Landscape genetics of alpine Sierra Nevada salamanders reveal extreme population subdivision in space and time

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Abstract
Quantifying the influence of the landscape on the genetic structure of natural populations remains an important empirical challenge, particularly for poorly studied, ecologically cryptic species. We conducted an extensive microsatellite analysis to examine the population genetics of the southern long-toed salamander (Ambystoma macrodactylum sigillatum) in a naturally complex landscape. Using spatially explicit modelling, we investigated the influence of the Sierra Nevada topography on potential dispersal corridors between sampled populations. Our results indicate very high-genetic divergence among populations, high within-deme relatedness, and little evidence of recent migration or population admixture. We also discovered unexpectedly high between-year genetic differentiation ($F_{ST}$) for breeding sites, suggesting that breeding groups vary over localized space and time. While environmental factors associated with high-elevation montane habitats apparently play an important role in shaping population differentiation, additional, species-specific biological processes must also be operating to account for observed deviations from temporal, among-year panmixia. Our study emphasizes the population-level insights that can be gained from high-density sampling in space and time, and the highly substructured population biology that may characterize amphibians in extreme montane habitats.

Keywords: Ambystoma, landscape genetics, least cost analyses, microsatellites, population structure, temporal resampling

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Introduction
The geography of landscape features can exert a strong structuring force on the probability of individual dispersal and species distributions (Slatkin 1987; Clobert et al. 2001; Avise 2004), particularly in extreme habitats at the margins of species’ ranges. Population genetic structure can result from both landscape and species-specific biological traits, and separating the two can be extremely challenging. However, such inferences are an essential component of understanding how habitat patches are locally colonized by migrants, how contemporary spatial distributions arise, and how sensitive, declining species can best be managed on complex landscapes.

Unless there is complete panmixia, we generally expect a pattern of genetic isolation by geographic distance (IBD; Wright 1943). In addition, natural landscapes tend to be heterogeneous with respect to suitable habitat corridors and contain additional barriers to movement at small spatial scales. This generally causes patterns of sharp genetic discontinuities over short distances (e.g. Spear et al. 2005; Giordano et al. 2007; Pérez-Espona et al. 2008). Habitat-specific migration probabilities (Wang et al. 2009), alone or in combination with species-specific ecological requirements (Clark et al. 2008), frequently work together to produce
significant genetic differentiation, even at short geographic distances (Fahrig 1998; Flather & Bevers 2002; McGarigal & Cushman 2002; Greenwald et al. 2009b).

One strategy for exploring the importance of landscape structure on population genetic structure is to focus on environmentally extreme habitats, particularly at the margins of species’ ranges. Landscape effects in these habitats should be pronounced, given that the species is at its ecological and physiological limit. Here, we examined spatial patterns of genetic variation among populations of the southern long-toed salamander (*Ambystoma macrodactylum sigillatum*), a species that occurs in high elevation alpine habitats in southern Oregon and the Sierra Nevada in California, USA (Pilliod & Fronzuto 2005). As with many amphibians, this species has a biphasic life cycle with aquatic larval and terrestrial post-metamorphic stages (Stebbins 2003). Breeding adults move to breeding ponds as snow and ice thaw, and larvae may metamorphose in late summer of their first year, or overwinter for up to 3 years before metamorphosis (Pilliod & Fronzuto 2005). Although terrestrial adults can move many hundreds of meters from a breeding site, the complex mountainous topography and seasonality of their high-elevation habitat has generally been thought to limit long-distance dispersal (Pilliod & Fronzuto 2005).

We focused on the southernmost portion of the long-toed salamander’s range in the Lake Tahoe region of the Sierra Nevada in California. This region is topographically complex, environmentally extreme, and includes the altitudinal limit of the species’ range (Stebbins 2003). We predicted that the topography and short migration season would limit gene flow among local populations of southern long-toed salamanders (Sheppard 1977; Howard & Wallace 1981, 1985; Giordano et al. 2007), resulting in disrupted gene flow attributable to environmental barriers. We further predicted that variation among years for the same breeding sites would be negligible, because landscape features play no role in among-year genetic variance. We sampled breeding populations at both broad and restricted spatial scales, integrating estimates of recent migration rates with least cost path (LCP) and corridor analyses to identify specific landscape features or routes that define gene flow among demes.

