low-altitude sites, with site BAM significantly differentiated from the remaining 10 sites within the Bull River drainage (Fig. 3). Seven genetic clusters were identified among the 10 high-altitude sites, with all sites distinct except for clusters formed by RM 2, 3 and 4 and MEL and LEL (Fig. 4); in each of these two clusters, sites were within 2 km of one another and found within the same basin. Thus, results of *BAPS* analyses support the results of *BAYESASS* that low-altitude populations only form two genetic clusters, while high-altitude populations form genetic clusters only within basins.

The topography of the population graph was analysed using binomial tests in order to detect distinct groups of sites. The population graph showed a significant deficiency of edges between high- and low-altitude sites (Fig. 5; $p(X \leq K_{\text{between high and low}}) = 0.0123$), suggesting significant overall divergence between high- and low-altitude sites. We were not able to detect a significant deficiency of edges within high- ($P = 0.96$) or low- ($P = 0.90$) altitude sites independently using this method. However, two basins of sites were distinct, Rock Meadow (RM) ($P = 0.002$) and Bull River ($P = 0.0004$) (Fig. 4).

There was a strong negative ($r^2 = 0.736$; $P < 0.001$) correlation between allelic richness and altitude and a significant negative correlation between expected heterozygosity and altitude ($r^2 = 0.623$; $P < 0.001$) (Fig. 5).

Discussion

By focusing on an organism with a broad geographical range and diverse life history, our study sheds light on which key factors limit species' dispersal and distributions. Our results contribute to a growing body of work that suggests landscape features, and specifically mountain ridges, limit dispersal and gene flow among amphibian populations (Lougheed *et al.* 1999; Shaffer *et al.* 2000; Monsen & Blouin 2003), including long-toed salamanders (Tallmon *et al.* 2000). Allozyme evidence also suggests that dispersal among populations of long-toed salamanders is limited by mountain ridges in the Bitterroot Mountains along the Idaho/Montana border (Tallmon *et al.* 2000). Herein, we found four key results that support our predictions: (i) there is significantly higher gene flow among low- vs. high-elevation sites; (ii) gene flow is restricted among high-altitude sites; (iii) when considered together, low-elevation sites are significantly subdivided from high-elevation sites; and (4) there is significantly higher genetic variation in low- vs. high-elevation sites. These four results are all consistent with the hypothesis that topography and altitudinal gradients have shaped the spatial distribution of genetic variation in long-toed salamanders.

Our analyses provide several lines of evidence that low-altitude sites appear to be similar genetically, while high-altitude sites are generally significantly more differentiated from one another. Low-altitude sites appear to have appreciable gene flow, as the majority of pairwise comparisons did not have F_{ST} values that were significantly greater than zero. This may be expected for sites that occupy the same drainage (Tallmon *et al.* 2000). All low-altitude sites (except BAM) are along the Bull River drainage, which is flanked by large flooded meadows, sloughs and many small ponds in which long-toed salamanders could potentially breed. In addition to the difference of low vs. high altitude, gene flow among these sites may be higher due to the fact that they are within the same drainage,

Table 2 Pairwise F_{ST} comparisons between all sampling sites above the diagonal as calculated by FSTAT version 2.9.3.2 (Goudet 1995). Non-significant F_{ST} values are bolded

