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| Complete List of Authors: | Moore, Jennifer; Michigan State University, Department of Fisheries and Wildlife
Tallmon, David; University of Alaska Southeast, Biology Program
Nielsen, Julie; University of Alaska Southeast
Pyare, Sanjay; University of Alaska Southeast, Biology Program |
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Effects of the landscape on boreal toad gene flow: does the pattern-process relationship hold true across distinct landscapes at the northern range margin?

Jennifer A. Moore (JAM)\textsuperscript{1,2}, David A. Tallmon (DAT)\textsuperscript{1}, Julie Nielsen (JN)\textsuperscript{1}, Sanjay Pyare (SP)\textsuperscript{1}

\textsuperscript{1} University of Alaska Southeast, 11120 Glacier Highway, Juneau, AK 99801, USA
\textsuperscript{2} Michigan State University, Department of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA

Corresponding author: Jennifer Moore

Current address: Michigan State University, Department of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA

Email: moore.jennifer@gmail.com

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ABSTRACT

Understanding the impact of natural and anthropogenic landscape features on population connectivity is a major goal in evolutionary ecology and conservation. Discovery of dispersal barriers is important for predicting population responses to landscape and environmental changes, particularly for populations at geographic range margins. We used a landscape genetics approach to quantify the effects of landscape features on gene flow and connectivity of boreal toad (Bufo boreas) populations from two distinct landscapes in Southeast Alaska (Admiralty Island, ANM, and the Chilkat River Valley, CRV). We compared two commonly used methodologies for calculating resistance distances in landscape genetics studies (resistance based on least-cost paths and circuit theory). We found a strong effect of saltwater on genetic distance of CRV populations, but no landscape effects were found for the ANM populations. Circuit theory methods outperformed least-cost path methods for both landscapes. Our discordant results show the importance of examining multiple landscapes that differ in the variability of their features, in order to maximize detectability of underlying processes and allow results to be broadly applicable across regions. Saltwater serves as a physiological barrier to boreal toad gene flow and affects populations on a small geographic scale, yet there appear to be few other barriers to toad dispersal in this intact region. Boreal toads may therefore be well suited to a northward range expansion, which could be imposed under future climate regimes.

INTRODUCTION

Landscapes strongly impact ecological and evolutionary processes, ultimately affecting gene flow, connectivity, and geographic range dynamics. Understanding how underlying landscape characteristics affect dispersal and population connectivity is a major goal in evolutionary
ecology and conservation. Molecular genetic data and high resolution spatial data now provide powerful tools to quantify how landscape and environmental features shape genetic variation in situations where traditional ecological methods may be inadequate (e.g., landscape genetics; Manel et al. 2003; Holderegger & Wagner 2006; Manel & Segelbacher 2009). Because these tools can operate on very fine scales, landscape genetic techniques are ideal for investigating functional connectivity in species with low vagility or relatively small ranges.

Amphibians are model candidates for studies of landscape effects on connectivity. Most amphibian species occur as metapopulations (Smith & Green 2005), they generally have low dispersal capabilities, and many are philopatric to breeding sites (Blaustein et al. 1994). These life history characteristics often lead to high genetic differentiation at small scales. Patchy distributions and high stochasticity of breeding site occupancy renders the use of traditional ecological methods (e.g., radio telemetry, capture-mark-recapture) alone insufficient for understanding gene flow in amphibians. Likewise, the impacts of certain landscape features (e.g., rivers and streams) may be difficult to predict based on field studies alone. For instance, small order streams can serve as corridors or assist movement for boreal toads (Adams et al. 2005; Schmetterling & Young 2008), but as waterways increase in width and flow they may become barriers to movement and dispersal. Furthermore, because amphibians are ectotherms with high evaporative water loss, they may be strongly impacted by landscape and environmental features due to thermal or moisture limitations on physiology.

