Elevated Genetic Structure in the Coastal Tailed Frog (Ascaphus truei) in Managed Redwood Forests

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Abstract

Landscape alterations have dramatic impacts on the distribution of genetic variation within and among populations and understanding these effects can guide contemporary and future conservation strategies. We initiated a landscape-scale genetic study of the coastal tailed frog (Ascaphus truei) on commercial timberlands within the southern range of the species in Mendocino County (CA, USA). In total, 294 individuals from 13 populations were analyzed at 9 microsatellite loci. None of the sampled populations departed from mutation–drift equilibrium, indicating recent population bottlenecks were not detected in contemporary samples. Fine-scale analysis indicated sampled populations were structured at the watershed level (mean $F_{ST} = 0.077$ and mean $G_{ST} = 0.425$). Landscape analyses suggested wet and moist areas may serve as significant corridors for gene flow within watersheds in this region ($r^2 = 0.32–0.54$ for moisture-related features). Results indicate populations of frogs may have persisted at this scale through intense periods of timber harvest, making southern range edge populations of coastal tailed frogs resilient to past land use practices.

Key words: conservation, landscape, microsatellites, timberlands

A central role of conservation biology is to understand how anthropogenic habitat alteration affects animal populations so that management solutions can be implemented in time to avoid local extinction (Fahrig and Merriam 1985; Cushman 2006; Lindenmayer and Fischer 2006). Habitat loss and fragmentation, associated with a range of land use practices, often produce a continuum of population responses dependent on the frequency and spatial scale of the disturbance events and their interaction with species’ life history, distributional patterns, and regional environmental factors (Fischer and Lindenmayer 2007). Although an extreme and direct result of habitat loss is extinction, less extreme forms of habitat alteration can influence populations over several generations in subtler ways not readily discernible by traditional demographic methods; these include smaller effective population size, decreased connectivity, lower genetic diversity, and inbreeding depression (Frankham et al. 2002). Conservation genetics research is being increasingly utilized to assess population metrics to improve the conservation and management of sensitive species associated with commercially valuable industries (Manel et al. 2003; Storfer et al. 2007) and simultaneously address fundamental issues of distributional changes and range limits of those species (Eckert et al. 2008).

The coast redwood (Sequoia sempervirens) forests of California occupy a limited geographic range along the Pacific Coast and are closely associated with maritime summer fog (Barbour et al. 2007). Most of these forests have experienced intense commercial logging for more than a century and consist of second- and third-generation trees. The remaining old-growth and late-seral stands, representing about 5% of the original forest, exist primarily in state and federal parks (Noss 2000). Although timber harvest volumes have declined in recent decades, the redwood region continues to be both a significant source of commercial wood products (Stewart 2007) and an area of high conservation interest (Noss 2000). Redwood forests harbor several sensitive terrestrial and aquatic animal species (Noss 2000), including 3 species of
co-occurring headwater stream amphibians (Petranka 1998; Adams and Pearl 2005). These forests and their associated amphibian populations not only face pressure from continued timber harvest and human encroachment but also from climate change that reduces the extent and duration of coastal fog (Johnstone and Dawson 2010).

The coastal tailed frog (Ascaphus truei) is a headwater stream amphibian distributed from southern British Columbia to northern California. In California, it is listed as a "species of special concern" and reaches its southern range terminus along a narrow band of mostly privately owned coast redwood forest in southern Mendocino County (Jennings and Hayes 1994). Tailed frogs have long lives, have a lengthy larval period, exhibit delayed reproductive maturity, and are highly philopatric to natal streams (Metter 1967; Brown 1975; Daugherty and Sheldon 1982a, 1982b; Wallace and Diller 1998; Burkholder and Diller 2007). They utilize cool, fast-flowing streams containing rocky substrates and low amounts of fine sediment for breeding, egg deposition, and larval development (Welsh and Ollivier 1998; Diller and Wallace 1999; Welsh et al. 2000). Both aquatic and terrestrial life stages have a narrow range of air- and water-temperature tolerance levels (Brattstrom 1963; Brown 1975). All these traits suggest a strong coevolutionary link with old-growth forest systems (Welsh 1990).

Concern over the conservation status of the coastal tailed frog has been raised because much of its range coincides with lands managed for commercial timber production (Adams and Pearl 2005; Kroll 2009), and several studies indicate that commercial logging can impact this and other headwater amphibian populations (Bury 1983; Corn and Bury 1989; Welsh 1990; Welsh and Lind 1991; Dupuis and Steventon 1999; Ashton et al. 2006). Timber harvest and associated activities are known to increase fine sediment deposition in streams, which can directly inhibit larval feeding, reduce periphyton growth, and fill interstitial spaces serving as cover (Corn and Bury 1989; Welsh and Ollivier 1998; Stoddard and Hayes 2005; Jackson et al. 2007). Removal of canopy cover and mature trees also reduces the insulative capacity of the forest to maintain cool and moist terrestrial microclimates, as well as suitable aquatic conditions for cold-water-adapted species (Chen et al. 1998; Welsh et al. 2005a). Such habitat alterations may not only depress the abundance of tailed frogs for a period of time (Ashton et al. 2006), but extreme forms of alterations (e.g., clear-cutting and forest conversion) may interact with topographic and regional climate to further isolate or extirpate frog populations across entire watersheds (Welsh et al. 2005b; Welsh and Hodgson 2008).

