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Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected vs. managed forests

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Abstract

Habitat loss and fragmentation are the leading causes of species' declines and extinctions. A key component of studying population response to habitat alteration is to understand how fragmentation affects population connectivity in disturbed landscapes. We used landscape genetic analyses to determine how habitat fragmentation due to timber harvest affects genetic population connectivity of the coastal tailed frog (*Ascaphus truei*), a forest-dwelling, stream-breeding amphibian. We compared rates of gene flow across old-growth (Olympic National Park) and logged landscapes (Olympic National Forest) and used spatial autoregression to estimate the effect of landscape variables on genetic structure. We detected higher overall genetic connectivity across the managed forest, although this was likely a historical signature of continuous forest before timber harvest began. Gene flow also occurred terrestrially, as connectivity was high across unconnected river basins. Autoregressive models demonstrated that closed forest and low solar radiation were correlated with increased gene flow. In addition, there was evidence for a temporal lag in the correlation of decreased gene flow with harvest, suggesting that the full genetic impact may not appear for several generations. Furthermore, we detected genetic evidence of population bottlenecks across the Olympic National Forest, including at sites that were within old-growth forest but surrounded by harvested patches. Collectively, this research suggests that absence of forest (whether due to natural or anthropogenic changes) is a key restrictor of genetic connectivity and that intact forested patches in the surrounding environment are necessary for continued gene flow and population connectivity.

Keywords: *Ascaphus truei*, bottlenecks, fragmentation, landscape genetics, tailed frog, timber harvest

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Introduction

Anthropogenic land-use change is the greatest threat to the conservation of biodiversity (Sanderson *et al*. 2002). Habitat fragmentation and loss challenge the majority of the world's species at the population level and it is thus central to organismal biology to investigate their response (Ferrière *et al*. 2004; Ewers & Didham 2005). On one hand, frequent disturbance of environments may select for increased dispersal ability as individuals try to locate more suitable habitat (Holt & McPeek 1996; Parvinen 2004). Conversely, dispersal may lead to population declines if movement through fragmented habitat leads to high disperser mortality or decreased fitness (Gibbs 1998; Casagrandi & Gatto 1999; Fahrig 2001). Thus, studies that estimate dispersal rates alone may lack sufficient insight into the evolutionary potential of fragmented populations. However, dispersal studies that assess the biotic and abiotic factors that influence dispersal can provide valuable predictions regarding which types of habitat alteration will maintain or reduce population connectivity, thereby influencing population genetic structure. Gene flow often occurs through dispersal and successful breeding, and as a result, is highly correlated with dispersal (Bohonak 1999). Comparative landscape genetic studies across fragmented and continuous landscapes will yield important insights into the habitat variables most important for facilitating or inhibiting dispersal and consequent gene flow.

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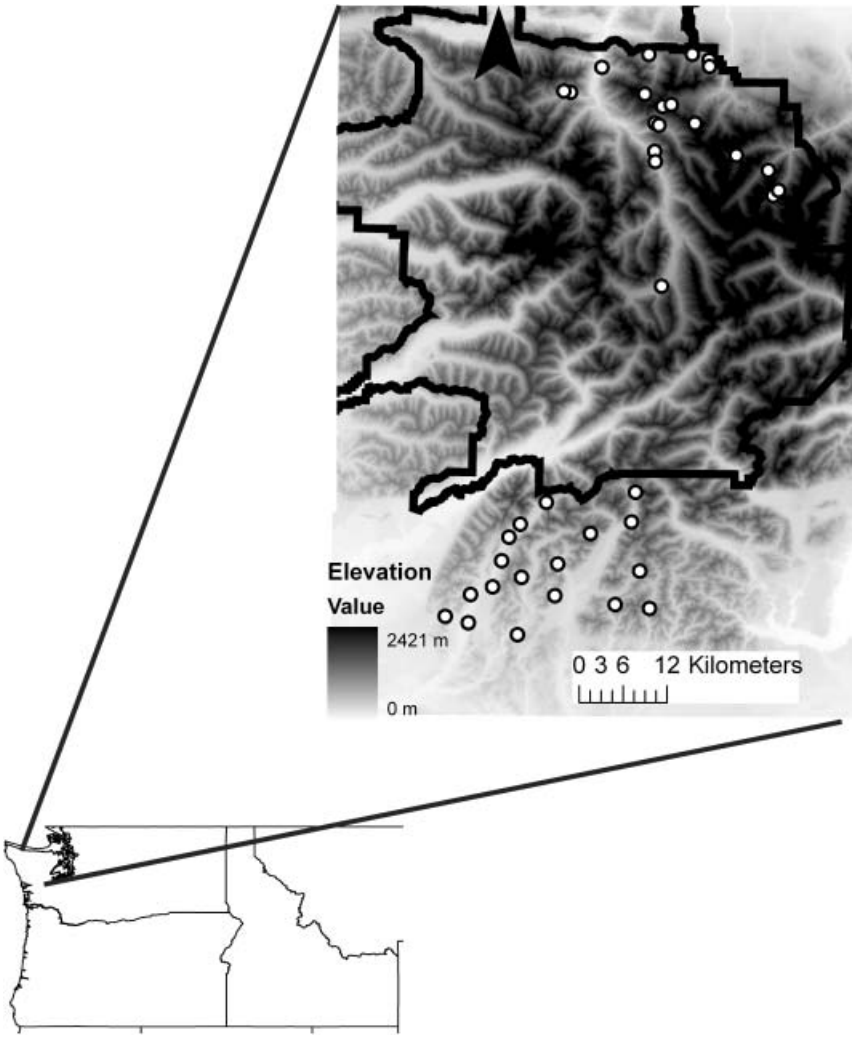

Fig. 1 Map of sampling sites across both study regions. Bold black line represents boundary of Olympic National Park. Inset indicates general location of study site on a map of the Pacific Northwest of the USA.

A major form of habitat alteration is timber harvest. Fragmentation effects of timber harvest can be complex, resulting in a landscape matrix composed of a mixture of forested and open areas at different stages of re-growth (Thiollay & Meyburg 1988). As a result, harvested landscapes may constrain evolutionary responses of forest species by reducing gene flow or genetic diversity (Singer & Thomas 1996). Connectivity among populations in a patchy, harvested environment is often necessary for population persistence due to the predominance of source-sink processes (Pulliam 1988). Recently harvested areas may represent population sinks, and therefore individuals may need to successfully immigrate to surrounding forested patches to survive or reproduce. The harvested forests on the Olympic Peninsula of Washington in the Pacific Northwest of the USA (see Fig. 1) are a well-studied example with regard to understanding the effects of fragmentation on the demographic dynamics of mammals (Lomolino & Perault 2001). Based on mark–recapture studies within a number of forest patches, species richness of mammals was positively correlated with both percentage of old-growth forest in the habitat matrix and distance to forested corridors among patches. Such studies suggest that landscape configuration and environmental variables are important for connectivity among patches, yet mark–recapture approaches cannot easily assess which specific factors are influencing population connectivity in fragmented landscapes.