The goal of this study was to understand the natural and anthropogenic landscape features and environmental factors that affect genetic connectivity in boreal toad (*Bufo boreas*) populations in southeast Alaska. Boreal toads are widespread across western North America, inhabiting altitudes up to 3600 m asl. Listed as near-threatened by the IUCN (Hammerson et al.
boreal toad populations have been nearly extirpated in large portions of western continental North America with the likely cause attributed to infection by the pathogenic chyrid fungus (*Batrachochytrium dendrobatidis*) (Carey 1993; Muths et al. 2003). In Alaska, declines have been reported anecdotally, and chytrid fungus has been confirmed in many populations (Adams et al. 2007), but the status of boreal toads is not well understood.

Southeast Alaska is a unique study area for a number of reasons. First, the varied and distinctive landscape provided an opportunity to test for the effects of dynamic landscape features (e.g., fiords, glaciers) that are rare but may be historically important in other parts of the boreal toad range. Further, because southeast Alaska is relatively pristine with fewer anthropogenic stressors like large scale habitat loss and widespread contamination, and populations there have higher breeding-site occupancy rates than currently exist elsewhere (S. Pyare, unpublished data), this study provides a rare opportunity to understand relatively natural patterns of gene flow among populations. Lastly, Southeast Alaska is a rapidly changing landscape at the very northern edge of the boreal toad range. Understanding current dispersal patterns and connectivity of these populations will better allow us to predict the impact of future climate change, particularly in assessing the potential for a northward range expansion.

Most landscape genetics studies examine population gene flow within a single landscape. However, analysis of multiple landscapes provides more robust results that are applicable across geographic regions, or allows for comparisons between regions that differ in key environmental or structural landscape features (Segelbacher et al. 2010, Short Bull et al. 2011). Thus, we compared two geographically distinct but structurally similar landscapes within the same region (southeast Alaska). We sampled boreal toad populations on Admiralty Island (ANM) and in the Chilkat River Valley (CRV) (Fig. 1). Admiralty Island National Monument is a 3,860 km²
federally protected, roadless wilderness area on Admiralty Island, part of the Alexander
Archipelago. ANM is composed of extensive old-growth temperate rainforest interspersed with
coastal mountains and areas of sparsely forested peatlands. Above timberline (at ~800 m asl) the
forest gradually changes to alpine-tundra with rock outcrops and permanent ice fields. Sampled
populations from CRV were located between two large, major river valleys (the Chilkat and
Chilkoot) near the town of Haines (population 1,800). The Chilkat River Valley and surrounding
areas have a rugged topography and are heavily influenced by glaciers and coastal mountains
(e.g., the Takshanuk Mountains, 1,200 m asl). The dominant habitat is spruce-hemlock rainforest
that is characteristic of the region, interspersed with areas of peatland (Fig. 1). CRV populations
encompassed the Chilkat inlet, which is the northernmost inlet of the Lynn Canal, one of the
deepest (610 m) and longest (140 km) saltwater fiords in the world. Including sites within the
developed portions of Haines allowed us to assess whether any low levels of anthropogenic
development, especially roads, affected connectivity of toad populations, and whether patterns
differed from the more pristine ANM landscape.

Landscape genetics is a rapidly developing field, with a need for more ev aluative
research on the best methodology for different ecological systems and sampling scenarios. We
compared two popular methods of calculating resistance distances for use in landscape genetics
studies – least cost path analysis (LCP, Cushman et al. 2006) and isolation by resistance based
on circuit theory (CT, Mcrae 2006; Mcrae & Beier 2007). These two methods calculate distances
based on least resistance of the matrix between two sampling points (populations or individuals).
However, they differ in that LCP only calculates one path between each pair of points and CT
allows for multiple paths depending on the dimensions of the underlying matrix between
sampling points. Because these methods are becoming increasingly popular (Spear et al. 2010), it is important to examine circumstances under which one method will outperform the other.

To better understand connectivity of a widespread amphibian in a unique environment, to aid conservation efforts for boreal toads, and to add to the growing body of literature addressing different landscape genetic methodologies, we addressed three main questions:

1. To what degree are boreal toad populations in southeast Alaska genetically differentiated, and how is the genetic variation spatially structured?

2. What specific landscape feature(s) affect gene flow and connectivity of boreal toad populations, and does the pattern-process relationship hold true for two distinct landscapes within the same region?