To date, only a few studies have examined population genetic characteristics of tailed frogs on landscapes experiencing timber harvest. These studies, conducted in British Columbia and Washington, found larval populations showed reduced genetic diversity and increased bottlenecks associated with clear-cuts (of ~10-year-old trees) compared with unlogged old-growth forests (Walhe et al. 2005), and significantly reduced gene flow in areas lacking forest cover (Spear and Storfer 2008). Spear and Storfer (2008) further noted that lag effects from timber harvest may lead to erroneous conclusions (i.e., timber harvest leads to higher gene flow) without additional analysis. Although these studies corroborate previous observational studies on the potential for timber harvest to negatively affect tailed frog populations, they may not be representative of tailed frog population genetic characteristics in other parts of the species’ range where timber harvest occurs, especially areas with different disturbance histories, timber harvest practices, climates, and forest types.

We initiated a population genetic study of coastal tailed frogs on privately owned commercial timberlands in the central part of the redwood zone. This study area has generated strong conservation interest because it has been intensively harvested for decades, lacks any significant old-growth forest, and is within the southern extent of the tailed frog’s geographic range. Based on these facts, we hypothesized that 1) extant tailed frog populations exhibit genetic patterns associated with small isolated populations (e.g. bottlenecks); 2) genetic connectivity among populations is primarily associated with vegetation characteristics affected by timber harvest (forest structure and/or canopy cover); and/or alternatively 3) if tailed frog populations are not genetically isolated, then migration corridors tied to other physiographic features may better explain genetic connectivity.

Materials and Methods

Study Area

Totally, 4 watersheds were sampled in the North Coast physiographic province of Mendocino County, California (Figure 1) based on known tailed frog locations from recent distribution surveys. These watersheds consisted of second- and third-generation coast redwood (S. sempervirens) forests, with Douglas-fir (Pseudotsuga menziesii) and tanoak (Notholithocarpus densiflorus) forming a significant component. Climate is maritime and marked by cool, mild summers and wet winters. Mean annual precipitation for study sites during 2003–2009 was 119.9 cm (standard deviation [SD] = 30.4, range = 90.8–139.7 cm), with more than 50% occurring from December to February. Mean weekly average temperature and mean weekly maximum temperature of streams during the summer stress period at the locations where samples were collected were 13.8 °C (SD = 0.47, range = 13.3–15.0 °C) and 14.5 °C (SD = 0.54, range = 13.5–15.9 °C), respectively, in the period 2007–2009. Elevation ranges from sea level to 600 m.

Forest structure patterns on the landscape have been heavily influenced by commercial timber harvests during the past 120 years. These timberlands have experienced at least 2 harvest entries and have been shaped by a regimen of clear-cutting and repeated burning that removed most of the large valuable trees (primary forest) and left a significant portion of conifer-dominated land to be overtaken by pioneering tanoak. Commercial harvests that occurred before the passage of the Z'Berg–Nejedly Forest Practice Act of 1973 provided scarce protection to watershed conditions important to fishes and other aquatic species. During this time, roads were built adjacent to watercourses and entire
watersheds were harvested with very little forest retention. Since 1998, however, the timber volume harvested has been dramatically reduced and the method of harvest has transitioned to greater reliance on uneven-aged techniques (i.e., group and single tree selection), with an increased focus on riparian stand management and restoration to improve in-stream habitat connectivity for aquatic species.

Sampling and DNA Extraction
Tailed frog larvae were sampled during the months of March–June in 2007, 2008, and 2009. Small tail clips (~2 mm²) were taken from each individual and placed immediately in 95% ethanol. DNA was extracted from a collection of 294 larval tail clips using a DNeasy extraction kit (Qiagen, Inc., Valencia, CA). A random sample of these extractions was then verified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) to ensure high-quality quantifiable DNA.

Polymerase chain reaction (PCR) was performed on extracted samples using microsatellites developed by Spear et al. (2008). Thirteen microsatellites were screened in these populations for variability within and among populations. Further, 3 multiplexes, consisting of either 4 or 5 microsatellites, were amplified using a Qiagen Multiplex Kit (Qiagen, Inc., Valencia, CA) following manufacturer’s specifications. Cycling conditions consisted of 95 °C for 15 min, followed by 27 cycles at 94 °C for 30 s, a locus-specific annealing temperature (see Spear et al. 2008) for 90 s, and finishing with an extension step at 72 °C for 60 s. An additional extension of 60° for 30 min was added to ensure reaction completion.

Before amplification, each microsatellite marker was individually labeled with a fluorescent dye found in the G5 set from ABI (Applied Biosystems, Inc., Foster City, CA). Amplified products were processed through an ABI 3130 × 1 Sequencer/Fragment Analyzer to allow for visualization of individual alleles. Allele size was determined with ABI GENEMAPPER version 3.7 software using the LIZ500 size standard to allow for uniform binning and range identification (Table 1). Final genotypic data were exported into CONVERT version 1.31 (Glaubitz 2004) for conversion to the most common genetic analysis programs.

Population Genetic Analysis
To account for possible family structure in our samples, we first used the method of Wang (2004) and Wang and Santure (2009) to identify full sibs within each of our sites with the
Table 1  Least-cost path variables used in landscape genetic analysis (see Materials and Methods), path criteria used for each variable, the resolution for each layer and the source of the data layers. Specific hypotheses that were tested with each variable are given.