A valuable approach to the problem of understanding the detailed effects of habitat fragmentation is to use landscape genetics, which integrates the fields of population genetics and landscape ecology to identify specific landscape variables that influence genetic structure (Manel *et al*. 2003; Storfer *et al*. 2007). Several landscape genetic studies have shown that least-cost paths based on resistance surfaces better describe gene flow than straight-line routes (Michels *et al*. 2001; Vignieri 2005; Cushman *et al*. 2006). Other studies demonstrate that genetic structure is different among different habitats or disturbance types within the same species (Hitchings & Beebee 1997; Jacquemyn

2004; Banks *et al*. 2005). For these reasons, a landscape genetics approach has great potential to address fundamental questions relating to connectivity of populations in fragmented habitat.

One species that serves as an excellent example for addressing effects of forest fragmentation is the coastal tailed frog, *Ascaphus truei*. This species is restricted to the forests of the Pacific Northwest coastal mountains and the Cascade Mountains of the USA. This limited geographic range is at least partially due to the physiological limitations of tailed frogs, as individuals require continual moisture, cool temperatures and low sedimentation in breeding streams (Claussen 1973; Brown 1975; Adams & Pearl 2005). Although no studies of fine-scale genetic connectivity of tailed frogs have yet been conducted, it has been assumed that dispersal and gene flow are low, due to low desiccation tolerance as well as mark–recapture studies that documented individual frogs only 100 m to 1 km from streams (Corn & Bury 1989; Wahbe *et al*. 2004). Due to these restrictions, harvest is expected to isolate frog populations because it is unlikely that frogs are able to disperse through habitat with reduced canopy cover and moisture.

In this study, we test three hypotheses on the effects of timber harvest on population connectivity of a forest-associated species: (i) genetic connectivity of tailed frog populations is positively correlated with the extent of forested habitat across the landscape; (ii) timber harvest should genetically isolate formerly connected populations, resulting in population declines and increased dispersal mortality; (iii) while forest cover is hypothesized as an important variable affecting dispersal, other landscape variables will also modify impacts of timber harvest. Based on previous studies of the biology of tailed frogs, we predict that areas with low solar radiation, low slope and high precipitation should be positively correlated with genetic connectivity. Collectively, testing these predictions will not only yield insight into the current ecology of tailed frogs, but will also allow for inference of population response to future anthropogenic disturbance.

Materials and methods

Study site and field sampling methods

We selected localities within the northeast corner of Olympic National Park (NP) on the Olympic Peninsula of Washington, USA (Fig. 1) as unharvested old-growth sites for this study. Previous surveys indicated that this region contained the highest known prevalence of tailed frogs across NP (Adams & Bury 2002). Harvested study sites are located in the nearby southern area of Olympic National Forest (F), which lies within the largest continuous block of national forest on the Olympic Peninsula (Fig. 1). We chose these sites because spatial data are freely available (as opposed to privately-owned forests) and because of the presence of some remaining old-growth stands for comparison to the unharvested area within NP. Despite higher elevation across NP, there are no large differences in average slope between the two areas. Within both the old-growth and harvested areas, we used a stratified random sampling design, with stratification by two classes of solar radiation [low (0–0.5) and high (0.51–1)] and river drainage. We used solar radiation because of the reported negative influence of high temperature and desiccation on tailed frogs (Claussen 1973; Brown 1975). Solar radiation was estimated based on aspect and using the following equation: $[1 - \cos(\{\pi/180\}\{\text{aspect} - 30\})]/2$ (Roberts & Cooper 1989). This creates a continuous variable from 0 to 1, with 1 representing highest solar radiation. Additionally, sampling streams in separate drainages was important for testing whether gene flow was restricted to occurring along stream corridors, as suggested by previous tailed frog studies showing close proximity of metamorphosed animals to streams. We obtained genetic material from 20–30 individuals per site nonlethally by collecting mouth swabs from adults (Goldberg *et al*. 2003) or tail clips from larvae. Samples were stored in 95% EtOH (tail clips) or a lysis buffer (mouth swabs).

DNA extraction and genotyping

We used QIAGEN DNeasy 96-well plate kits (QIAGEN Inc.) to extract DNA from tissue or mouth swabs. Using polymerase chain reaction (PCR), we amplified 13 polymorphic microsatellite DNA markers developed for *Ascaphus truei* (Spear *et al*. 2008) to obtain indices of genetic diversity and gene flow. Specific PCR conditions for each locus are described in Spear *et al*. (2008) and negative controls were included within each PCR run. Microsatellite products from each PCR were run on an ABI 3730 automated sequencer (Applied Biosystems, Inc.) at the Washington State University LBB1 core facility and genotyped using GeneMapper 3.7 software (Applied Biosystems, Inc.). Because larvae were primarily sampled, we identified potential family groups using the maximum-likelihood algorithm in the program Colony (Wang 2004). We used Colony results to ensure that number of individuals per family group were equal at each site, thus minimizing influence of any particular family group on genetic structure.

Genetic data analysis

We tested for significant deviations from Hardy-Weinberg equilibrium and the presence of linkage disequilibrium using GenePop version 3.4 (Raymond & Rousset 1995). Allelic diversity and expected heterozygosity were calculated using FSTAT 2.9.3 (Goudet 2001). To investigate the extent of gene flow, we estimated the level of genetic differentiation

among populations using G'_{ST} (Hedrick 2005). G'_{ST} is a standardized measure of genetic distance based on Weir & Cockerham's (1984) adjustment of F_{ST} that divides the estimated F_{ST} by its upper limit [the value if the two populations were maximally differentiated (i.e. shared no alleles)]. This correction is useful when there is high allelic diversity and the upper bound of F_{ST} is < 1, as is common with microsatellites. We used RecodeData version 0.1 (Meirmans 2006) to create an FSTAT file with maximally differentiated populations, and ran both the original and recoded file in FSTAT to calculate G'_{ST}. Other genetic distance measures (D_{ps}, Nei's D and chord distance) gave similar results as G'_{ST}, but we chose G'_{ST} because it generally had greater support in models we tested.

We estimated population clusters based on two methods. The first was the Bayesian algorithm in the program Structure (Pritchard *et al*. 2000). We used the admixture model with correlated allele frequencies and for each potential K (number of clusters), we conducted five runs consisting of 1 million simulations with a 100 000 burn-in period (which was sufficient for convergence). We evaluated the most likely number of populations using the posterior probability of each K using the average value of the ln Pr (X/K) generated by Structure, as suggested by the program authors.

We also used a Bayesian clustering algorithm that included spatial information in the form of hidden Markov random fields (François *et al*. 2006) using TESS version 1.1 (Chen *et al*. 2007). Hidden Markov random fields are used to model spatial dependence among individuals and therefore incorporate the a priori assumption that nearby individuals are more likely to have similar allele frequencies than more distant individuals. TESS was run for 50 000 simulations (10 000 burn-in) to estimate K, as well as assign individuals to clusters. We chose 50 000 simulations because convergence was always reached at this level after five independent runs. As suggested by the manual, we used the parameters of no F model and no admixture. We used a spatial interaction value (which determines the degree of spatial dependence) of 0.6 (as suggested by the authors), but trials at other interaction parameters (0.3 and 0.9) produced consistent results. For both clustering methods, we assigned each site to the cluster that the majority of individuals at that site were assigned to. Individuals were assigned to the cluster with the greatest proportion of membership, although we recognize that low membership probabilities may indicate weak structure or unsampled populations.