	Low-altitude sites										High-altitude sites										
	Wpt35	410 M	BAM	BLH	BOHS	MM 26	PRT	RCM	RCP	TBP	CG	SP	Cliff	RM1	RM2	RM3	RM4	SL	BEAR	MEL	LEL
Wpt35	0.04	0.055	0.04	0.047	0.041	0.009	0.043	0.022	0.021	0.077	0.048	0.159	0.065	0.09	0.097	0.075	0.063	0.047	0.117	0.116	
410 M		0.084	0.018	0.004	0.018	0.045	0.022	0.038	0.055	0.122	0.062	0.186	0.063	0.102	0.118	0.1	0.057	0.085	0.102	0.113	
BAM			0.075	0.088	0.063	0.054	0.083	0.057	0.073	0.102	0.081	0.157	0.062	0.097	0.088	0.096	0.068	0.073	0.113	0.105	
BLH				0.011	0.024	0.034	0.007	0.035	0.056	0.109	0.071	0.146	0.076	0.093	0.126	0.118	0.066	0.092	0.144	0.147	
BOHS					0.036	0.052	0.009	0.057	0.075	0.139	0.078	0.175	0.098	0.126	0.156	0.144	0.07	0.115	0.145	0.146	
MM26						0.048	0.018	0.041	0.049	0.116	0.087	0.143	0.07	0.111	0.133	0.114	0.05	0.102	0.145	0.156	
PRT							0.045	0.036	0.032	0.087	0.042	0.157	0.077	0.086	0.102	0.091	0.045	0.044	0.133	0.125	
RCM								0.045	0.058	0.132	0.1	0.155	0.095	0.124	0.152	0.142	0.061	0.111	0.162	0.167	
RCP									0.026	0.059	0.067	0.1	0.067	0.086	0.086	0.076	0.061	0.066	0.117	0.13	
TBP										0.109	0.091	0.164	0.087	0.103	0.115	0.094	0.092	0.071	0.14	0.151	
CG											0.094	0.069	0.116	0.117	0.119	0.106	0.119	0.119	0.167	0.155	
SP												0.186	0.077	0.086	0.09	0.089	0.065	0.051	0.107	0.086	
Cliff													0.193	0.186	0.213	0.206	0.177	0.216	0.247	0.258	
RM1														0.05	0.051	0.048	0.088	0.086	0.072	0.087	
RM2															0.045	0.021	0.124	0.1	0.125	0.125	
RM3																0.038	0.127	0.089	0.096	0.097	
RM4																	0.107	0.089	0.11	0.121	
SL																		0.087	0.156	0.139	
Bear																			0.136	0.115	
MEL																				0.023	
LEL																					

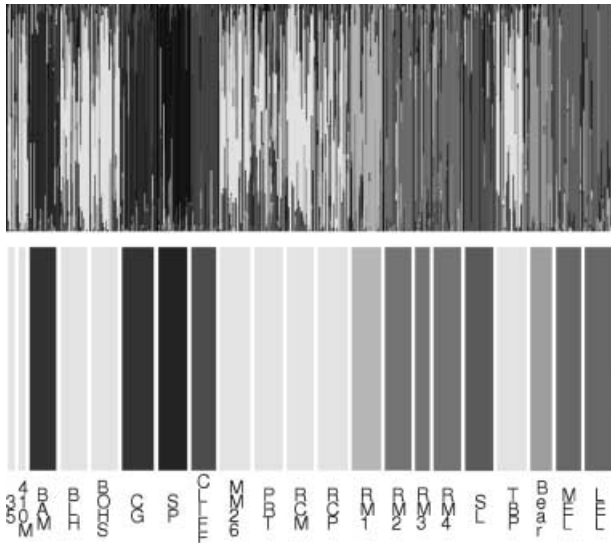


Fig. 2 (a) Assignment probabilities of samples from individual genotypes among the 21 samples sites using BAPS (Bayesian analysis of population structure; Corander *et al.* 2003). (b) Delineation of 10 genetic clusters using BAPS; each shade of grey indicates sampling localities belonging to each cluster.

despite their relatively large geographical distance relative to high-altitude sites. In spring, high volume thaws lead to flooding of the plains and sloughs adjacent to the Bull River, probably facilitating high gene flow among long-toed salamander populations.