**Materials and methods**

**Study landscape and population sampling**

Lake Tahoe is a basin lake encircled by mountains with elevations ranging from 1900 to 3050 m (Elliott-Fisk et al. 1996; Erman et al. 1996). Land cover in the basin is dominated by mixed coniferous forest, brush fields and frequent granite patches at higher elevations, with wet and dry meadows and riparian areas in valley floors (Erman et al. 1996). Southern long-toed salamanders breed in high mountain ponds and lakes (Anderson 1967), environments that are not uniformly distributed in the Lake Tahoe basin. Recent surveys by the US Forest Service found that *A. m. sigillatum* has a patchy distribution in the basin, which is likely due to topographic (they presumably avoid bare rock and heavy snowpack) and ecological features of this high elevation landscape.

We sampled southern long-toed salamander breeding habitats at broad- and fine-scales across three drainages (Fig. 1). The fine-scale distances between individual breeding sites were generally less than 1 km within drainage basins, a distance that other ambystomatid salamanders can traverse in a single migration event (Searcy & Shaffer 2008). Tail clips were collected from 1142 southern long-toed salamander larvae from 2000 to 2004. Our sampling included 47 discrete breeding ponds ranging in elevation from 1938 to 2728 m in the Lake Tahoe basin and a smaller neighbouring watershed in the Eldorado National Forest. Two additional sites (EBB and HLL) were located in more distant watersheds to the south of the study region (Fig. 1; see Appendix). Sites were separated by up to 44 km within the Lake Tahoe basin (sites 1–29), and up to 77 km across the entire study (site 1–HLL). Within the Lake Tahoe basin and Eldorado watershed, sampled ponds were a minimum of 81 and 54 m apart, respectively. In all cases, we sampled larvae haphazardly from throughout each breeding pond to ensure that our sample was a representative cross-section of the year’s breeding effort. We included all individuals in our genetic sample, given that close and distant relatives were presumably sampled at random.

**Temporal sampling**

Because *A. m. sigillatum* adults live for many years and may not breed every year (Petranka 1998), a single year sample probably does not include all of the breeding individuals utilizing that site. Given our interest in detecting patterns of population structure, we felt that it was important to establish the amount of among-year, within-pond genetic variance that could be confounded with among-pond, within-year variation. We therefore sampled five separate breeding ponds in the Lake Tahoe basin each for two consecutive breeding seasons in 2003 and 2004 (Appendix). This means that while we discuss 29 geographically discrete breeding sites, five of these were resampled and raise the total number of sampled breeding populations to 34 in Lake Tahoe.
Molecular methods

We isolated genomic DNA by proteinase K and cell lysis (Sambrook & Russell 2001), followed by purification with Gentra Puregene tissue kits (QIAGEN). DNA samples were suspended in 10 mM Tris–Cl (pH 8.0), diluted to a concentration of 10 ng/µL, and used as template in touchdown PCR to amplify 18 tetranucleotide microsatellite loci. Fourteen were developed specifically from *A. macrodactylum* (Savage 2009), one from *A. maculatum* (Julian et al. 2003), and three from *A. californiense* (Savage 2008). Amplification and fragment analysis protocols follow methods described by Savage (2008, 2009). Five percent of the samples were reamplified for each locus with a high fidelity polymerase (TaKaRa LA Polymerase, or Phusion Polymerase, Finnzymes) to verify consistency of scored alleles at each locus. We checked for genotyping errors using Microsatellite Toolkit version 3.1 (Park 2001), and estimated null alleles and large allele dropout in Micro-checker version 2.2.3 (Van Oosterhout et al. 2004).

Population genetics and summary statistics

Standard descriptive summary and population genetic statistics were calculated in GENEPOP version 3.4 (Raymond & Rousset 1995), FSTAT version 2.9.3.2 (Goudet 1995, 2001), and MSA version 4.0 (Dieringer & Schlotterer 2003). Multilocus estimates of $F_{ST}$ (Weir & Cockerham 1984) were calculated in FSTAT, and FREENA (Chapuis & Estoup 2007) to account for the potential effects of null alleles on estimates of population subdivision. For all summary statistics of population differentiation we evaluated confidence with 10 000 bootstrap replicates over all loci.