<table>
<thead>
<tr>
<th>Least-Cost Path Variables</th>
<th>Least-Cost Path Criteria</th>
<th>Resolution</th>
<th>Source</th>
<th>Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Redwood vegetation (1986)</td>
<td>Cost ratio: 10:5:1 (other vegetation: small conifer: medium: large conifer)</td>
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<td>California Department of Forestry and Fire Protection</td>
<td>Paths selected have larger conifers</td>
</tr>
<tr>
<td>Canopy cover&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Maximize canopy</td>
<td>30 × 30 m</td>
<td>2001 National Land Cover Database</td>
<td>Paths selected have higher canopy cover</td>
</tr>
<tr>
<td>Heat load index (HLI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Minimize HLI on path</td>
<td>10 × 10 m</td>
<td>DEM</td>
<td>Paths selected have lower heat load</td>
</tr>
<tr>
<td>Composite topographic index (CTI)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Maximize CTI</td>
<td>10 × 10 m</td>
<td>DEM</td>
<td>Paths selected have higher wetness</td>
</tr>
<tr>
<td>Stream</td>
<td>Cost ratio: 10:1 (terrestrial: stream)</td>
<td>30 × 30 m</td>
<td>—</td>
<td>Path selected follow streams</td>
</tr>
</tbody>
</table>

<sup>a</sup>Homer et al. 2007.
<sup>b</sup>McCune and Keon 2002; McCune 2007.
<sup>c</sup>Yang et al. 2005; Sorensen et al. 2006.

program COLONY (Jones and Wang 2010). Full sibs were removed before any further analysis as they may distort allele frequencies and create inflated levels of Hardy–Weinberg and linkage disequilibrium. GENEPOPO07 (Rousset 2008) was used to estimate the observed and expected heterozygosity. The methods of Guo and Thompson (1992) and Raymond and Rousset (1995) were, respectively, used to determine within-population deviations from Hardy–Weinberg equilibrium (HWE) and to estimate linkage disequilibrium (LD) across loci. Due to HWE deviations, the remaining analyses were performed with only 9 loci (see Results). Within-site allelic richness was estimated using the approach of Kalinowski (2005).

Population Structure

We tested for deviations in mutation–drift equilibrium using heterozygosity excess (Luikart and Cornuet 1998) and the method of Garza and Williamson (2001). The Wilcoxon test (Piry et al. 1999<cb>) was used to determine whether recent population bottlenecks have led to increases in expected heterozygosity, relative to a population with a constant effective population size (Ne) at drift–migration equilibrium, across sampled sites. In total, 1000 simulations were performed (2-phase model, 10% variance in multistep mutations, and 90%, single-step mutations) to determine mutation–drift equilibrium expectations. Deviations from equilibrium conditions using M-ratios were assessed through 10 000 simulations assuming θ (=4Neμ) values of 0.2, 0.5, 1.0, 2.0, and 5.0. These values assume a mutation rate of 5 × 10<sup>-8</sup> and a pre-bottleneck Ne of 100, 250, 500, 1000, and 2500 for each value of θ. Additionally, an average size of non-single-step mutations of 3.5 and 22% of non-single-step mutations were assumed (see Peery et al. 2012, for review of parameters). Analyses were performed with the software packages BOTTLENECK version 1.2 (Piry et al. 1999<cb> and CRITICAL_M (Garza and Williamson 2001).

Pairwise measures of genetic differentiation were estimated as $F_{ST}$ (Weir and Cockerham 1984), $G_{ST}$ (Hedrick 2005), Jost’s $D$ (Jost 2008) and Dps (Bowcock et al. 1994). $G_{ST}$ is a correction of standard $F_{ST}$ measures (Weir and Cockerham 1984), which standardizes $F_{ST}$ by the maximum possible value between 2 populations. A data set to estimate the maximal $F_{ST}$ among population pairs was created in RECODE version 1.0 (Meirmans 2006). Values for the original and recoded data sets were run through FSTAT version 2.9.3 (Goudet 1995) to generate pairwise $F_{ST}$ values. Original values were divided by the recoded $F_{ST}$ values to obtain $G_{ST}$. Jost’s D was estimated using the online software SMODG version 1.2.5 (Crawford 2010) and Dps was estimated with MSA version 4.05 (Dieringer and Schlötterer 2003). Analysis of molecular variance (AMOVA) was used to assess the degree of population structure at different hierarchical levels using 2 different sets of groupings in our AMOVAs. The first included all populations and the grouped sites were based on watershed (Cottaneva, Navarro, Elk {Upper and Lower}, and Alder—see Figure 1), whereas the second analysis was similar except for the exclusion of the Cottaneva Creek sites. This latter analysis was done to examine patterns of isolation by distance at smaller spatial scales. Significance of the AMOVAs was determined with 10 000 permutations using the program ARLEQUIN version 3.5 (Excoffier and Lischer 2010). The method of Wilson and Rannala (2003) was used to determine whether directional gene flow is occurring among the sampled sites. BAYESASS+ version 1.3 was run for 6 × 10<sup>7</sup> iterations, by sampling every 2 × 10<sup>3</sup> iterations and discarding information from the first 1 × 10<sup>7</sup> iterations as burn in. Delta values for $F$, $P$, and $m$ were adjusted to ensure that the acceptance ratios fell within the recommended 40–60% range suggested by the BAYESASS+ users’ manual. The analysis was run 3 independent times to assure convergence.