3. Which method of calculating resistance distances (least-cost path vs. circuit theory) performs best, and how can this information contribute to future landscape genetics studies?

METHODS

Sample collection

Boreal toad samples were collected from tadpoles at 21 known breeding sites (10 from ANM, 11 from CRV) during the summer breeding seasons of 2005-2007, and 2009. Euclidean distances between sites ranged from 33 m to 50 km for both landscapes. Sampling site locations were recorded using a handheld GPS (Garmin GPSMAP® 76CSx, Olathe, KS, USA). Tissue samples were collected as tail clips (2-3 mm) from tadpoles. Samples were stored in cryotubes in 95% ethanol at room temperature prior to DNA extraction. A sterile field protocol was maintained to minimize spread of disease and contamination of samples.
Genetic data and analyses

Genomic DNA was extracted using Qiagen DNeasy tissue kits and protocols (Qiagen Inc., Valencia, CA, USA). Eleven species-specific microsatellite loci (BBR45, BBR36, BBR233, BBR29, BBR86, BBR87-b, BBR34-2, BBR4, BBR281, BBR292, BBR293; Simandle et al., 2006) were amplified using PCR and scanned on an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Inc.). Fragments were analyzed and visualized using ABI Peak Scanner software (v 1.0, Applied Biosystems) and allele sizes were manually scored. PCR conditions and multiplex panels followed Murphy et al. (2010a). We amplified approximately 9% of samples twice to screen for genotyping and/or human error and to reamplify any rare alleles. Concordance between runs was high with an error rate of less than 0.75%.

Larvae samples can be problematic due to allele frequency bias from sampling siblings (Allendorf and Phelps 1981). Thus, we used a maximum likelihood approach in the program COLONY (Wang 2004) to identify full sibling clusters (Goldberg and Waits 2010). Samples were then filtered to only include one individual from each full sibling family, in order to avoid biasing allele frequencies from sampling kin (Goldberg and Waits 2010).

We tested loci for significant deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium (LD) at each locus for each sampling locality in GenePop 4.0 (Raymond & Rousset 1995). We used a Monte Carlo chain method (1000 dememorizations, 100 batches, 1000 iterations) following the algorithm of Guo and Thompson (1992) and applied a Bonferroni correction for a table-wide significance level of 0.05. We calculated observed and expected heterozygosities, as well as the number of alleles per locus in GenAlEx v. 6.3 (Peakall & Smouse 2006). We also determined allelic richness per locus for each sampling site using Fstat 2.9 (Goudet 1995).
To assess the level of genetic differentiation among sites for each landscape, we calculated global and pairwise $F_{ST}$ estimates in GenAlEx v. 6.3 (Wright 1931; Weir & Cockerham 1984; Peakall & Smouse 2006), and tested for significant deviations from zero based on 999 permutations. We also calculated pairwise Cavalli-Sforza and Edwards chord distance ($D_c$; Cavalli-Sforza & Edwards 1967) in Microsatellite Analyzer (MSA; Dieringer and Schlötterer 2002) for use in landscape analyses. $D_c$ weighs mutation as insignificant compared to genetic drift, so this measure may be particularly suitable for microsatellites and for the fine scale of most landscape genetic studies (Cavalli-Sforza & Edwards 1967, Takazaki & Nei 1996). In order to qualitatively assess other factors that may be affecting genetic variation or levels of genetic differentiation, we estimated effective population sizes ($N_e$) and 95% confidence intervals for all sampled populations using the single-sample linkage disequilibrium method in LDNe (Waples & Do 2008) and approximate Bayesian method in ONeSAMP 1.2 (Tallmon et al. 2008). For species with overlapping generations (e.g., toads), these estimates actually reflect the effective number of breeders in the year in which they were sampled ($N_b$; Waples 2005). The priors for $N_b$ estimates in ONeSAMP (i.e., the upper and lower bounds of $N_b$ for the population) were set at 2 and 500 for all populations. Significance is assumed at $p<0.05$ for all analyses, unless otherwise noted.