Population clusters

We used STRUCTURE version 2.2 (Pritchard et al. 2000; Falush et al. 2003) to estimate the number of genetic clusters represented by the assayed genotypes. We used a uniform prior for population of origin in STRUCTURE to estimate population assignment likelihoods using three different data partitions: (i) the combined dataset of 52 sampled populations (18 Eldorado and 34 Lake Tahoe basin sites only) and 1102 genotyped individuals; (ii) the Eldorado dataset containing 18 populations and 387 individuals; and (iii) the Lake Tahoe sample of 34 populations and 715 individual genotypes.

We performed 10 independent trial runs for values of $K$ from 3 to 18 for Eldorado, and 12–22 for Lake Tahoe. We ran each for $10^6$ repetitions following a burnin of 200 000 with an admixture model, alpha estimated from the data, and correlated allele frequencies. For initial runs we found incremental increases in the value of ln$[Pr(X \mid K)]$ with increasing $K$, a pattern that can lead to overestimating the true number of genetic clusters (Evanno et al. 2005). We therefore used the $\Delta K$ correction (Evanno et al. 2005) to choose the optimal number of clusters. Simulations suggest that $\Delta K$ yields a significant improvement in cases where population structures do not conform to the island model (Waples & Gaggiotti 2006), and where there is strong population genetic differentiation.
Recent gene flow

Assignment tests were used to estimate recent gene flow. We used the probability of the genetic assignment of an individual to a population other than that where it was actually collected as evidence of recent migration (Paetkau et al. 2004). We conducted the assignment tests in GENECLASS2 version 2.0.9 (Piry et al. 2004) using the Bayesian probability method (Rannala & Mountain 1997) and Monte-Carlo resampling for probability testing with 10,000 simulated individuals (a = 0.01). Because our genotype dataset included only larvae, admixture of larval genotypes is assumed to result primarily from recent parental migration.

We then used the program BIMR version 1.0 to simultaneously estimate recent gene flow (N_{gen} ≤ 2) and the environmental factors that influence it (Faubet & Gaggiotti 2008). BIMR implements a Bayesian MCMC method that relaxes Hardy–Weinberg (H–W) equilibrium assumptions by using population-specific inbreeding coefficients, assumes migration-drift equilibrium in the prior generation, and estimates the probability that genotypes originate from a different deme (here defined as a breeding pond) than the sampled one from the last generation (Faubet & Gaggiotti 2008). We estimated recent gene flow among populations in the Eldorado and Lake Tahoe basins, and used a cost-distance matrix (see below) to examine environmental factors that might lead to observed population structure. Five replicates were performed with the F-model (Falush et al. 2003) for each of the four datasets (Eldorado and Lake Tahoe basins, each with and without an environmental cost matrix) with a sample size of 50,000 iterations following a burnin of 50,000 and a thinning interval of 50.

Relatedness and effective sizes

If populations are highly inbred or yearly larval cohorts are the product of only a few mating pairs, this will lead to a signal of small effective population sizes and potentially high among-pond differentiation. Pairwise relatedness among larvae in each pond was performed using the r_{st} estimator (Queller & Goodnight 1989). We tested for a significant deviation from random mating by permuting this matrix 999 times to generate 95% confidence intervals for the expected relatedness under random mating within populations. We then used a linkage disequilibrium method implemented in the program LDNE version 1.31 (Waples & Do 2008) to estimate effective population sizes (N_e) under a sample size bias correction (Waples 2006). For each sampled population, the harmonic mean N_e and jackknife-adjusted 95% confidence intervals were calculated for allele frequencies above or equal to the critical value (P_{crit} = 0.05).