Self-assignments were performed using the Rannala and Mountain (1997) method in GENECLASS version 2.0 (Piry et al. 2004). The program STRUCTURE version 2.2 (Pritchard et al. 2000; Hubisz et al. 2009) was used to characterize the genetic ancestry of sampled populations. We ran STRUCTURE with the following parameters: 50 000 burn-in steps, 150 000 recorded Markov chain Monte Carlo (MCMC) steps, and use of the admixture model with no previous information on...
population of origin. A total of 10 independent runs were performed varying \( K \) from 1 to 8. The approach of Evanno et al. (2005) was used to find the optimal \( K \) over all runs. Outputs were exported to Clump version 1.1.2 (Jakobsson and Rosenberg 2007) and combined using a greedy algorithm with 10,000 random permutations. Results were exported to Distuct version 1.1 (Rosenberg 2004) for viewing.

Landscape Analysis

We examined the relationship between several landscape variables and genetic connectivity by analyzing genetic data with geographic information system (GIS) data in a model selection framework. Following the development of a priori hypotheses and selection of landscape variables, we created least-cost paths (LCPs) in a GIS representing potential gene flow routes between sites (Spear and Storfer 2010). Totally, 6 LCPs were generated between 10 sites in 3 nearby watersheds (Lower Navarro, Elk, and Lower Alder), yielding 45 unique routes (3 sites in Cottaneva Creek watershed were excluded due to the >40-km separation distance from the other sites). LCPs were based on 5 variables hypothesized to directly or indirectly influence temperature and moisture regimes critical to tailed frog life-history patterns: composite topographic index (CTI; a measure of “wetness”), heat load index (HLI; a measure of incident solar radiation), presence or absence of streams, canopy cover, and forest structure (Table 1).

Because we were limited by a small number of sampling locations, we developed simple paths to evaluate the relative importance of these landscape variables on genetic connectivity. Thus, 5 LCPs were based on single variables, and a sixth LCP was derived by combining 2 variable rasters minimizing HLI and maximizing CTI. HLI and CTI were derived from the 1986 Coastal Redwood Vegetation Database 2001 (version 1.0; Homer et al. 2007) and was maximum over the LCP. Finally, the forest structure variable raster was derived from the 1986 Coastal Redwood Vegetation layer developed by Fox (1998). We chose this coarse-scale layer because it represented a lag time of 20 years before our sampling and might better explain contemporary genetic patterns (Spear and Storfer 2008). For this path, 13 unique forest structure classes spanning the study area were condensed into 3 distinct attribute categories in the Coastal Redwood Vegetation layer based on species, size class, and density and these were assigned a cost ratio of 10:5:1 (other vegetation: small conifer: medium-to-large conifer).

A cost raster was developed for each of the 5 variable rasters, where a cell was allocated a weighted value between 1 and 10 (1 equals the least cost or most favorable conditions, and 10 equals the greatest cost) to estimate the cost to traverse a given cell for that variable (Table 1). Canopy cover was the exception as it was given a weighted value between 1 and 100 (the inverse of canopy cover percentage). A cost-distance function calculates the LCP traveling through the most favorable cells for a given variable from a source cell (the location of a sample site) to all other sampled sites. This resulted in 2, often nonidentical, paths for each pairwise comparison, effectively doubling the number of paths for each LCP. Because gene flow directionality between sites was not determinable, we partitioned the LCP data set into 2 groups of 45 pairwise comparisons and analyzed each group separately. Independent variables such as topographic distance, average canopy cover, average forest structure, average CTI, and average HLI were calculated for each LCP between sites. LCPs were created using ARCGIS version 10 and the LCP’s topographic distance was calculated using Surface Tools extension (Jenness 2008) for ARCVIEW 3.2, version 1.6b.

Linear regression was used to examine relationships between several independent variables for a given LCP and each genetic distance measure (dependent variable). A second-order Akaike Information Criterion (AICc = \( n \times \log ((\text{RSS})/n) + 2K[n/(n - K - 1)] \)), correcting for small sample size, was calculated for each regression model and used to determine the most parsimonious model in the set representing our a priori hypotheses (Burnham and Anderson 2002). The AICc was calculated using the maximum likelihood estimate (MLE) approximated from the linear regression output (formula: MLE = \( \text{RSS}/n \)), where RSS = residual sum of squares and \( n \) = sample size; Burnham and Anderson 2002). The number of parameters (\( K \)) used in each model was determined by the number of estimated regression parameters, plus intercept and variance. LCP models were compared with traditional isolation-by-distance (IBD) models and a null model to determine whether specific landscape variables were more informative predictors of genetic connectivity than a resistance-free landscape. Landscape variables were retained in the model only if their estimated coefficient’s 95% confidence intervals were nonoverlapping with zero.

Regression diagnoses were performed on each linear regression model to ensure that assumptions of normally distributed residuals and constant variance were met before calculating the MLEs used in model selection. Similarly, because our data were distributed in geographic space, it was also necessary to ensure that linear regression was the appropriate statistical method to model landscape variables and genetic distance. Failing to account for spatial autocorrelation in statistical models may result in biased parameter estimates and spurious model fit and, thus, lead to poor inference (Legendre 1993). To assess whether spatial autocorrelation in the residuals was present in our data, we calculated Global Moran’s I for each candidate model and determined whether it was significantly different from the random result (\( P < 0.05 \)). We employed the inverse-distance weighting method and based our matrix on single (straight-line) midpoint values between site...
pairs (see Spear and Storfer 2008, 2010). Finally, after ruling out the presence of any spatial dependencies in our models, we employed the nonparametric bootstrap to assess model uncertainty, resampling the original data set with replacement ($N = 45$) and determining the top model in each run of 10 000 iterations. All statistics were performed with Stata/SE version 11.2 for Windows. Stata modules `spmatrix` and `ankest` were used to generate the spatial weights matrix and calculate Global Moran’s $I$, respectively (Jeanty 2010a, 2010b).