Three genetic tests for reductions in effective population size were implemented to determine whether timber harvest is leading to potential population declines. These included tests for heterozygosity excess relative to equilibrium expectations (Cornuet & Luikart 1996), shifted allele distributions (Luikart *et al*. 1998) and M-ratios relative to a threshold expected value (Garza & Williamson 2001). We used the program Bottleneck (Cornuet & Luikart 1996) to test for both heterozygosity excess and shifted allelic distributions. We assessed significant heterozygosity excess using a Wilcoxon sign-rank test, with correction for multiple comparisons using the false discovery rate method (FDR; Benjamini & Hochberg 1995). Finally, the M-ratio is the ratio of k/r, with k representing number of alleles and r represents the allelic size range. As rare alleles are lost, k is reduced faster than r, and therefore, a low M-ratio relative to a critical value indicates population declines. We used the critical value of 0.68 provided by Garza & Williamson (2001). All three tests were used because they may give insight into the timing of the population declines. For example, a study of tiger salamanders (*Ambystoma tigrinum*) found that the heterozygosity excess test was sensitive to only very recent disturbances, whereas shifted allele distributions and M-ratios should retain bottleneck signatures for a longer time (Spear *et al*. 2006).

Spatial analysis

To test the influence of landscape and habitat variables on genetic structure, we used spatial autoregression with several potential paths of connectivity. The first path was topographic straight-line distance between sites, which would be expected if the population structure is due solely to distance rather than to landscape characteristics. Second, we developed a least-cost path that maximized movement through intact, unharvested forest. Forest cover data for Olympic National Park was derived from a vegetation layer with 25×25 m resolution that was developed specifically for the park using both ground-truthed data and LandSat Thematic Mapper satellite imagery (Pacific Meridian Resources 1996). We used a polygon layer based on forest age class from a database maintained by the Olympic National Forest to identify harvested patches (Olympic National Forest 2001). This layer classified patches as one of six age classes (years 0–20, 21–40, 41–60, 61–80, 81–160 and 160+). We considered patches 160 years or older as unharvested forest, as these patches have no record of harvest by the US Forest Service. We converted the polygon coverage to a grid with 10×10 m resolution. As the forest data were categorical, they were assigned cost values. Because we had no empirical data to guide cost assignment, we tested three different potential cost ratios (2:1, 10:1 and 100:1), with all non-forest (for NP) or non-forest/harvested (for F) patches assigned the higher cost and unharvested patches given a cost of 1. Additionally, within the F study area, to test whether there was a temporal lag in genetic response to land change (as demonstrated by Holzhauer *et al*. 2006), we created least-cost paths minimizing movement only through harvest older than 20 years (i.e. harvest less than 20 years

was grouped with unharvested stands and given a cost of 1) and paths only avoiding harvested areas greater than 40 years old. We did not create least-cost paths based on only harvest greater than 60 years because the 61–160 age class made up a very small percentage of the total harvested area. As with the previous forest least-cost paths, we used the three different cost ratios to create the 20+ year and 40+ year least-cost paths.

The next pair of least-cost paths minimized solar radiation and slope, respectively. Both slope and aspect were derived from a USGS digital elevation model (DEM) with 10×10 m resolution. For computational efficiency, we reclassified both solar radiation and slope into five categories with cut-offs between classes based on natural breaks. These categories were then assigned a cost value of 1–5, with 5 indicating the highest slope or solar radiation. However, these five categories were a simplification of a continuous gradient, and therefore, we did not assign any alternative cost values (i.e. we assumed a linear relationship with cost to gene flow). Additionally, we created a least-cost path based on the multiplied effect of cover and solar radiation. As we were testing the hypothesis that solar radiation was primarily important in areas with reduced or disturbed cover (non-forest or harvest), we set the cost of all forested areas to 1, regardless of the solar radiation. Therefore, the combined cover/solar radiation path was primarily influenced by solar radiation in patches without undisturbed forest. Our final least-cost paths tested whether gene flow primarily occurred along riverine corridors. However, because Olympic rivers primarily flow into salt water, it is impossible to connect all sites by rivers without moving across land. Therefore, we created a cost surface maximizing movement along rivers by giving a cost to terrestrial movements. Once again, we used the same three different relative cost ratios as with the forest paths, with rivers always at a cost of 1 and land the higher cost. We used countywide stream layers available through the Washington Department of Natural Resources. All least-cost paths were created using the 'cost distance' function in ArcGIS 9.2 (Environmental Systems Research Institute). This function calculates a single line between two sites that has the lowest cumulative cost value.

For each path of gene flow, we calculated several independent variables along the route. These included total topographic distance, topographic distance through non-forest patches created either by natural processes (NP) or harvest (F), and the weighted averages of solar radiation, slope and precipitation along the path. We calculated these averages by first multiplying each individual value by the per cent of the overall route that passed through pixels with that value, and then adding individual calculations together to produce a weighted average. Precipitation data were taken from a data layer created by the PRISM Group (Oregon State University, http://www.prismclimate.org). Among the independent variables, there was no correlation of non-forest or harvest with any of the other variables. While there is some correlation ($r^2 = 0.05$–0.15) between solar radiation and slope and between slope and precipitation along some paths, this relationship is relatively weak, and therefore, no independent variable strongly predicts the other.

To analyse the influence of the independent landscape variables described above on gene flow (estimated using G'_{ST}) along each path, we used spatial autoregression, implemented in the program Geoda (Anselin 2004). Spatial autoregression is similar to ordinary least-squares (OLS) regression, except that autocorrelation among the dependent variable (common in gene flow measures, as each site is included in multiple paths) is explicitly incorporated into the regression equation as a spatially-lagged dependent variable (O'Loughlin & Anselin 1992). The spatially-lagged dependent variable measures how similar values of a variable are to nearby values. We expect that including a spatially-lagged variable accounts for the non-independence of pairwise genetic data because paths involving the same sites are likely to be close spatially. The spatial autocorrelation component is defined based on a spatial weighting matrix (Haining 2003), which is computed using a variable that is expected to lead to autocorrelation in the dependent measure (i.e. gene flow). We tested autocorrelation variables using several spatial weighting matrices based on drainage contiguity or distance between route midpoints. The midpoint for each route was the point halfway between the two sites involved in a comparison. Thus, we are testing the hypothesis that site pairs within the same drainage and path routes that are close to one another (and thus connect nearby sites) will have similar rates of gene flow. We created spatial weighting matrices for midpoint distance at six distance thresholds (1 km, 3 km, 5 km, 10 km 15 km and 20 km). Any variable was only included in a particular least-cost path model if it was statistically significant through a stepwise procedure in which all variables are initially included and then excluded based on significance. We then evaluated the best regression model among the different least-cost paths using three criteria suggested by the author of Geoda: r^2, log likelihood and Akaike information criterion (AIC).