Individual relatedness across temporal samples

We resampled five separate breeding populations in 2 years to examine between-year reproductive patterns in the absence of physical barriers to gene flow. The primary goal was to ask whether individuals that utilize local breeding sites (ponds) are a random draw from the local mating pool from one season to the next. We approached this by calculating relatedness coefficients (r) and relationship categories for each breeding pond across years. For each sampled pond, we examined relatedness separately for each pond and year (within-pond, within-year analysis), as well as by pooling temporal cohorts (within-pond, between-year analysis). We assigned relatedness as follows: first-order (full sibs, FS), second-order (half sibs, HS), third-order (e.g. cousins), and unrelated (U; r < 0.05). We used MLRELATE (Kalinowski et al. 2006) to calculate maximum likelihood estimates of pairwise relatedness and relationships between individuals, with confidence intervals set at 0.95 for 5000 randomizations. If equally probable relationship categories were encountered, we categorized relationships as follows: (i) the parent–offspring (PO) relationship is not possible between larvae, and thus we considered these cases to be FS; (ii) if HS relationships had equal probability as either FS or PO, we assumed the relationship was that of HS; and (iii) if U had equal probability as HS and FS, we grouped these as third order (e.g. cousins) because a relationship between individuals could not be explicitly determined.

Landscape genetics and least cost analyses

LCP analyses are often used to quantify the ecological distance, or cost, between locations over the frictional landscape (Adriaensen et al. 2003). A few landscape genetic studies appear to have successfully applied the LCP approach to measuring the ecological distance (Spear et al. 2005; Broquet et al. 2006; Wang et al. 2009). A potential improvement on the LCP is the least cost corridor (LCC). The LCC incorporates the frictional area between locations, resulting in a two-dimensional spatial structure of the cost surface that measures the structure of landscape elements in a measured area, or corridor (Fremier & Savage, in prep.). Although using the same basic logic as a LCP, a two-dimensional corridor may more accurately reflect the biological realities for organisms on landscapes.

To create cost surfaces, we selected landscape variables that potentially influence salamander movement, including distance to the nearest pond, landcover, slope, and elevation (Howard Wallace 1981; Trenham 2001). Surfaces were derived from two datasets of mean sea-level elevation and regional land cover (CaSIL 2006;
FRAP 2008). Input datasets and analyses were completed with 30 × 30 m cell sizes. Distance to the nearest pond is the Euclidean distance from each cell on the landscape to the nearest pond or lake (excluding Lake Tahoe); this surface represents the network of ponds in the areas, and therefore stepping-stone movement between breeding sites. The landcover layer was reclassified into the following values on a scale from 1 to 10, where 10 was the most costly: (1) water, herbaceous, (2) agriculture, shrubland, (5) hardwood, (7) barren, (9) mixed conifer, and (10) urban. These landcover values were based on our best estimates of the natural history preferences of the species and resistance values proposed by Compton et al. (2007). For the elevation and slope surfaces, higher elevations and steeper slopes corresponded to greater costs.

For each cost surface the value at each cell represents a frictional value and the distance to cross the cell. We calculated the LCC and LCP between all pairwise combinations of breeding sites \( \left( \frac{n(n-1)}{2} \right) \) with 16 separate cost surfaces. Each of the 16 cost surfaces represented all possible combinations of four selected predictor variables without varying the between-variable multipliers. That is, for every run each variable has equal weight on the final cost estimate. In addition, all but the landcover surface (only one vegetation surface) was of continuous data type, which allowed us to avoid weighing within-surface friction values (e.g. one step in elevation is same cost over the entire surface). We defined the LCC as the lowest 2% of cells by cost (e.g. Fig. 2). This definition limits the cost analysis to only those cells between two breeding ponds, but allows for multiple potential paths between ponds. We evaluated the performance of the 2% threshold by running the model with values between 1 and 10%. At 1%, the model performed similarly to the LCP, and for values between 2 and 10%, results were all similar to the 2% threshold. IBD, LCP and LCC metrics were then evaluated against normalized genetic differentiation, \( F_{ST}/1 - F_{ST} \) (Rousset 1997) values, using the partial Mantel statistic (only \( P < 0.05 \) shown). While \( F_{ST} \) is by no means ideal for estimating movement patterns (notably critiqued by Whitlock & McCauley 1999), we could not reliably identify gene flow by other measures. All GIS analyses were completed in ArcGIS ver. 9.2 (ESRI, Inc.) using the Python ver. 2.5.2 scripting language. All statistics were calculated in R version 2.7.2 statistical software package.