Results

Population Genetic Analysis

A total of 294 individual tailed frogs were genotyped at 12 loci; however, 3 loci were removed due to HWE deviations (see the following sections; Table 2). Moreover, 33 individuals were removed due to the identification of full-sib relationships. The HWE analysis produced 25 out of 156 significant tests, whereas the LD analysis produced 14 out of 858 significant tests (both taking into account Bonferroni corrections for multiple comparisons). A majority of significant departures from HWE were found at 3 loci (A31, A17, and A3–14 tests), a problem rectified by removing these loci from subsequent analyses. Moreover, 1 final locus (A1) did not consistently amplify across samples, leaving a total of 9 microsatellite loci. Allelic richness did not vary substantially across sample sites (Table 2), though the sites from the Cottaneva Creek drainage had slightly lower levels of allelic richness when compared with other collection sites. Only 1 site showed significant departure from mutation–drift equilibrium (CA1008) with the heterozygosity excess test. Further, 2 sites contained significantly lower $M$-ratio values (Table 2). These were dependent on which pre-bottleneck $t$ was assumed.

Population Structure

Levels of pairwise genetic differentiation were significant in all but 2 comparisons (Supplementary Material). Lower levels of pairwise differentiation were evident from within-watershed comparisons. Mean overall $F_{ST}$ and $G_{ST}$ were 0.077 and 0.425, respectively. The AMOVA indicated that, for both sets of groupings, substantial genetic structure existed at all hierarchical levels examined (Table 3). A lower amount of genetic variation is partitioned among groups when the Cottaneva Creek sites are removed (Table 3; Group 2).

Individual-based assignments successfully assigned 96.1% of individuals back to their original site of collection (Table 4). When misassignments did occur, individuals were always assigned to a site within the same watershed (Table 4). The Bayesian analysis of migration rates revealed that significant migration events only occurred at the within-drainage level, with single-source populations found in the Cottaneva Creek, Navarro River, and Elk River catchments (Table 4). The Evanno method suggested that $K = 2$ was the most appropriate level of clustering (not shown). However, inspection of the likelihood values for all values of $K$ indicated a plateau at $K = 4$ (Supplementary Material). We, therefore, also report results of the STRUCTURE analysis for $K = 3$ and $K = 4$ (Figure 2). There was shared ancestry between the lowermost site in the Navarro watershed (WL1003) and the Cottaneva Creek sites and the uppermost sites in the Navarro watershed (WL1008 and WL1009) and the sites from Elk and Alder Creeks across all reported $K$ values (Figure 2).

Landscape Analysis

Landscape modeling, using different measures of genetic distance, consistently found that LCP models based on HLI + CTI or stream path ranked higher than models based on IBD, canopy, forest structure, CTI, and HLI. These 2 models had substantial support and were indistinguishable from one another (ΔAIC$_C$ values: 0–2; Burnham and Anderson 2002) in analyses when $F_{ST}$ and $G_{ST}$ were used, whereas HLI + CTI was the top overall model when Jost’s $D$ and Dps were used (Table 5). If we include models with ΔAIC$_C$ values 2–3 in the candidate set, $F_{ST}$ had 7 highly supported models, $G_{ST}$ 2, Jost’s $D$ 4, and Dps 3 (Table 5). The LCP based on HLI + CTI ranked high across all genetic distances; Stream and IBD had 3 genetic distances; and IBD ranked high for 3 genetic distances. All remaining models had moderate levels of support (ΔAIC$_C$ values 3–7) and all LCP and IBD models were more informative than the null model (Table 5). Topographic distance was the only independent variable retained in the regression analysis because its 95% confidence interval did not include zero. All distance measures were positively correlated with genetic distance (data not shown), and 23–54% of the variation was explained by any single model for all 4 genetic distance measures (Table 5). Model rankings based on bootstrap selection frequencies ($π$) for 10 000 bootstrap samples mirrored AIC$_C$ rankings for the top models based on moisture variables. These frequencies represented a confidence set ranging from 80–93% for all measures of genetic distance (Table 5).

Discussion

Amphibians are often touted as strong indicators of environmental change and stress due to various physiological and ecological characteristics (Welsh and Ollivier 1998; Welsh and Hodgson 2008). Although long-distance dispersal events are rare for amphibian species (Funk et al. 2005b), a majority of studies often find geographically limited gene flow and dispersal and a strong role for topography in mitigating gene flow (Funk et al. 2005a; Wang 2009; Savage et al. 2010). Our study demonstrates that tailed frogs in the southernmost portion of their range exhibit significant fine-scale population structure at the watershed level and that gene flow is correlated with environmental features that relate to the biology of the species. We also found that despite a history of land use alterations in the region, a majority of the sites did not show signs of a recent population bottleneck. These results suggest that although habitat alteration in the study region has been pronounced, populations of coastal tailed frogs in the Mendocino coast region appear to have remained viable despite widespread disturbance.
Table 2  Site codes, sample sizes ($N_{full} = \text{total collected at each site;} N_{trim} = \text{total remaining after removal of highly related individuals [see Materials and Methods for details]), estimates of observed and expected heterozygosities ($H_O$ and $H_E$) for each of the sample sites. Mean allelic richness ($A_R$) and results from heterozygosity excess and $M$-Ratio tests are also presented.}