Results

Population genetic structure

We obtained sufficient numbers of genetic samples from 20 sites across NP (mean = 26) and 18 sites across F (mean = 28). All loci and populations were in Hardy–Weinberg equilibrium with the exception of site EL2 at locus 14A and site S1 at locus 4A. Additionally, only two pairs of loci (out of 78 pairwise comparisons) were significantly out of linkage equilibrium; this is no greater than at random using an

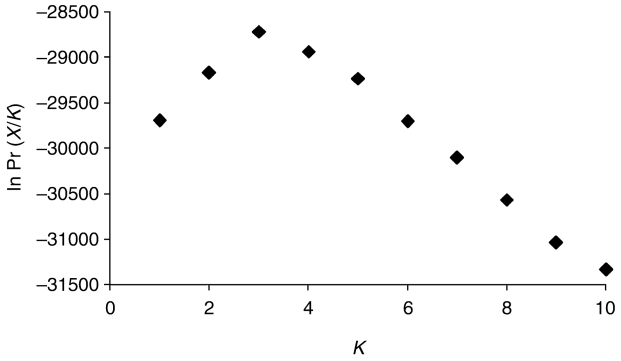

Fig. 2 Plot of ln Pr (X/K) vs. K (number of populations) for Structure analysis of sites across Olympic National Park. The greatest ln Pr (X/K) ($K = 3$) has a posterior probability of 1.

$\alpha = 0.05$. There were very few sibling pairs detected at each site, and therefore, on average only one or two individuals were excluded per site (i.e. there were at least 20 family groups for every site). Overall, both genetic diversity and gene flow were high among sampling sites in both regions. Number of alleles was high (21 alleles/locus in NP, 25 alleles/locus in F) and heterozygosity was also very high (0.855 in NP, 0.912 in F). Without standardization, there was little apparent population subdivision in either area with an F_{ST} of 0.03 across NP and a value of 0.004 across F. However, the high heterozygosity of these markers reduced the maximal F_{ST} to a value far less than 1 (0.09–0.17). Standardizing these values yielded a G'_{ST} of 0.16 (0.11–0.22, 95% CI) for NP and 0.04 (0.03–0.05, 95% CI) for F. Therefore, there is higher overall genetic differentiation across NP relative to F.

The NP sites clearly separated into three genetic clusters, based on both Structure (Fig. 2; posterior probability = 1) and TESS results. While these three clusters were spatially continuous and tended to group by drainage, sites EL4, EL5 and EN1 all group with the Morse Creek sites (Fig. 3A). This suggests dispersal is not limited to stream corridors. Examination of pairwise G'_{ST} estimates are consistent with the clustering results, but also give insight into the degree of differentiation within and among clusters (Table 1). The Gray Wolf sites had the greatest divergence from the other two clusters, with all comparisons exhibiting high differentiation. Second, the cluster consisting of the majority of the Elwha sites appears to contain substructure between the northern and southern sites not detected through Bayesian clustering. Finally, the cluster dominated by Morse Creek sites has the greatest genetic connectivity despite the fact that it includes three separate drainages and has sites separated by up to 12 km. Overall, the greatest distance between two sites that were genetically connected was 24 km (between sites EL6 and EL10).

In contrast, across F, the two clustering approaches gave different results, although both methods show low genetic differentiation (Fig. 3B). Structure has the greatest likelihood for a single cluster that included all individuals (Fig. 4; posterior probability = 1). TESS indicated two clusters, but these two clusters were highly admixed, with several sites evenly split into two clusters. Even sites assigned to one of the two clusters only had 60–70% of individuals assigned to the cluster. The border of the two clusters lies within the western half of the study area. Interestingly, the pairwise distance measures, while supporting low differentiation, were not entirely consistent with the clustering results. The cluster represented by the squares (Fig. 3B) did display pairwise differentiation from most of the sites in the Satsop and Wynoochee cluster (represented by stars). However, sites H9 and S1 each demonstrated moderate differentiation with nearly all comparisons, yet these sites were included with other sites in the clustering results. The pairwise distance matrices indicate that these two sites might belong in individual clusters (Table 2). The maximum distance at which pairwise comparisons showed low differentiation was 30 km, between sites H8 and W2.

There was no evidence of recent declines in population size across NP (Table 3) as evidenced by lack of heterozygosity excess, lack of shifted allele distributions, and the fact that no M-ratio values were below the critical value. On the other hand, there were seven of 18 sites across F that showed significant heterozygosity excess after correction for multiple comparisons (Table 4). However, all F allele distributions were normal, and all M-ratio values exceeded the critical value.

Spatial analysis

Across NP, there was one model that alone explained the greatest variation in gene flow (Table 5). The model with the most support ($r^2 = 0.65$, AIC wt = 0.98) was a least-cost route that minimized travel through areas of non-forest and high solar radiation. This best model included significant spatial autocorrelation at a spatial scale of 3 km, as well as the variables of total topographic distance, slope and solar radiation. Distance, slope and solar radiation all had a positive relationship with genetic distance, and therefore, were negatively correlated with gene flow. Although not a strongly supported model, the model with a 2:1 non-forest cost performed better than either the 1:10 or 1:100 ratio. Lastly, there was no evidence that gene flow primarily occurred along stream corridors based on the low support for these models, no matter the cost ratio.

There were three best-supported models across F (Table 6) and they explained less variation than the NP models. Additionally, there was no significant spatial autocorrelation based on drainage contiguity or spatial proximity. Therefore, all F regression models are based on OLS