Results

Population genetics and summary statistics

Our full dataset consisted of 18 microsatellite loci scored for 1142 individuals drawn from 54 breeding populations (including the five resampled populations) representing 34 populations in the Lake Tahoe basin, 18 in the Eldorado basin, and two more distant populations to the south (Fig. 1). No significant pairwise linkage disequilibrium was detected for the 18 loci. Potential null alleles were detected in locus AcroD330 due to homozygote excess for most of the allele size classes in four Lake Tahoe basin populations (1, 5, 11, and 21). Over the entire dataset the number of alleles per locus ranged from nine (AcroD231) to 25 (AcroD098, AcroD167, and AcroD190), with an average of 17.6; individual population means ranged from 2.2 (site 12) to
6.3 (site 28). Private alleles were detected in 31 of the 56 sampled populations, with frequencies ranging from 1.9 to 27.3%.

Populations generally conformed to H-W proportions for each locus. Although some populations (26 out of 54 tested) exhibited locus-specific deviations, no single population was out of H-W equilibrium for a majority of loci (Table S1, Supporting information). Population differentiation across all 54 populations was high ($F_{ST} = 0.27$, CI: 0.25–0.30, $P \leq 0.0001$; after null allele correction, $F_{ST} = 0.27$, CI: 0.25–0.29), indicating substantial population subdivision across the sampled region (Tables S2 and S3, Supporting information). All pairwise $F_{ST}$ estimates for which we had reasonable sample sizes ($N > 10$) were significant regardless of the distance among breeding sites. Within the Lake Tahoe basin, null allele corrected $F_{ST}$ levels ranged from 0.01 (sites 25 and 26) to 0.48 (sites 1 and 6), with an average of 0.26 (CI: 0.24–0.30) across 34 populations. Results for the Eldorado basin were virtually identical (mean $F_{ST} = 0.26$, CI: 0.23–0.29, range 0.01–0.33). In the five temporally resampled ponds, $F_{ST}$ estimates between sample years were all significant, ranged from 0.07 (site 16) to 0.17 (site 1), and were similar in magnitude to values among breeding sites within years (Table S3, Supporting information).

Population clusters

STRUCTURE clustered individuals from the Eldorado and Lake Tahoe watershed regions into two non-overlapping population clusters (Fig. 3). No admixture was inferred between Eldorado and Lake Tahoe indicating no inter-basin movement. Clustering for Lake Tahoe and Eldorado populations resulted in incremental increases in log-likelihoods with the number of populations up to the total number surveyed ($N = 34$, including the five resampled ponds for Lake Tahoe and $N = 18$ for Eldorado). Overall, very little evidence of admixture among breeding sites was found in either Lake Tahoe or Eldorado. The highest likelihood scores were 14 populations for Eldorado and 25 for Lake Tahoe, and using $\Delta K$, the best estimate was eight Eldorado and 15 Lake Tahoe clusters (Fig. 3).

Gene flow

Assignment tests computed in GENECLASS2 indicated that the majority of individuals (91.3%; 1043 of 1142) were genetically assigned to their respective sampling sites. All individuals from Eldorado or Lake Tahoe were assigned to their drainage of origin. Assignments of individuals to sites other than their collection locality generally involved assignment of the temporarily resampled sites to the alternate year (Tables S4 and S5, Supporting information). In Lake Tahoe there were only two individual salamanders with a greater than 80% probability of assignment to a different breeding site, whereas there were nine such individuals in Eldorado (Tables S4 and S5, Supporting information). Consistent with these assignment test results, recent migration rates estimated in BIMR version 1.0 resulted in no detectable recent gene flow among sampled ponds in either Eldorado or Lake Tahoe.
Relatedness and effective sizes

Across the entire dataset individuals were often related within, but not between, local breeding populations. Relatedness was moderately high in both the Lake Tahoe basin (mean = 0.37, range = 0.13–0.72), and Eldorado (mean = 0.28, range = 0.11–0.45), and relatedness was significantly higher than expected for all populations (Fig. 4a,b). Effective population sizes \((N_e)\) were often small, with nearly one-quarter of populations estimated to have effective sizes of 20 or fewer individuals, and approximately half having effective sizes of 50 or less. \(N_e\) estimates ranged from 2.3 (site 18) to 228.9 (site EBB) breeding individuals across all 54 populations (Appendix).