BAPS analyses upheld our prediction that low-altitude sites are behaving as a panmictic population. Low-altitude sites only formed two distinct clusters – one in the Bull River drainage and the second (BAM), which was geographically disjunct from the rest of the populations (Fig. 4). High-altitude sites formed eight distinct clusters, with each basin forming a distinct cluster relative to other basins. However, high-altitude sites in the Cabinet mountains are apparently not as genetically differentiated as those in the Bitterroots (Tallmon *et al.* 2000); this difference may be due to the fact that the prior study used allozymes, as opposed to microsatellites, as the genetic marker (Dhuyvetter *et al.* 2004). Allozymes evolve more slowly than microsatellites and may thus represent more ancient events than those represented by microsatellites in this study. Nonetheless, basin level structuring in long-toed salamanders is consistent with that found in Columbia spotted frogs (*Rana luteiventris*) in the inland northwest, where genetic populations are often comprised of multiple sites within a basin (Funk *et al.* 2005). But, mountain ridges still restrict gene flow among basins (Funk *et al.* 2005), despite the capacity for long-distance dispersal in Columbia spotted frogs described by Pilliod *et al.* (2002).

We detected several cases of directional migration in our analysis using BAYESASS. As expected, downstream dispersal was found between the high elevation Rock Meadow ponds. However, migration between populations within basins was not always downstream, in contrast to studies of other amphibian species (Kraaijeveld-Smit *et al.* 2005). However, a recent study showed upstream dispersal bias

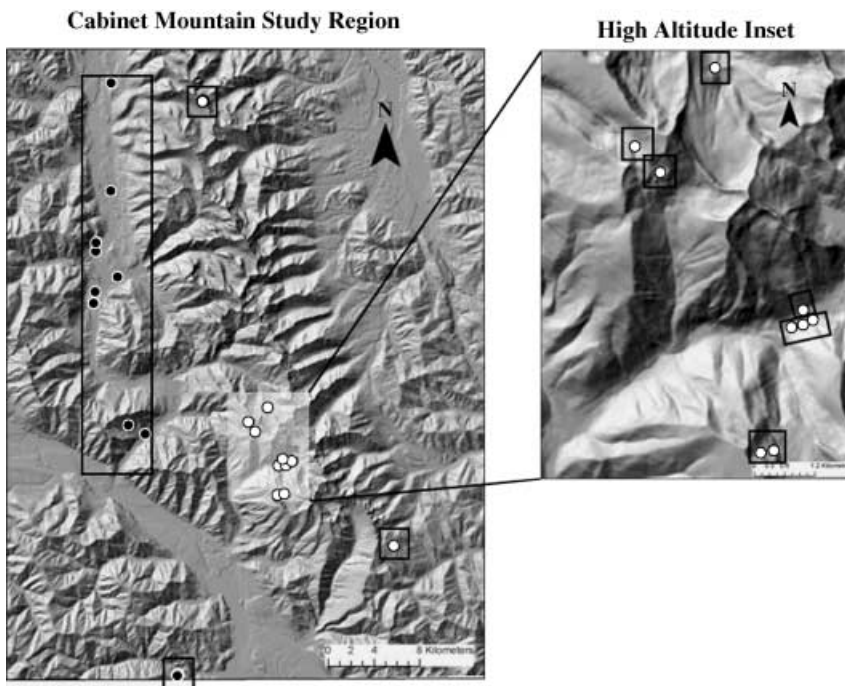


Fig. 3 Map of study area with genetic populations identified by BAPS (Corander *et al.* 2003) clustering algorithm over all sampling sites. High-altitude populations are represented by white circles and low-altitude populations are represented by black circles. Dark squares around single or groups of different sampling sites indicate distinct genetic clusters.