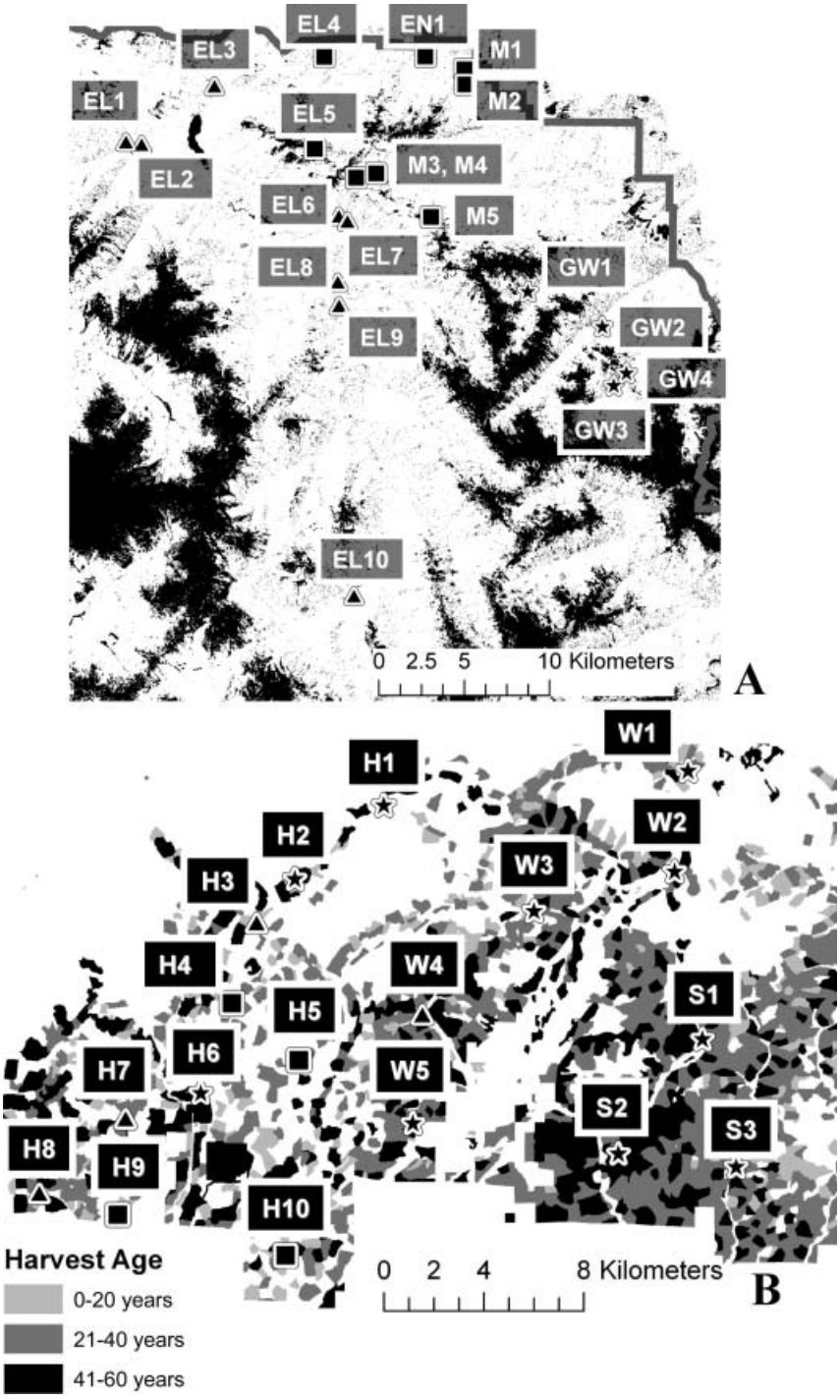

Fig. 3 Population clustering results from Structure and TESS output. Labels next to symbols are site names. (A) Sites within Olympic National Park; shapes represent different distinct clusters supported by both Structure and TESS. EL, Elwha River drainage; EN, Ennis Creek drainage; GW, Gray Wolf River drainage; M, Morse Creek drainage. Black patches represent non-forested areas. Thick grey border is park boundary. (B) Sites within the Olympic National Forest: squares and stars represent different clusters, triangles represent sites with equal membership in either cluster as identified by TESS. Structure grouped all F sites into one cluster. H, Humptulips River drainage; S, Satsop River drainage; W, Wynoochee River drainage. Background represents patches of harvest of the three age classes of 0–20, 21–40, and 41–60. White areas represent forest older than 60 years.

regression. The three models with the strongest support (total AIC wt = 0.92) were two least-cost paths that minimized movement through harvested areas of all age classes with cost ratios of 1:2 and 1:10, and a third path based on the combination of harvest of age class 20+ years and solar radiation, and all included total topographic distance, slope and solar radiation (except for the third path, which excluded the latter). Topographical distance and solar radiation had a positive relationship with genetic distance. However, per cent slope was negatively correlated with genetic distance across F, in contrast to NP. Overall, no single variable was present in every model, but a variable related to harvest was included in the least-cost path or as an independent variable in every tested model except one (Table 6). In general, different cost ratios produced models of similar support, with the exception of

Table 1 Pairwise G'_{ST} for sampling sites across Olympic National Park (NP). Site names are as in Fig. 3A. G'_{ST} values in bold indicate moderate differentiation (> 0.05) and values bold italics in represent high differentiation (> 0.15) (Wright 1978)

	EL1	EL2	EL3	EL4	EL5	EL6	EL7	EL8	EL9	EL10	EN1	GW1	GW2	GW3	GW4	M1	M2	M3	M4
EL2	0																		
EL3	**0.06**	0.01																	
EL4	0.04	0.03	**0.06**																
EL5	0.04	0.04	**0.09**	0.03															
EL6	**0.12**	**0.05**	**0.06**	**0.14**	**0.13**														
EL7	**0.11**	0.03	**0.08**	**0.14**	**0.12**	0													
EL8	**0.11**	0.03	0.04	**0.1**	**0.13**	0	0.02												
EL9	***0.18***	**0.12**	**0.12**	***0.17***	***0.21***	0.04	0.03	**0.06**											
EL10	***0.15***	**0.07**	**0.07**	***0.17***	***0.18***	0.03	0.02	–0.02	0.01										
EN1	**0.1**	**0.08**	**0.12**	0	**0.06**	***0.18***	***0.21***	**0.15**	***0.22***	***0.21***									
GW1	***0.3***	***0.28***	***0.27***	***0.25***	***0.28***	***0.33***	***0.34***	***0.31***	***0.36***	***0.38***	***0.29***								
GW2	***0.19***	**0.14**	***0.21***	***0.16***	***0.15***	***0.23***	***0.25***	***0.22***	***0.26***	***0.26***	***0.2***	0.05							
GW3	***0.31***	***0.26***	***0.3***	***0.23***	***0.23***	***0.34***	***0.35***	***0.33***	***0.33***	***0.34***	***0.25***	0.05	**0.05**						
GW4	***0.28***	***0.26***	***0.29***	***0.24***	***0.26***	***0.32***	***0.33***	***0.32***	***0.31***	***0.34***	***0.28***	**0.09**	**0.06**	0.02					
M1	**0.14**	**0.13**	***0.17***	0.03	**0.05**	***0.2***	***0.22***	***0.16***	***0.23***	***0.24***	–0.02	***0.21***	***0.14***	***0.17***	***0.22***				
M2	**0.07**	**0.09**	**0.11**	0.02	0.04	**0.15**	***0.18***	**0.13**	***0.23***	***0.22***	–0.01	***0.27***	***0.19***	***0.26***	***0.27***	0.03			
M3	**0.14**	**0.15**	**0.13**	0.03	**0.09**	***0.21***	***0.25***	***0.19***	***0.26***	***0.29***	0.03	***0.3***	***0.24***	***0.28***	***0.3***	0.01	0.05		
M4	**0.12**	**0.12**	***0.18***	0.03	**0.06**	***0.25***	***0.25***	***0.22***	***0.32***	***0.31***	0.05	***0.36***	***0.24***	***0.34***	***0.35***	0.02	0.03	0.01	
M5	**0.06**	**0.06**	**0.09**	–0.01	0.04	**0.13**	***0.19***	**0.13**	***0.21***	***0.2***	0	***0.25***	**0.14**	***0.24***	***0.28***	–0.01	–0.02	–0.03	–0.03