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Cottaneva Creek</th>
<th>Navarro River</th>
<th>Elk Creek</th>
<th>Alder Creek</th>
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<td>RC1005</td>
<td>WL1003</td>
<td>WL1005</td>
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<td>$H_E$</td>
<td>0.508</td>
<td>0.742</td>
<td>0.857</td>
<td>0.926</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.613</td>
<td>0.742</td>
<td>0.857</td>
<td>0.613</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.914</td>
<td>0.742</td>
<td>0.926</td>
<td>0.914</td>
</tr>
<tr>
<td>$H_O$</td>
<td>1.000</td>
<td>0.742</td>
<td>0.926</td>
<td>1.000</td>
</tr>
<tr>
<td>Mean $H_E$</td>
<td>0.755</td>
<td>0.882</td>
<td>0.808</td>
<td>0.742</td>
</tr>
<tr>
<td>Mean $H_O$</td>
<td>0.738</td>
<td>0.722</td>
<td>0.778</td>
<td>1.000</td>
</tr>
<tr>
<td>$A_R$</td>
<td>7.41</td>
<td>7.41</td>
<td>7.41</td>
<td>7.41</td>
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<td>ns</td>
<td>ns</td>
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<td>ns</td>
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<tr>
<td>$M$-Ratio</td>
<td>0.764</td>
<td>0.819</td>
<td>0.843</td>
<td>0.765</td>
</tr>
</tbody>
</table>

ns, not significant at $\alpha = 0.05$; *, significant with $\theta = 0.2$; **, significant with $\theta \leq 2.0$. 

P < 0.05
Population Genetic Analysis

Equitable amounts of allelic richness and heterozygosity were observed across sites, with slightly lower amounts in Cottaneva Creek. This equitable amount of variation, coupled with the lack of any consistent bottleneck signatures across sites, indicates that populations studied here have comparable demographic histories. The lack of any reduction in variation across sites is unusual given the history of timber harvest in the region. Spear and Storfer (2008) found a limited incidence of recent bottlenecks for coastal tailed frogs in forested regions from Washington State, whereas Spear and Storfer (2010) did not find any bottleneck signatures in Rocky Mountain tailed frog (Asaphus montanus) populations from harvested sites in north-central Idaho. In either case, they attributed the lack of bottleneck signatures, for both the Heterozygosity Excess test and the M-Ratio test, to a demographic rebound from historical reductions in effective population size and/or increased migration. Given that our study sites have been intensively harvested during the past century (Noss 2000; Stewart 2007) and that harvest volumes have declined and practices changed over the past decade (Douglas R, personal observation), it is conceivable our study populations have recovered from initial declines, thus obscuring any bottleneck signatures. Likewise, elevated within-watershed migration rates may also account for the lack of recent reductions in effective population size throughout our study sites (see following section and Busch et al. 2007). In all cases, it appears that most coastal and Rocky Mountain tailed frog populations are capable of genetically rebounding from large-scale environmental perturbations.

Population Structure

The major unit that governs genetic structure in this study appears to be the watershed. AMOVA results indicated a significant degree of genetic variation partitioned at the watershed level, which is also reflected in the STRUCTURE analysis. Local dynamics appear to be important in structuring populations of tailed frogs in the southern portion of their range. Assignment “tests” indicate a high degree of assignment back to the initial site of collection, with any misassignments occurring at the within-watershed scale. Likewise, the Bayesian analysis for recent gene flow only found significant instances within watersheds. The STRUCTURE analysis predominately grouped samples by watershed, with the exception of individuals from WL1008 and WL1009 (Navarro River), who displayed mixed ancestry with samples from the Elk/Alder Creek watershed. Interestingly, the Bayesian analysis of recent migration did not detect any migratory events between these sites. This discrepancy could be due to historical connections and contemporary isolation between the 2 watersheds. Moreover, a single intervening watershed does exist between the Navarro River and Elk Creek, Greenwood Creek, though we did not sample this region. Further sampling in this area would allow differentiation between the relative roles of gene flow versus historical structure. Regardless, this observation is restricted to a single case and does not discount the high amount of population structure observed in our study.

High site fidelity is a common pattern documented in tailed frogs in our study region (Burkholder and Diller 2007), which would account for the significant population structure observed at the watershed level. In coastal tailed frogs from the Olympic Peninsula, relatively high amounts of gene flow were found across 2 study regions (5–20 km; Spear and Storfer 2008). A similar result was also found for Rocky Mountain tailed frogs (Spear and Storfer 2010). In contrast, our southernmost sites (Navarro, Elk, and Alder) are within the southern extent of the geographic range of the coastal tailed frog; yet, we find a relatively higher degree of differentiation when compared with these other studies. This degree of site fidelity could account for resilience to past disturbances, which would have a much greater effect on species with higher dispersal capabilities, or could be a result of adaptation to habitat fragmentation or warmer regional climate. This also suggests larger-scale environmental changes (like those from regional and global climate change) would have a much greater effect on this species.