**Landscape resistance models**

To examine the impact of the landscape on functional connectivity, we first calculated resistance distances based on a series of models using either the Circuitscape program (for CT analyses, Shah & Mcrae 2008) or the Landscape Genetics extension for ArcGIS 9.3 (for LCP analyses, Etherington 2010). While both of these methods are based on the concept of movement surfaces,
input as coded grids, the methods differ in allowing for a single path of least resistance (LCP) versus total resistance based on multiple potential paths of least resistance (CT) between populations. Grid cells correspond to a conductance (for CT) or resistance (for LCP) value that reflects the ability of an organism to move through the habitat in that cell.

To derive the grids, we used landscape variables that we predicted would have possible relevance to boreal toad habitat selection, vagility, dispersal, and gene flow based on relevant literature, expert opinion, and occupancy models (S. Pyare, unpublished data). The habitat structure layer reflected the structural complexity and permeability of the landscape and was based on the following elements: terrestrial vegetation type, non-vegetative landcover (e.g. rocks, moraines, ice fields), lentic and lotic waterbody size, and wetlands (Table 1). We developed five alternative habitat structure grids with different resistance classifications and values based on hypotheses about the functional importance of various landscape elements on boreal toad movement (Bartelt et al. 2004; Adams et al. 2005; Murphy et al. 2010a; Table 2).

Assigning resistance values to different structure types can be problematic (Spear et al. 2010). However, for each habitat structure grid, we attempted to mitigate any subjectivity by inflating the values of the landscape elements we were specifically testing relative to the other elements in the model (see Appendix S1 for resistance values).

We also tested for the influence of the following five variables: 1. Insolation, which reflects the thermal properties of the landscape, and is important for toad connectivity due to their high rates of evaporative water loss and temperature sensitivity (Bartelt et al. 2004; Bartelt & Peterson 2005); 2. Rugosity, a measure of landscape “ruggedness” derived from the ratio between actual-surface and planar areas, which reflects where there is a higher energetic cost when moving through more topographically complex landscapes; 3. Permanent snow or ice
fields, as this affects breeding phenology (Corn 2003) and may be a thermal barrier to dispersal; 4. Saltwater, which may be a physiological barrier (Taylor 1983); and 5. Roads (for CRV only), which are known to limit amphibian dispersal and movements (Carr & Fahrig 2001; Arens et al. 2007; Bull 2009). Insolation and rugosity grids were coded as continuous variables, while permanent snow/ice fields, saltwater, and road grids were coded as presence/absence. All grids were rescaled between 0 and 1.

We first computed resistance distances for the single-variable models. We tested all single factor models as well as all two-factor models including only the best fitting habitat structure model resulting in 21 different resistance surfaces for ANM and 27 for CRV (Appendix S2). We also created a ‘flat’ landscape surface (e.g., Lee-Yaw et al. 2009), in which all grid cells had the same value. This is equivalent to testing for isolation-by-distance using Euclidean distances, but it takes into account the fact that the underlying landscape is bounded and not infinite.

Resolution of all grids was standardized to 50-m grid cell size. Due to computational limits of the Circuitscape program and the extreme variation of southeastern Alaska landscapes, we limited the extent of our analysis to within 50km of sampling sites and all the area in between. For comparative purposes, we used the same extent and resolution for the CT and LCP analyses. Grids were exported from ArcGIS for Circuitscape analysis using the ‘Export to Circuitscape’ tool (J. Jenness, http://www.circuitscape.org/Circuitscape/ArcGIS.html).

Landscape analyses

We conducted a series of simple Mantel tests to examine correlations between matrices of genetic against geographic or resistance distances for each pair of populations within each
landscape. New methodologies are rapidly emerging in the landscape genetics literature, and with further simulation studies and greater utility, standardization of new analytical techniques may develop. We chose to use Mantel tests because they are still one of the most powerful, widely used, and easily interpretable tests that are most appropriate for distance data (Legendre & Fortin 2010). Pairwise resistance distances were calculated in Circuitscape 3.5 and ArcGIS 9.3. We first tested for a pattern of isolation by distance (IBD) whereby genetic differentiation increases with geographic distance. Isolation by distance is expected under mutation-migration-drift equilibrium, and requires a stepping-stone migration model. Thus, we compared matrices of pairwise Euclidean and ‘flat’ distances to pairwise $D_c$ with simple Mantel tests (Mantel 1967) using the ecodist package (Goslee & Urban 2007) in R (R Development Core Team 2006). Significance of Pearson correlations was assessed based on 10,000 random permutations of the data and 95% confidence intervals were calculated based on 10,000 bootstrapped iterations. We then tested for patterns of isolation by resistance (IBR) for each of our landscape models. We calculated pairwise resistance values for each model using both LCP and CT, and compared these matrices to the matrix of pairwise $D_c$ using simple Mantel tests and significance testing (as above). We applied a Bonferroni correction for multiple tests for a corrected alpha level of 0.002.