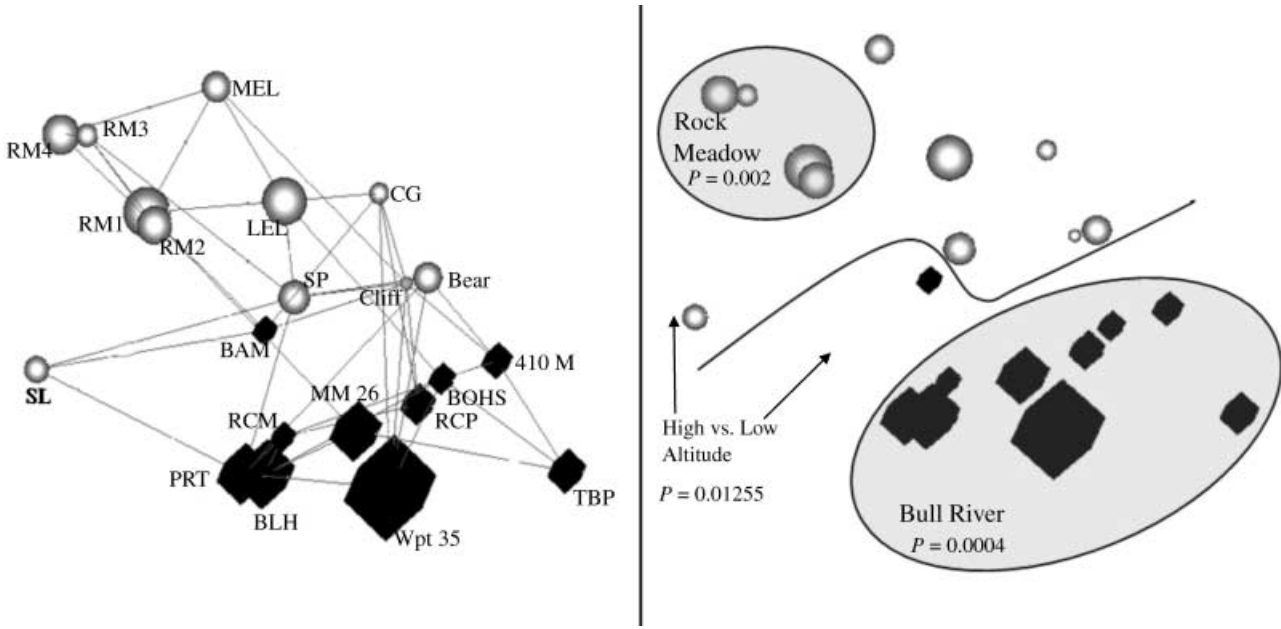


Fig. 4 Population graphs for study sites. Nodes represent sites with diameter equivalent to within-site microsatellite variation; edges represent among site variation ascribed to the connected sites, length is proportional to site similarity (Dyer & Nason 2004; Krasfur *et al.* 2005). (a) Population graph with edges between sites, spherical nodes represent high-altitude populations, cube nodes are low-altitude populations. (b) Populations graph displaying the significance of the genetic divergence between high- and low-altitude groups, and the genetic isolation of two major groups of sites: Rock Meadow and Bull River.

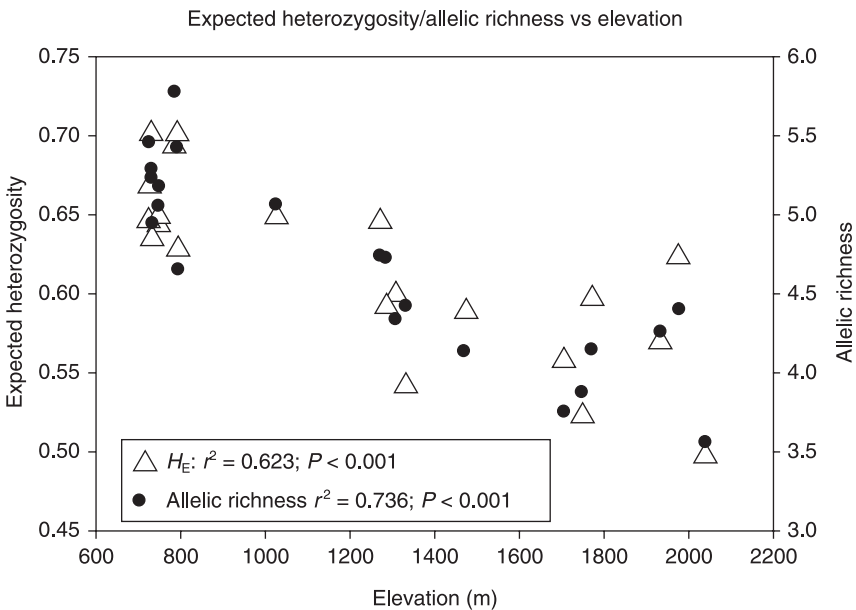


Fig. 5 Plot of expected heterozygosity (H_E , hollow triangles) and allelic richness (filled circles) vs. altitude by sampling site.