Relatedness across temporally sampled sites

Our analysis of within-year relatedness indicated that very few individuals within years, or when years were pooled, were half or \((FS; \text{Fig. 5})\). STRUCTURE analyses for each pond pooled across 2 years indicate that in each

![Graphs showing relatedness estimates for populations in Eldorado and Lake Tahoe.](image)

**Fig. 4** Mean pairwise relatedness estimates for populations in (a) Eldorado and (b) Lake Tahoe. Bars near the zero equilibrium value are the 95% upper and lower expected values estimated by permutation procedures, and bound the values expected if breeding at each site is panmictic. All populations differ from expected mean relatedness, which are likely due to inbreeding and/or family structure at each site (possibly also correlated with very small effective population sizes).

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case $K = 2$ breeding units exist, with high probabilities of individual assignment to their respective breeding year (Fig. 5).

**Landscape genetics and least cost analyses**

Mantel tests indicated that three of the 32 ecological distance models performed better than the IBD model (1–4% more variance explained) in the Eldorado dataset, whereas predictive features for the Lake Tahoe basin were a combination of landcover, elevation and pond distance.

Partial Mantel tests between genetic distance and ecological distance returned similar results for both for the Eldorado and Lake Tahoe datasets. The IBD model (straight-line distance) explained 56% and 52% of the variation in the Eldorado and Tahoe datasets respectively ($P < 0.001$). The remaining variation was explained by a combination of other variables, and in general models with fewer variables performed better (Fig. 6). Elevation was a good predictor for both datasets, followed by pond network distance and slope. The LCC performed better than the LCP in all cases, although they were highly correlated. In general, the two basins showed similar patterns of landscape effect, but the genetic differentiation was so high that no significant spatial autocorrelation was detected among nearest neighbor sampled ponds.

**Discussion**

The single most striking result from our analysis is the very high levels of genetic differentiation (average $F_{ST} = 0.27$) for *A. m. sigillatum* populations in the rugged, high elevation habitat of the Sierra Nevada. We found uniformly high within-population individual relatedness, small effective population sizes with no detectable recent migration, and little among-pond admixture based on individual assignment tests and Bayesian clustering analyses. All of these analyses suggest that gene flow is highly restricted in this species and landscape compared to other studies of congeneric salamanders in more moderate environments (e.g. Giordano et al. 2007; Zamudio & Wieczorek 2007; Greenwald et al. 2009a; Purrenhage et al. 2009; Wang et al. 2009).

**Environment and population structure**

High elevation is associated with severe topographical, climatic, and ecological conditions in the mountains of western North America. Suitable amphibian habitat in these regions is generally patchy, and salamander breeding demes may often be small compared to lower elevation populations (Funk et al. 1999; Giordano et al. 2007). The duration and intensity of cold temperatures, snow cover, and pond freezing/drying at high elevation may limit population sizes and among-pond dispersal in most years, leading to high levels of genetic dispersal, and seven out of 32 in the Tahoe basin (1–7%; Table S6, Supporting information). Slope and pond network distance (stepping stone distance) were better predictors of genetic distance in the Eldorado dataset, whereas predictive features for the Lake Tahoe basin were a combination of landcover, elevation and pond distance.

![Fig. 5 Relative relatedness measures per sample year (2003 & 2004) for the five resampled breeding ponds.](image-url)
subdivision (Funk & Dunlap 1999; Tallmon et al. 2000). This was the case in an allozyme study of A. m. krausei, where high genetic differentiation ($G_{ST} = 0.30$) among 34 populations in the Bitterroot Mountains (Montana, USA) was attributed to an elevation limit on dispersal imposed by mountain ridges (Tallmon et al. 2000). However, the harsh landscape does not appear to be the whole story, because other pond-breeding Ambystoma uniformly display higher levels of gene flow across both similarly harsh (Spear et al. 2005; Giordano et al. 2007) and more permeable landscapes (Zamudio & Wieczorek 2007; Purrenhage et al. 2009; Wang et al. 2009). Taken together, our data suggest that the extreme habitats associated with high elevation salamander populations may be driving the population substructure seen in A. m. sigillatum, but that population and species-specific attributes are also contributing to the extreme levels of substructure in the Lake Tahoe region.