**RESULTS**

We genotyped 663 samples from 21 breeding sites (11 from CRV and 10 from ANM) (Fig. 1). We then removed all but one full sibling from each family, which reduced the dataset to 426 individuals for all further analyses. No locus or population showed consistent deviations from HWE, or consistent linkage disequilibrium after Bonferroni correction so all 11 loci were retained for further analyses. All loci were polymorphic with the number of alleles per locus
ranging from 2-16 (Table 3). Mean observed heterozygosity across all loci and populations was 0.51 (SE=0.02). Global $F_{ST}$ was 0.049 (p=0.001) for ANM and 0.052 (p=0.001) for CRV. Pairwise $F_{ST}$ between sites, within each landscape, ranged from 0.004 - 0.21 and 0.00 – 0.21 for ANM and CRV respectively (Table 4). Mean pairwise $D_c$ was 0.26 for ANM and 0.23 for CRV. Pairwise Euclidean distances between populations averaged 12.55 km and 11.4 km for ANM and CRV respectively (Table 3).

Estimates of the effective number of breeders were small. ONeSAMP (Tallmon et al. 2008) estimates averaged 40 breeders for CRV populations and 26 for ANM populations, and LDNe (Waples & Do 2008) estimates averaged 30 breeders for CRV and 46 for ANM (Table 3). However, these estimates may be somewhat inaccurate due to small sample sizes for some of the populations.

There was no significant pattern of IBD for either CRV or ANM populations, based on Euclidean distances (CRV $r = 0.55$, $p = 0.45$; ANM $r = 0.27$, $p = 0.14$) or the ‘flat’ landscapes (CRV $r = 0.56$, $p = 0.03$; ANM $r = 0.44$, $p = 0.13$), based on an adjusted alpha value. No landscape models were statistically significant for ANM populations, based on CT or LCP analyses. The top model for ANM (based on CT analyses, and containing the insolation and permanent snow/ice variables) did explain more of the variation in the genetic data than the landscape-free models, although it did not provide a particularly good fit with an $r$ value of 0.44.

For CRV populations, five CT landscape models and one LCP landscape model were significant (Fig 2). The best fitting LCP model contained the structure 4 and saltwater variables and explained 73% of the variation in the data ($p = 0.0006$). The top CT model contained the saltwater and permanent snow/ice variables and explained 74% of the variation in the genetic data ($p = 0.0005$). The top five significant models for CRV all contained the saltwater variable,
but the single factor model containing only saltwater explained 72% of the variation in the
dataset (p = 0.001). Roads provided a particularly poor fit for the data, only explaining 7% of the
variation. It appears that saltwater is the strongest landscape variable driving the variation in the
CRV data, and this variable explains at least 18% more of the variation in the genetic data than
the landscape-free models. However, if models are compared based on the conservative criterion
of non-overlapping confidence intervals, the saltwater model is not significantly better than
many other landscape or landscape-free models (Fig. 2). Overall, the CT models provided a
much better fit for the data than the LCP models.

DISCUSSION

We examined the effects of the landscape on patterns and distribution of genetic variation in
boreal toad populations from two distinct landscapes in southeast Alaska. Our study provides the
first landscape genetic analysis of an amphibian in Alaska, and adds to the growing number of
landscape genetic studies comparing multiple landscapes (Spear and Storfer 2010, Short Bull
2011). We found discordant results from general population genetic parameters and geographic
analyses between the two landscapes. First, populations from CRV had lower genetic variation
and were more strongly differentiated than populations from ANM, even though straight-line
distances between populations and effective population sizes were not different. Second, while
we found a significant effect of the landscape for boreal toad populations in CRV, no such
pattern was found for populations on ANM.