in the salamander *Gyrinophilus porphyriticus* (Lowe *et al.* 2006). We detected upstream dispersal in the high-altitude Engle lake basin between MEL and LEL, and along the low-altitude Bull River drainage between several populations. Two alternative hypotheses for upstream dispersal are either long-toed salamanders are capable of moderate

amounts of terrestrial dispersal, or flooding events in the spring create adequate aquatic upstream dispersal corridors for adult or larval individuals.

High-altitude sites, taken together, were also significantly differentiated from low-altitude sites, as supported by *F*-statistics, BAPS analyses and population graphing.

Topographical relief between low- and high-altitude sites and between most high-altitude sites can exceed 1000 m. Ridges between high-altitude sites in the Cabinet Mountains are typically steep and characterized by rocky talus slopes, lack of water, and little vegetation. Although low-altitude sites begin to thaw in March and April of most years, the surface of high-altitude ponds remains frozen as late as July in the highest ponds (A. Giordano, unpublished data). Deep snow piles up in high-altitude cols and cirques, further isolating high-altitude sites from potential migrants for as much as 9 months of the year. While upstream gene flow from low-altitude sites to these cirques seems unlikely, our data suggest gene flow throughout the mountain range between high-altitude basins is even more unlikely.

Restricted gene flow between breeding populations generally contributes to genetic differentiation and can allow for local adaptation (Endler 1977; Conover & Schultz 1995; Huey *et al.* 2000; Palo *et al.* 2003; Skelly 2004; Weitere *et al.* 2004). Long-toed salamanders inhabit a wide geographical range and display different life histories across an altitudinal gradient throughout northwestern North America. As a result, genetic differentiation between high- and low-altitude sites could be further magnified by life-history differences between high- and low-altitude sites. Clutch sizes are larger with smaller eggs at low-altitude sites, with larger eggs and smaller clutch sizes at high-altitude sites (Kezer & Farner 1955; Howard & Wallace 1985). The difference in time to metamorphosis at high altitude (> 1200 m) and at low altitude is often as much as 12–16 months (Kezer & Farner 1955; Anderson 1967; Howard & Wallace 1985). Larvae from low-altitude sites generally metamorphose in 2–3 months likely due to habitat ephemerality, while larvae at high altitudes most often overwinter, probably due to much colder temperatures and limited food resources. These life-history differences could either be a result of divergent selection pressures or phenotypic plasticity. If they result of divergent selection pressures, they could result in local adaptation, and genetic differentiation between high- and low-altitude sites. Thus, by assessing genetic connectivity between populations at different altitudes, we address issues pertaining to how species persist and spread in diverse and dynamic environments and the potential for local adaptation.

Restricted gene flow among high-altitude populations relative to low-altitude populations may explain the lower genetic variability found among high-altitude sites relative to low-altitude sites. That is, high-altitude sites exhibit lower genetic variation due to lower gene flow among sites, post-Pleistocene colonization, or a combination of both. Further research with broader geographical sampling and additional genetic markers (e.g. mitochondrial DNA) would be necessary to test the latter hypothesis.

Conclusions

Our data suggest that long-toed salamanders likely conform to the predictions of Funk *et al.*'s (2005) mountain-valley population structure model. Lower levels of gene flow were found among high-altitude sites relative to low-altitude sites, which is most likely due to the harsher terrain, topography and climate among high-altitude sites. Several analyses showed higher gene flow among low-altitude sites than high-altitude sites and restricted gene flow between low-altitude sites and high-altitude sites. As a result of decreased gene flow, smaller effective population sizes are expected as altitude increases. Indeed, significant negative correlations were found between altitude and allelic richness and expected heterozygosity, suggesting effective population sizes are smaller in high-altitude populations than in low-altitude populations.