Table 2 Pairwise G'_{ST} for sampling sites across the Olympic National Forest (F). Site names are as in Fig. 3B. G'_{ST} values in bold indicate moderate differentiation (> 0.05) and values in bold italics represent high differentiation (> 0.15) (Wright 1978)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	S1	S2	S3	W1	W2	W3	W4
H2	–0.01																
H3	–0.01	0.01															
H4	0.04	–0.01	0.03														
H5	0.03	0.01	0	0.04													
H6	0.04	0.01	0.03	0.02	0.03												
H7	–0.02	–0.03	0.01	0.02	0	0.02											
H8	0	0.02	0.02	0.04	0	**0.08**	0										
H9	**0.09**	**0.08**	**0.1**	**0.1**	**0.11**	**0.1**	**0.1**	**0.12**									
H10	0.03	0.04	0.01	0.05	–0.01	0.03	0.01	–0.01	**0.11**								
S1	**0.06**	**0.07**	**0.11**	**0.11**	**0.1**	**0.1**	**0.06**	**0.11**	***0.24***	**0.12**							
S2	0.03	0.05	0.04	**0.09**	**0.06**	0.05	0.04	**0.07**	**0.12**	**0.06**	**0.06**						
S3	0.04	–0.03	**0.07**	**0.07**	**0.06**	**0.07**	0.02	**0.09**	***0.19***	0.05	0.02	0.02					
W1	0.02	0.04	0.03	**0.05**	0.05	0.03	0.02	**0.09**	**0.08**	**0.08**	**0.07**	0	0.01				
W2	–0.01	0.04	0.05	**0.1**	**0.07**	**0.1**	0.04	0.04	***0.18***	**0.1**	0.05	0.04	0.03	0.01			
W3	0.02	0.04	0.03	**0.05**	0.03	0.04	0.05	0.03	**0.14**	**0.05**	**0.07**	0.01	0.02	0	**0.05**		
W4	–0.01	0.01	–0.01	0.01	0.01	0.01	–0.01	–0.02	**0.1**	0.03	**0.1**	0	0.04	0.01	0.04	0.03	
W5	0.01	0.02	0.03	**0.08**	0.01	**0.06**	0.01	0.01	**0.13**	**0.06**	**0.08**	0.01	0.05	**0.05**	0.02	**0.05**	0.04

the 1:100 cost ratio for all harvest, which had the lowest support of any model. Additionally, there was some evidence for a temporal lag in the effect of timber harvest on gene flow. One of the best-supported models was based on the interaction of solar radiation with harvest older than 20 years. Additionally, six of the models tested included distance through older harvested areas (either 20+ or 40+ years) as the most significant independent variable.

Discussion

The results of this study revealed several insights of general importance to population genetic studies. These include the importance of incorporating landscape analyses in comparative genetic studies, the observed temporal lag in genetic response within harvested areas and the presence of population bottlenecks across the landscape. Additionally,

Site	$H_E - H_{EQ}$	*P* value	Allele distribution	M-ratio	M-ratio variance
EL1	–0.005	0.905	Normal	0.826	0.014
EL2	–0.007	0.5	Normal	0.819	0.015
EL3	–0.007	0.953	Normal	0.824	0.023
EL4	–0.006	0.393	Normal	0.78	0.022
EL5	–0.003	0.393	Normal	0.793	0.035
EL6	–0.006	0.632	Normal	0.755	0.024
EL7	–0.001	0.42	Normal	0.697	0.031
EL8	–0.008	0.812	Normal	0.755	0.031
EL9	–0.003	0.682	Normal	0.775	0.022
EL10	–0.002	0.473	Normal	0.762	0.015
EN1	–0.005	0.682	Normal	0.763	0.02
GW1	–0.007	0.863	Normal	0.712	0.045
GW2	–0.002	0.095	Normal	0.787	0.03
GW3	–0.007	0.98	Normal	0.796	0.034
GW4	–0.008	0.863	Normal	0.779	0.026
M1	–0.012	0.905	Normal	0.794	0.02
M2	–0.004	0.658	Normal	0.814	0.021
M3	–0.012	0.847	Normal	0.771	0.011
M4	–0.005	0.936	Normal	0.816	0.02
M5	0.001	0.393	Normal	0.713	0.028

Table 3 Results from tests of population size reductions across NP sites. Sites are as in Fig. 3A. $H_E - H_{EQ}$ represents the difference between actual expected heterozygosity and expected heterozygosity under the stepwise mutation model and *P*-value estimates the probability of no heterozygosity excess. Allele distribution is either normal or shifted

Site	$H_E - H_{EQ}$	*P* value	Allele distribution	M-ratio	M-ratio variance
H1	0.004	**0.011**	Normal	0.874	0.015
H2	0.008	0.047	Normal	0.862	0.017
H3	0.006	0.108	Normal	0.881	0.007
H4	0.01	**0.011**	Normal	0.806	0.025
H5	0.015	**0.002**	Normal	0.837	0.02
H6	0.006	0.029	Normal	0.745	0.012
H7	0.006	0.047	Normal	0.838	0.015
H8	0.006	0.055	Normal	0.796	0.018
H9	–0.004	0.916	Normal	0.766	0.02
H10	0.006	0.095	Normal	0.841	0.022
S1	–0.012	0.878	Normal	0.811	0.021
S2	–0.001	0.207	Normal	0.792	0.022
S3	0.009	**0.016**	Normal	0.736	0.024
W1	0.008	**0.02**	Normal	0.809	0.012
W2	–0.002	0.446	Normal	0.825	0.02
W3	0.003	0.04	Normal	0.788	0.017
W4	0.01	**0.001**	Normal	0.81	0.023
W5	0.012	**0**	Normal	0.768	0.024

Table 4 Results from tests of population size reductions across F sites. Sites are as in Fig. 3B. $H_E - H_{EQ}$ represents the difference between actual expected heterozygosity and expected heterozygosity under the stepwise mutation model and *P* value estimates the probability of no heterozygosity excess. Values in bold indicate statistical significance after FDR correction. Allele distribution is either normal or shifted

this study provided new information regarding the population structure of tailed frogs and its implications for conservation and management.

Importance of landscape analysis

We demonstrated that a comparison of gene flow among different regions may produce misleading conclusions unless multiple landscape variables are specifically tested. Typically, studies exploring land-use change compare only genetic diversity or gene flow between a continuous region and a fragmented region (examples include Hitchings & Beebee 1997; Millions & Swanson 2007; Noel *et al*. 2007). In our study, we found greater genetic subdivision across the unharvested region (NP) than in the harvested region (F); this could lead to a conclusion that harvest increased genetic connectivity for tailed frog populations, counter to our initial hypothesis. However, our landscape analysis

Model	Variables	r^2	Log-likelihood	AIC	AIC weight
Straight-line	3 km midpt Distance Solar radiation	0.61	577.065	–1144.1	0
Forest (2:1)	3 km midpoint Non-forest dist Solar radiation Slope	0.63	583.773	–1155.5	0.02
Forest (10:1)	3 km midpoint Non-forest dist	0.52	556.491	–1105	0
Forest (100:1)	3 km midpoint Non-forest dist	0.53	557.533	–1107.1	0
Forest/solar	**3 km midpt Distance Slope Solar radiation**	**0.65**	**587.758**	**–1163.5**	**0.98**
Solar	3 km midpoint Distance Slope Solar radiation	0.62	580.47	–1148.9	0
Slope	3 km midpoint Distance Slope	0.63	581.412	–1152.8	0
Stream (2:1)	3 km midpoint Non-forest dist	0.49	548.778	–1089.6	0
Stream (10:1)	3 km midpoint Distance Slope	0.5	550.146	–1090.3	0
Stream (100:1)	3 km midpoint Non-forest dist	0.51	550.187	–1092.4	0