Are these differences between populations in the two landscapes the result of natural or
anthropogenic factors? Populations in more fragmented landscapes are generally expected to
have lower genetic variation and be more genetically structured than those in pristine landscapes
(e.g., Knutsen et al. 2000; Berry et al. 2005; Arens et al. 2007; Dixo et al. 2009). In the absence of a landscape-based analysis, we might conclude that the differences between our populations are the result of human impacts fragmenting the landscape and causing greater isolation of CRV populations. Our landscape modelling showed that genetic structure of CRV populations was not affected by roads, which would most likely be the strongest anthropogenic impact in this landscape. Human population density is low in CRV, which means that the roads in this landscape are few, with very low-volume vehicle traffic. The Haines highway, which is the only major road, is still only two lanes wide and has very little traffic due to the remoteness of the region (e.g., traffic coming from the south arrives via marine ferry). Roads may have a much greater impact in more heavily populated regions, with larger and denser road networks (Murphy 2010). It is possible that other human activity (e.g., development, habitat modification) is reducing the number of breeding populations or causing reduced survival of dispersing metamorphs or juveniles resulting in decreased genetic connectivity (Cushman 2006).

We found an effect of natural landscape features for CRV and not for ANM populations, which indicates that natural variation in the CRV landscape may be driving our ability to detect the effect of landscape features. The CRV populations occur along either side of a major river valley and saltwater fiord, and are bounded to the east and west by coastal mountains and extensive ice fields. Suitable wetland habitat is patchy and occurs mostly at low elevation sites that are found primarily along the edge of the river or canal (Fig. 1). In contrast, although interspersed with high elevation mountains and permanent ice fields, ANM populations encompass large stands of semi-forested peatland wetlands which are interconnected by numerous freshwater lakes (Fig. 1). Chan and Zamudio (2009) showed that genetic differentiation is lower in amphibian species in habitats that are more homogeneous versus those
that are more variable (e.g., arid-adapted desert toads versus pond-breeding temperate amphibians). Our results suggest that this pattern holds true for intraspecific comparisons of populations from different landscapes as well.

The lack of a good fitting IBD or landscape-resistance model for ANM populations could be due to 1) populations not having reached migration-drift equilibrium (MDE), or 2) connectivity being affected by a landscape or environmental feature that we did not quantify, or one that was not sufficiently variable to detect its effects on gene flow of populations inhabiting this landscape. In certain areas in southeast Alaska, deglaciation provides new breeding habitats that could easily be colonized by toads. However, once colonized, populations are likely to reach MDE rather quickly due to their small effective population sizes (Allendorf & Phelps 1981). Although boreal toads are at the edge of their geographic range in southeast Alaska, it is unlikely that populations occurring on islands in the Alexander Archipelago, which is known for its high levels of endemism (MacDonald & Cook 1996, Cook et al. 2006), have been recently founded. Juvenile toads are capable of dispersing long distances on land (e.g., >2700 m, Bull 2009), but swimming across an expanse of saltwater is probably unlikely, thus limiting migration or further colonization of these islands. Future work is needed to address historic patterns of amphibian colonization and connectivity in this region.

Admiralty Island (ANM) populations may be affected by a landscape feature that we did not include in our analyses. The use of multivariate resistance surfaces quickly becomes complex (Spear et al. 2010), so researchers are limited in the number of factors and combinations of factors they are able to examine (Manel et al. 2010). We chose to include only landscape features that we deemed important based on expert opinion, relevant literature, and breeding site surveys (S. Pyare, unpublished data), and deliberately limited the number of factors and multivariate
models we examined in order to provide clear, easily interpretable results. Therefore, we may have unknowingly overlooked a critical landscape feature that was not deemed important for habitat selection, vagility, dispersal, and gene flow in previous studies of boreal toads where habitats and ecological conditions differ from southeast Alaska.