Landscape Analysis

Recent genetic analyses of tailed frog populations reveal that a combination of physiographic, vegetative, and climate variables may influence gene flow over different landscapes that exhibit a variety of natural and anthropogenic disturbances (Spear and Storfer 2008, 2010; Spear et al. 2012). Our results indicate that tailed frog gene flow on commercially managed coast redwood forests near their southern range terminus likely occurs along riparian corridors and other areas of

Table 3: Results of the AMOVA analysis for the 2 grouping scenarios. Scenario ‘1’ grouped all sampled sites by watershed and scenario ‘2’ grouped all sites by watershed after the removal of the Cottaneva Creek sites

<table>
<thead>
<tr>
<th>Group</th>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Variance Component</th>
<th>Percentage Variation</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Among groups</td>
<td>136.724</td>
<td>0.2990</td>
<td>8.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Among sites within groups</td>
<td>52.017</td>
<td>0.0688</td>
<td>1.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Within sites</td>
<td>1836.593</td>
<td>3.514</td>
<td>90.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2025.334</td>
<td>3.713</td>
<td>—</td>
<td>—</td>
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<tr>
<td>2</td>
<td>Among groups</td>
<td>32.210</td>
<td>0.0917</td>
<td>2.58</td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td>Among sites within groups</td>
<td>38.091</td>
<td>0.0748</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Within sites</td>
<td>1209.013</td>
<td>3.3866</td>
<td>95.31</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1279.314</td>
<td>3.5531</td>
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</table>
Table 4 Results of the individual-based assignments and Bayesian analysis for recent gene flow. Numbers on the diagonal indicate self-assignment back to the original source population. Mean estimates of gene flow, followed by 95% confidence intervals (in parentheses) are given only for cases where elevated gene flow was found and are presented after individual assignments. All values are greater than would be expected for 13 populations. Numbers in bold indicate misassignments.

<table>
<thead>
<tr>
<th>Source</th>
<th>RC1004</th>
<th>RC1005</th>
<th>RC1006</th>
<th>WL1003</th>
<th>WL1005</th>
<th>WL1007</th>
<th>WL1008</th>
<th>WL1009</th>
<th>CL1003</th>
<th>CE1001</th>
<th>CE10025</th>
<th>CE1009</th>
<th>CA1008</th>
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</thead>
<tbody>
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<td>4</td>
<td>0.272</td>
<td>(0.202, 0.323)</td>
<td>0.273</td>
<td>(0.198, 0.323)</td>
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<td>15</td>
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</tbody>
</table>
high moisture with low solarization (streams and wet areas on north-facing slopes). Although these results run counter to our initial hypothesis that forest structure and canopy cover would be more informative predictors of gene flow, we are unable to dismiss these alternative hypotheses as fully uninformative as there is a range of factors that could have influenced this outcome. Furthermore, we also found that univariate regression models relating topographic distance to genetic distance best explained variation over all the LCPs without any contribution from additional landscape covariates. This contrasts with previous studies that routinely identified several landscape variables as informative predictors of gene flow—a result that may be attributed to regional differences in physiography and forest management.

Previous studies of tailed frog genetics on managed landscapes appear to have greater topographic relief than our study area, in addition to larger blocks of distinct even-aged stands and nonforest areas due to clear-cutting (Spear and Storfer 2008, 2010; Spear et al. 2012). In contrast, our study area was located in a low-elevation, coastal redwood forest that had a past history of large-scale clear-cutting. Even though we used canopy cover and forest structure layers in our GIS analyses to represent past landscape conditions, the different management regimes may have had the effect of homogenizing the landscape with respect to these variables. This could make it difficult to distinguish their effect on gene flow relative to a landscape with a detectable history of intensive even-aged management, and this possibly explains why they consistently ranked lower than moisture-related and resistance-free surfaces in the model selection analyses (Anderson et al. 2010). Future studies should pursue the use of data that more accurately represent fine-scale landscape
features conducive for tailed frog migration corridors, unlike the scale and resolution of the forest structure and canopy cover GIS layers used here.

Overall, these results suggest that tailed frog migration corridors in this region occur along areas that provide cool and moist habitat, a finding consistent with this species’ known biology. Tailed frogs have low tolerance for elevated air and water temperatures (Brown 1975; Welsh 1990; Welsh et al. 2005a), especially in the larval stage (Altig and Brodie 1972), and are subject to desiccation at all stages of their life cycle. Although several studies have documented that post-metamorphic tailed frogs move through upland habitats in harvested areas (Matsuda and Richardson 2005), others suggest they exhibit low dispersal ability, remain close to the stream environment, and move through riparian corridors (Daugherty and Sheldon 1982b; Burkholder and Diller 2007). Spear and Storfer (2010) found that stream paths were associated with genetic connectivity for Rocky Mountain tailed frogs and suggested this pattern was a result of both regional climate and habitat disturbance. A similar pattern with stream path was also detected in coastal tailed frog populations in managed areas lacking significant forest cover near Mount St. Helens (Spear et al. 2012). Reduced annual precipitation and increased summertime temperatures associated with more southerly latitudes likely impose further physiological stress on tailed frog populations and may explain regional differences in their behavior, habitat use patterns, and ultimately, their southern range distribution. We propose that

Table 5 Model selection results relating genetic distance to topographic distance for least-cost path (LCP) and isolation by distance (IBD) models (N = 45). Bootstrap selection frequencies (πi) are based on the number of times a model was selected as the top model out of 10,000 bootstrap samples of the original data set. Bold lines indicate modes with ΔAICc < 3