Table 5 Spatial regression results for Olympic National Park models. Model refers to hypothesized route of gene flow and relative costs (see Methods). Variables are all significant parameters included in best model (3 km midpt represents the spatially lagged dependent variable). We used the three criteria of r^2, log-likelihood, and AIC. AIC weights are also included for each model to demonstrate the comparative level of support. All variables had a positive relationship with the dependent variable. Bolded indicates best supported models

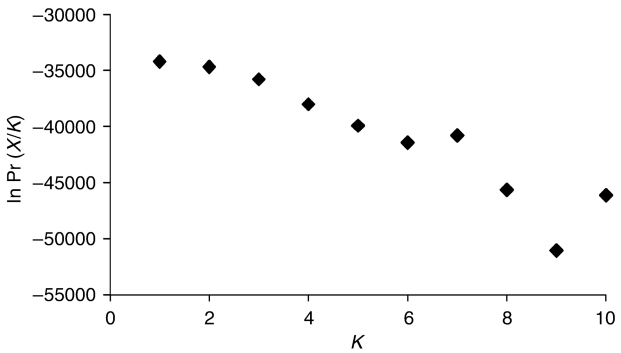

Fig. 4 Plot of ln Pr (X/K) vs. K (number of populations) for Structure analysis of sites across the Olympic National Forest. The greatest ln Pr (X/K) ($K = 1$) has a posterior probability of 1.

strongly suggested that subdivision across NP was primarily due to the presence of non-forest (primarily high-elevation meadows) and high solar radiation. These alpine areas are entirely absent from the F study region. In fact, the only natural non-forest found within our F study sites is a lake and a few riparian areas, which account for less than 2% of the area (based on GAP land cover data). Additionally, we detected a negative correlation between gene flow and harvested patches, suggesting that timber harvest leads to decreased gene flow. As a result, our landscape analysis indicates that the difference between the genetic differentiation across NP and F is primarily due to the higher elevations found in NP and not due to human management. The historic condition of continuous late-successional forest across F would allow for extensive movement and likely explains the lack of spatial autocorrelation. Furthermore, as harvest is relatively recent in this area, there may have not been sufficient time to structure populations as seen across NP, and this thus explains the weaker landscape correlation. Overall, this result supports our hypothesis that landscape features, particularly forest cover, have strong influence on tailed frog genetic population structure. However, despite the strong correlation of gene flow with forest, its absence does not seem to be an absolute barrier, as the higher cost of 100:1 always had weaker support compared to cost ratios of 2:1 or 10:1.

Furthermore, we discovered that one variable, slope, had an inconsistent relationship with genetic distance between the two regions; per cent slope is positively correlated with genetic distance within NP, but is negatively

Model	Variables	r^2	Log-likelihood	AIC	AIC weight
Straight	41–160 distance	0.18	636.41	–1266.8	0
Harvest (1:2)	**Distance**	**0.27**	**645.062**	**–1280.1**	**0.37**
	Slope (–)				
	Solar radiation				
Harvest (1:10)	**Distance**	**0.27**	**644.497**	**–1279**	**0.21**
	Slope (–)				
	Solar radiation				
Harvest (1:100)	Distance	0.14	632.315	–1254.6	0
	Slope (–)				
	Solar radiation				
Harvest 20+ (1:2)	Distance	0.22	639.741	–1271.5	0
	Slope (–)				
Harvest 20+ (1:10)	Distance	0.24	641.137	–1274.3	0.02
	Slope (–)				
Harvest 20+ (1:100)	Distance	0.24	641.406	–1272.8	0.01
	Solar radiation				
	Slope (–)				
Harvest 40+ (1:2)	Distance	0.23	640.467	–1270.9	0
	Slope (–)				
	Solar radiation				
Harvest 40+ (1:10)	Distance	0.23	640.747	–1271.5	0
	Solar radiation				
	Slope (–)				
Harvest 40+ (1:100)	Distance	0.21	638.054	–1268.1	0
	Slope (–)				
Harvest/solar	Distance	0.22	639.823	–1271.6	0.01
	Slope (–)				
Harvest 20+/ solar	**Distance**	**0.26**	**643.992**	**–1280**	**0.34**
	Slope (–)				
Harvest 40+/solar	41–60 length	0.21	638.732	–1269.5	0
	Slope (–)				
Solar	Distance	0.24	642.194	–1274.4	0.02
	Solar radiation(–)				
	Slope (–)				
Slope	41–60 distance	0.21	639.209	–1272	0
	Slope (–)				
Stream (1:2)	21–40 distance	0.23	640.818	–1271.6	0.01
	Slope (–)				
	Solar radiation				
Stream (1:10)	21–160 distance	0.22	639.316	–1268.6	0
	Precip				
	Slope (–)				
Stream (1:100)	41–160 distance	0.21	638.532	–1269.1	0
	Slope (–)				

Table 6 Spatial regression results for Olympic National Forest models. Model refers to hypothesized route of gene flow and relative costs (see Materials and methods). Variables are all significant parameters included in best model. We used the three criteria of r^2, log-likelihood, and AIC. AIC weights are also included for each model to demonstrate the comparative level of support. All variables had a positive relationship with the dependent variable unless indicated by a (–) symbol. Bold indicates best-supported models

correlated across F. This discrepancy may be explained by the differences in the spatial distribution of slope values between the two study areas. Several studies have documented a positive association of stream gradient with larval abundance (Corn & Bury 1989; Diller & Wallace 1999; Adams & Bury 2002). Therefore, tailed frogs likely emigrate from (and immigrate to) areas of higher slope to breed. While both NP and F have regions of higher slope, there is higher positive spatial autocorrelation (i.e. clustering) of slope across F (Moran's I = 0.14; z = 201) than NP (Moran's I = 0.02; z = 30). This suggests that an F frog is more likely to encounter higher slope habitat after leaving breeding areas, and thus may have no choice but to cross high-slope habitat to locate breeding areas across F. In contrast, the negative relationship between slope and gene flow in NP may be due to greater proximity between high- and low-slope areas. If this explanation is accurate, it implies that tailed frogs prefer to move through gentler slopes if available, but that the frogs are capable of successfully moving through steeper slopes.

Temporal lag in genetic response

Our data suggest that there is a temporal lag in full genetic response to timber harvest. Although only one of the three best-supported models across F included a lag (20 years), this was the model that included an interaction with solar radiation, which was the best-supported model across NP. Furthermore, whenever distance through harvest was included as an independent variable, it was always represented by 20+ or 40+ year age class. Therefore, we suggest that while recent harvest does have some initial effect on gene flow, it is not representative of the full response to harvest and its interaction with other variables such as solar radiation. Although other genetic studies have addressed temporal effects by testing variables separately (Keyghobadi *et al*. 2005; Holzhauer *et al*. 2006), our results are valuable in that they suggest that the interaction among landscape variables may not be immediately detected in the genetic response, even if the individual variables alone are.