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This study was conducted as part of Andrew Giordano's master's research at Washington State University. He has analyzed population genetic patterns in various northwestern amphibian species and has performed research on the effects of altitude on life history dynamics of long-toed salamanders. He is currently a science and math teacher at Sugarbowl Ski Academy in Norden, California. Benjamin Ridenhour is a post-doctoral researcher at the University of Idaho. He is primarily interested in how multiple species interact and coevolve over broad geographic ranges. He is particularly interested in how microevolutionary forces, such as gene flow, shape antagonistic interspecific interactions. Andrew Storfer studies limits to species' ranges and uses population and landscape genetics as a tool to understand the factors that shape distributions of species. He is also interested in host-pathogen coevolution and conservation of amphibians.

Appendix I

Basic population information: site name, N = number of individuals, UTM (NAD 27, Zone 11), site elevation, expected heterozygosity (H_E), allelic richness and f , the coefficient of inbreeding

Site	N	UTM	Elev. (m)	H_E	Allelic richness	f
Wpt 35	10	589019E 5324643N	749	0.644	5.181	0.179
410 M	10	586540E 5338251N	731	0.701	5.230	0.230
BAM	27	591576E 5303550N	795	0.628	4.656	-0.033
BLH	28	584518E 5336395N	787	0.693	5.780	0.331*
BOHS	28	584673E 5337042N	733	0.635	4.944	0.277*
MM26	31	586104E 5355707N	792	0.701	5.426	0.236*
PRT	29	587572E 5325515N	1027	0.650	5.061	0.220
RCM	28	584698E 5340807N	732	0.669	5.287	0.201
RCP	31	584784E 5341151N	726	0.646	5.460	0.194*
TBP	30	585927E 5345966N	748	0.649	5.058	0.153*
CG	33	598000E 5325493N	1935	0.570	4.259	0.271*
SP	29	599617E 5327043N	1474	0.589	4.135	0.0774*
Cliff	26	598537E 5324975N	2042	0.498	3.565	0.138
RM 1	30	601384E 5322227N	1272	0.646	4.743	0.104
RM 2	27	601484E 5322098N	1310	0.600	4.339	0.124
RM 3	17	601310E 5321980N	1334	0.542	4.425	0.109*
RM 4	28	601172E 5321927N	1286	0.592	4.725	0.187
SL	29	593889E 5353944N	1978	0.623	4.401	0.118*
Bear	23	610671E 5314814N	1772	0.597	4.145	-0.006
MEL	26	600548E 5319460N	1707	0.558	3.752	0.251*
LEL	29	600731E 5319479N	1751	0.523	3.873	0.201*

Appendix II

Characterization of loci: locus name, # of observed alleles, annealing temperature, observed and expected heterozygosities, and Weir and Cockerham's F , Θ , and f , respectively. All loci but Aje D422 were amplified using touchdown PCR (annealing temperature start: 57 °C, end: 47 °C over 2 cycles) with the reaction conditions: 1.5 mM $MgCl_2$, 0.2 mM dNTP's 0.2 μ M forward and reverse primer, 0.025 U/ μ L *Taq* polymerase and 1 ng/ μ L DNA in a total volume of 25 μ L. Aje D422 annealed at 55 °C with 2.0 mM $MgCl_2$ concentration in the PCR

Locus	No. of observed alleles	Annealing temperature	H_O	H_S	F	Θ	f
AJED03	17	57 °C– 47 °C	0.81	0.823	0.108	0.092	0.018
AJED16	35	57 °C– 47 °C	0.498	0.857	0.463	0.079	0.417
AJED422	15	55 °C	0.644	0.744	0.245	0.129	0.133
AJED94	31	57 °C– 47 °C	0.67	0.873	0.289	0.082	0.226
AMAC40	4	57 °C– 47 °C	0.03	0.05	0.401	0.021	0.388
AMAD18	18	57 °C– 47 °C	0.634	0.664	0.151	0.122	0.032
AMAD95	11	57 °C– 47 °C	0.291	0.304	0.086	0.039	0.049
Overall					0.247	0.094	0.169