What role does landscape play in structuring high elevation populations?

If the high levels of population genetic differentiation we observed for this high-elevation salamander taxon result exclusively from landscape and climatic factors associated with extreme environments, then we would also expect to find a strong landscape signal in our estimates of dispersal and migration. That is, some breeding ponds should be relatively more connected by favourable habitat corridors leading to high levels of between-site dispersal. We did find significant partial correlations between genetic and landscape measures, including a significant effect of elevation, slope and pond density on dispersal. However, geographic distance remained the strongest single indicator of genetic differentiation, and itself explained over half of the among-pond variance in both watersheds. We interpret this result to indicate that, in the environmentally extreme habitat of the high Sierra Nevada, dispersal is a rare event, which leads to extremely high population differentiation regardless of the intervening habitat. That is, dispersal risk may be so high that it renders local populations semi-isolated over ecological and evolutionary time scales.

Philopatry and its consequences in the Sierra Nevada

Amphibians are generally considered to be poor dispersers with highly philopatric tendencies (Vences & Wake 2007; Gamble et al. 2007; Calhoun & deMaynadier 2008; Semlitsch 2008) leading to deep phylogeographic breaks (reviewed in Vences & Wake 2007). Although philopatry (adults returning to their natal breeding sites with great precision) is a biological attribute of species, it can lead to deeply subdivided population genetic structure that mimics a pattern of isolation-by-distance. Interestingly, salamander species in the genus Ambystoma are often capable of dispersing great distances to/from breeding sites, particularly at lower elevations in California (Trenham et al. 2001; Trenham & Shaffer 2005; Searcy & Shaffer 2008). Teasing apart the relative contribution of limited dispersal driven by harsh terrestrial conditions and strong philopatry regardless of environmental barriers requires detailed population studies of multiple ponds over many years (e.g. Trenham et al. 2001). Unfortunately, such studies have not been conducted for any high-elevation amphibian system in western North America. Given the apparent role that environmental factors play in explaining genetic variance in this study, we predict...
that dispersal is not exclusively limited by intrinsic biology (i.e. philopatry), but that the proportion of successful dispersers is extremely low because of extrinsic factors such as landscape permeability and/or dramatic seasonal climate changes. However, this is an empirical question that requires long-term field data for complete resolution.

Population structure, turnover, and temporal sampling
Given the very high levels of population structure observed in this study, we expected that the small local breeding populations at each site would be relatively inbred and genetically consistent from year to year. Instead, we found very high year-to-year genetic differentiation for five ponds sampled in sequential breeding years. One possible explanation for this among-year genetic variation is high asymmetry in reproductive success among members of a deme. If only a few individuals successfully breed each year, such variance in mating success could explain the exaggerated temporal $F_{ST}$ differences, and may contribute strongly to low overall effective population sizes for local demes that accentuates geographic patterns of isolation-by-distance.

Consistent with this interpretation, our data suggest that relatively few, generally unrelated individuals bred in sequential sample years, possibly because of a strong negative correlation in individual mating success and/or year-to-year variation in larval survivorship. One or a few breeding pairs per year, and/or reproductive skew could inflate average relatedness within ponds relative to the local deme. The among-year differences ($F_{ST}$) in the resampled ponds are consistent with this or similar scenarios, and the combination of harsh landscape conditions, small census populations, and limited breeding in any given year may all contribute to the patterns of spatial and temporal genetic structure we observed.

The consequences for small population sizes and local demography
The fate of small, semi-isolated populations is of basic interest in population genetics as well as applied conservation problems, because there are critical threshold expectations for population viability and maintenance of adaptive genetic diversity (Tallmon et al. 2004; Hogg et al. 2006). Such small populations can be at the edge of viability in extreme environments due to the pronounced effects of drift, the inability to respond to selection for locally adaptive traits, and limited opportunity for genetic rescue from other populations. The data presented here suggest that even in relatively undisturbed habitats, high-elevation long-toed salamander populations are connected by rare migration events that allow a strong role for genetic drift and fine-scale local adaptation. Because of its population trends, vulnerability to projected climate change, and the inherently unstable demography seen in many pond-breeding amphibians, $A. m. sigillatum$ is currently being considered for inclusion as a Species of Special Concern by California (http://www.dfg.ca.gov/wildlife/nongame/ssc/amphibian-reptile.html), and our genetic data in the Lake Tahoe region support this decision. Our data also emphasize the important role drift and small population dynamics could play for other species in this group, (e.g. the endangered Santa Cruz long-toed salamander which exists in a few fragmented population clusters) and for sensitive, high-elevation amphibians (e.g. the Yosemite toad, Southern Mountain Yellow-legged frog, and Sierra Nevada Yellow-legged frog).