The time lag observed suggests that timber harvest does not necessarily lead to immediate differentiation, but rather requires multiple generations to begin to change genetic population structure. Therefore, abundance studies may not accurately reflect the dynamics of the system. A study by Findlay & Bourdages (2000) illustrates this point. Species richness of several taxa (reptiles, amphibians, birds and vascular plants) was more strongly associated with historic road density than current road density. Ultimately, a lag may provide support for the theoretical idea of an 'extinction debt' (Tilman *et al*. 1994), in which populations do not go extinct until years after the disturbance that led to the decline.

Therefore, long-term genetic monitoring (e.g. Schwartz *et al*. 2007) should be used to understand the viability of populations in harvested landscapes. While the full response of genetic structure to landscape change may take several generations to detect, a genetic monitoring programme would be especially useful in determining whether connectivity is re-established following forest recovery. For example, in the future, if there is no longer any significant correlation with the older age classes, then this would strongly suggest renewed connectivity across regenerated forest.

Evolutionary response to forest fragmentation

We have demonstrated that fragmentation due to loss of cover (either natural or anthropogenic) limits gene flow (and presumably dispersal) in a forest-associated species. However, it is unclear from the above result whether reduced gene flow is due to reduced movement or disperser mortality. Our detection of significant heterozygosity excess at 7 of the F sites suggests recent population bottlenecks across the region. Although we did not detect bottlenecks with either of the other two tests (M-ratios and allele frequency shifts), we believe that bottlenecks have occurred across F for two reasons. First, there were no indications of heterozygosity excess across NP. If the significant heterozygosity excess across F were due to some other factors, then we would expect significant excess at NP as well. Second, both the allele frequency distribution and M-ratio tests are strongly influenced by the number of alleles per locus. Our loci were highly variable, and therefore, a loss of a few rare alleles may not have greatly changed the frequency distribution or the M-ratio. The observed bottlenecks cannot be solely attributed to degradation of breeding habitat, as four of the sites with declines are located in intact old-growth forest. Instead, individuals dispersing from streams in closed forest into the surrounding secondary growth or clear-cut forest may be subject to higher mortality and/or there is an overall reduction in emigration from breeding sites across the entire area.

These results are consistent with a recent empirical study on a herbivorous insect that demonstrated the surrounding habitat matrix was a better indicator of individual emigration than internal patch quality (Haynes *et al*. 2007). Sites with successful reproduction (birth rate > death rate) were identified, but they had high emigration with limited immigration due to the inhospitable surrounding matrix. These types of patches have been called 'sieves' (Thomas & Kunin 1999) and represent areas where stable populations are unlikely to exist as long as emigration is high. If timber harvest has indeed led to sites becoming sieves, then there should be selective pressure for lower dispersal rates from these patches. Accordingly, Baguette & Van Dyck (2007) suggest that reduced movement across fragmentation boundaries is an expected evolved response.

Further support for the hypothesis that organisms will evolve a tendency to avoid moving through inhospitable habitat comes from the results across NP. This area has patches of natural forest fragmentation due to the presence of alpine meadows. However, while the lack of forest reduced gene flow, there was no evidence of population size declines at any NP sites. Additionally, the significant spatial autocorrelation due to midpoint distance among gene flow paths across NP suggests that individuals are using similar routes across the landscape. This indicates either individuals are genetically predisposed to move in certain directions through continuous forest, or that behaviour has been altered to avoid open areas.

Tailed frog population structure and conservation

Our results suggest that tailed frog gene flow is common and extensive through overland forested habitat, contrary to previous expectations of strong stream association with metamorphosed individuals. Surprisingly, population connectivity occurs at a scale of up to 25–30 km. This

long-distance gene flow occurs terrestrially, as the clustering algorithms group sites not connected by rivers or streams and there was little support for a least-cost path based on stream connectivity. Previous studies have differed in their conclusions regarding tailed frog movement. Daugherty & Sheldon (1982) reported very low movement in tailed frogs, but this study investigated the closely related Rocky Mountain tailed frog (*Ascaphus montanus*) across a drier environment. The authors found that juvenile frogs had the lowest recapture rates and highest degree of movement. In coastal tailed frogs, there has been some evidence of longer movements, with frogs caught in pitfall traps up to 100 m from streams (although the average movement was only 14–37 m) (Wahbe *et al*. 2004) and were encountered up to 1 km from water (Corn & Bury 1989). Therefore, long-distance movement likely occurs via a small number of individuals and may be difficult to track using mark–recapture techniques.

The regression analyses clearly supported our hypothesis that landscape and environmental variables had important influence on gene flow in this system. In particular, topography and land cover strongly affected population connectivity in both study regions. As expected, amount of forest cover, amount of solar radiation and degree of slope all significantly influenced gene flow. Precipitation was a significant variable in only one model that had low support, but this is probably due to a narrow gradient of precipitation change within the scale of each study region. Finally, the autocorrelation across NP suggested that paths of gene flow were most similar when in relatively close proximity (within 3 km). The narrow autocorrelation is somewhat unexpected, as genetic connectivity between some sites ranged up to 20 km. The strong similarity of genetic values among movement paths within a few kilometres does suggest that tailed frog movement is highly nonrandom with corridor use through closed forest habitat. However, it must be noted that midpoints only represent a very small portion of the overall path, and therefore, we cannot make definitive conclusions about the scale of autocorrelation along the entire path.

Our study has several important implications for the conservation and management of tailed frogs and potentially for forest-associated species in general. Protection of breeding sites (such as through the use of stream buffers), while undoubtedly important for successful reproduction, may only be partially sufficient to maintain viable populations. It is also important to protect terrestrial corridor zones of appropriate habitat to allow for movement between sites. The weak subdivision observed within the managed forest suggests that differentiation due to harvest is beginning to occur. This pattern may eventually lead to loss of genetic diversity due to disperser mortality and genetic drift from isolation, potentially compromising evolutionary potential. However, if continuous patches of intact forest are maintained between streams, then we suspect that connectivity will be maintained across harvested forests. Our data also appear to be consistent with findings that forest-associated mammals are most common near corridors of intact forest (Lomolino & Perault 2001). Additionally, recent reduction in gene flow due to forest disturbance has occurred among capercaillie (grouse) populations in Europe (Segelbacher *et al*. 2008). Therefore, the implications for population genetic structure may not only be relevant to stream amphibians, but more generally to a taxonomic variety of forest-associated species.

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This research was conducted as part of Stephen Spear's dissertation research. The overall focus of Stephen's doctoral research is to use landscape genetic techniques to understand the role of disturbance on tailed frog genetic structure at multiple spatial and temporal scales. Andrew Storfer studies limits to species' ranges and uses population and landscape genetics as tools to understand the factors that shape distributions of species. He is also interested in host-pathogen co-evolution and conservation of amphibians.