<table>
<thead>
<tr>
<th>Genetic Distance</th>
<th>Model Rank</th>
<th>Model</th>
<th>No. of Parameters</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Akaike Weights, wi</th>
<th>Bootstrap Selection Frequency, πi</th>
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</thead>
<tbody>
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<td>–374.43</td>
<td>0.00</td>
<td>0.30</td>
<td>0.35</td>
<td>0.41</td>
</tr>
<tr>
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<td>0.84</td>
<td>0.20</td>
<td>0.32</td>
<td>0.34</td>
</tr>
<tr>
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<td>–372.99</td>
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<td>0.18</td>
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<tr>
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<td>–372.55</td>
<td>1.88</td>
<td>0.12</td>
<td>0.32</td>
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<tr>
<td></td>
<td>5</td>
<td>LCPTopographic b 3</td>
<td>–371.89</td>
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<td>0.30</td>
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<tr>
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<td>0.42</td>
<td>0.52</td>
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<tr>
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<td>0.01</td>
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<td>5.73</td>
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<td></td>
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<td>6.90</td>
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<td>0.46</td>
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<td>32.68</td>
<td>0.00</td>
<td>—</td>
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</tr>
</tbody>
</table>

*Euclidean distance path accounting for topography (slope).

Minimum straight-line distance between sites.

All-constants model.
tailed frogs in the extreme southern part of their range are more likely to be confined to stream corridors, seeps, springs, and other wet areas because refugia are limited in upland habitats and suitable conditions for overland movement vary seasonally. Watercourse buffers and wet area protections are therefore likely to be highly beneficial to the tailed frog as the maintenance of suitable water temperatures has been a key determinant in their ability to persist in a region with a long history of timber harvesting now currently dominated by young redwood forests.

Study Limitations

Landscape genetic studies have the potential to shape conservation policy and land management decisions, yet researchers have cautioned that modeling techniques can be sensitive to numerous factors that can lead to erroneous conclusions (Anderson et al. 2010; Cushman and Landguth 2010; Landguth et al. 2010; Spear et al. 2010). Our analysis may suffer from 1 or more of these issues. First, the number of tailed frog sites sampled in this study may be too small to accommodate the modeling of landscape variables in a way that distinguishes competing a priori hypotheses. The considerable unexplained variation in all of our models may be due to the small sample size but could also indicate that there were additional variables or higher resolution GIS features that should have been included in the analysis. Second, our analysis showed that $F_{ST}$ contained the least amount of power to distinguish among numerous landscape models when compared with other genetic distance measures. It has been argued that $F_{ST}$ may not adequately reflect genetic differentiation for highly variable markers like microsatellites (Hedrick 2005; Jost 2008; Meirmans and Hedrick 2011; Whitlock 2011). The genetic distances presented here have different underlying assumptions about genetic differentiation, and their inclusion in the model selection analyses was able to identify a single model (HLI + CTI) with strong support across distances. However, it is unclear if the inability to distinguish among numerous models using $F_{ST}$ is a shortcoming of this particular metric, our sampling design, or the scale and quality of the GIS layers used in the analysis. Therefore, we advocate using these results as working hypotheses requiring further refinement and evaluation. Future work in the region should focus on increasing sampling intensity and consider additional variables (GIS data and more complex cost surfaces) to better evaluate these landscape hypotheses.

Conclusions and Conservation Implications

Understanding population structure at range edges is of high interest to the conservation of the entire species as range-edge populations may contain highly divergent populations, distinct pools of genetic variation, and may suffer increased susceptibility to local extinction in the face of global climate change (Hampe and Petit 2005). Recent work has suggested that summer fog coverage may decline in the coast redwood region (Johnstone and Dawson 2010), which could impose a further physiological stress on southern range-edge amphibian populations that depend on the cool, maritime climate. In all, given the high amount of population structure and potential negative impacts from warming scenarios predicted by climate change models, it appears that southern edge tailed frog populations may be highly susceptible to localized extinction, even in the face of presumed resiliency to past habitat alteration and loss. Conservation efforts should consider the potential impacts of future climate change scenarios and how best to mitigate their negative effects.

Previous work on the relationship between amphibian population genetic parameters and timber harvest practices in western North America has ultimately been aimed at determining the detrimental effects of timber harvest. We were unable to assess the impacts of fragmentation on tailed frog differentiation because suitable reference sites (i.e., old-growth or late-seral forest) are absent from this region. However, we found no evidence for recent reductions in effective population size for any of our sites. Spear and Storfer (2008) found evidence for long-distance gene flow and movement over forested habitats for tailed frogs in coastal Washington (United States). Our results differ in that recent migration events were only found within watersheds, but we did find evidence for migration corridors along streams and moist areas. This contradiction may underscore the difference in regional climates and forest types between coastal northern California and Washington (United States).

Clearly this work, and that of others (Welsh et al.), suggests that conservation of headwater stream amphibians in the redwood region of northern California is a highly complex and important issue. We suggest this region should be of high conservation importance given the narrow physiological tolerances of amphibians, the general feature of high genetic structure, and a history of large-scale landscape alteration. Conservation and management strategies should not only aim to establish riparian buffers in harvested regions but also identify restoration projects that will hasten the development of suitable habitats in degraded areas conducive for tailed frog breeding and overland movement. Likewise, maintaining areas of upslope habitats, which provide linkages between headwater streams, would also be beneficial for improving connectivity within and between watersheds (Olsen and Burnett 2009). Furthermore, detailed estimates of direct dispersal, coupled with a more intensive landscape approach, will provide a more accurate assessment of movement corridor requirements for this species.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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