Conclusion
The identification of a straightforward relationship between the landscape and population genetic structure is complicated by several factors including habitat complexity, species-specific life histories, and demography. Our results indicate that populations in the Lake Tahoe region of the Sierra Nevada are isolated by geographic distance, as well as by elevation and the pond network structure. Even in a finely sampled breeding pond network such as we reported for the Eldorado dataset, the role of individual features and how they structure populations is challenging to evaluate. We propose two factors that together may be driving these results. First, the effect of the landscape is so strong that gene flow among sites is essentially zero, and population genetic patterns primarily result from mechanisms such as drift and local adaptation (Slatkin 1993; Koizumi et al. 2006; Dionne et al. 2008). Second, high temporal variation in reproductive success may further contribute to—or even obscure landscape effects on—average among-site genetic differentiation, resulting in high among-year genetic differentiation for individual ponds. Overall, our results highlight the importance of sampling populations both spatially and temporally to more fully characterize migration-drift dynamics, particularly in extreme landscapes.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Heterozygosity estimates at the eighteen microsatellite loci genotyped for each population of the Southern long-toed salamander (*Ambystoma macrodactyulum sigillatum*). For each breeding site, *N* is the number of individuals genotyped and from which the observed (HO) and expected (HE) heterozygosity were estimated. Heterozygosity values in bold indicate populations that do not conform to Hardy-Weinberg expectations for that particular locus (Bonferroni corrected *P*-value <0.000001 for table-wide significance level of *α* = 0.05). Grey cells indicate cases where populations with no information for heterozygosity estimates are cases of monomorphic loci.

Table S2 Pairwise FST estimates for populations sampled in the Eldorado subwatershed. Over all sampled populations in this localized area, FST = 0.259. Above the diagonal are FST values estimated after null allele correction and uncorrected FST distances below. Non-significant genetic differences are in bold (*P* = 0.000327, 3,960 permutations).

Table S3 Pairwise FST estimates for the Lake Tahoe populations (“global” FST = 0.269). Above the diagonal are FST values estimated after null allele correction and uncorrected FST distances below. Non-significant genetic differences are in bold (*P* = 0.000089, 11,220 permutations). Five breeding ponds (= populations) that were resampled in 2004 are noted with an asterisk; population numbers in parentheses refer to Figure 1 and the Appendix.

Appendix

<table>
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<th>Elevation</th>
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<th>Ne (% CI)</th>
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Table S4 Assignment of individuals to populations of origin and admixture proportions to other sites in the Eldorado populations dataset. The majority of assignments are to populations of sample origin, and even partial assignments are always less than 30% admixture proportion (numbers in parentheses).

Table S5 Assignment of individuals to populations of origin and admixture proportions to other breeding ponds sampled in the Lake Tahoe basin population dataset. The majority of assignments are to populations of sample origin, and even partial assignments are always less than 30% admixture proportion (numbers in parentheses indicate assignment proportions less than 80%).

Table S6 Mantel test results with linear geographic distance and corridor analyses. In the Eldorado populations, results show that three models using the least cost path (LCP) metric perform better than isolation by distance (IBD). Specifically, slope (S) and pond distance (D) were highly significant predictors of genetic distance among sampled breeding sites. For the Tahoe dataset, seven models performed better than IBD, with the least cost corridor (LCC) performing slightly better than the LCP. Elevation (E), vegetation (V) and pond distance are in many of the best explanatory landscape genetic models. For both datasets, IBD is an equally likely explanation for genetic distances, with little improvement from inclusion of landscape variables.

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## Appendix (Continued)

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