The extent of sampling for ANM populations, which encompassed less variation in the saltwater surface than sampled CRV populations, may have also contributed to the different results obtained from the two landscapes. Of the nine single-factor models that we tested for both landscapes (excluding roads), the saltwater variable yielded the greatest difference in mean and variance of pairwise resistance values (from CT analyses) between CRV and ANM populations. Pairwise resistance values based on the saltwater grid were 1.3 times higher on average for CRV than ANM populations. The CRV resistance values from the saltwater model also had the highest variance of any of the single factor models for both landscapes. Short Bull et al. (2011) showed that features were only supported in landscape models across sites where the landscape features were most variable. Our results support the assertion that landscape analyses should be replicated to capture the range of variability in specific features and thus better understand underlying processes (Arens et al. 2007; Constible et al. 2009; Manel & Segelbacher 2009; Segelbacher et al. 2010; Short Bull et al. 2011). Differences between landscapes should be identified a priori for appropriate hypothesis testing and comparative analysis. A multiple landscape approach may yield more robust results that are more broadly applicable, at least on a regional scale, than analysis of a single landscape.

Unlike boreal toad populations elsewhere (Manier & Arnold 2006), distance was not a strong predictor of gene flow in southeast Alaska. Likewise, we tested five alternative hypotheses about the effects of various structural elements on gene flow in boreal toad
populations and none of these models fit the data well. Boreal toads in southeast Alaska therefore appear to be broadly tolerant of the habitat, moisture and temperature regime that occurs there. For instance, toads in this region are known to breed in extreme thermal environments ranging from glacially fed lakes to warm thermal pools. In other parts of their range, toad dispersal and connectivity are strongly limited by cover and moisture (Bartelt et al. 2004; Murphy et al. 2010a). Southeast Alaska is a large coastal temperate rainforest that averages 3-4 meters of precipitation annually, so breeding ponds are not at risk of drying up before larvae metamorphose and many natural habitats are moist enough for toads to avoid desiccation (Carstensen et al. 2003). Thus, although amphibians have narrow environmental tolerances in moisture-limited regions and can be susceptible to habitat and hydrological alterations that are typical elsewhere, boreal toads in southeast Alaska may be able to exhibit more plasticity in breeding-site selection and dispersal, even in situations where habitats are altered. For instance, boreal toads have been found in and adjacent to recent clearcuts and access roads in other parts of southeast Alaska where timber harvesting is more common (S. Pyare, pers. obs.).

Most studies of amphibian connectivity base hypotheses on what is known about dispersal and movement of adults. However, amphibian dispersal often occurs at the metamorph or juvenile stage (Guerry & Hunter 2002; Rothermel 2004; Roznik & Johnson 2009), and regional persistence is more strongly affected by post-metamorphic dispersal than adult dispersal (Sinsch 1992, 1997). Thus, for more accurate predictions, information on movement patterns and habitat preferences of metamorph and juvenile amphibians is needed. Basing future landscape-modelling hypotheses on this information might prove more meaningful and strengthen the results of amphibian landscape genetics studies (e.g., Stevens et al. 2004, Stevens et al. 2006).
In our study system, circuit theory outperformed least-cost path methods for calculating landscape resistance distances (McRae & Beier 2007). Previous studies comparing these two methods have found conflicting results. McRae and Beier (2007) showed that CT outperforms LCP for population-based studies of mahogany and wolverines across their broad geographic ranges. On the other hand, Schwartz et al. (2009) found that LCP outperformed CT for an individual-based analysis of wolverines in the western United States. Schwartz et al. (2009) attributed this to the narrow, often linear habitat bands inhabited by the wolverines (e.g., between two mountain ranges), and suggested that LCP would outperform CT methods in systems where populations are narrowly distributed along linear bands of suitable habitat. Our toad populations are extensively distributed and intervening suitable habitat is not narrow and linear, resulting in the better fit of the CT models. For amphibians, LCP may outperform CT methods where dispersal occurs primarily in narrow waterways (e.g., streams; Schmetterling & Young 2008).

Further comparison of these methods, and other newly emerging methods (e.g., gravity models, Murphy et al. 2010b), would be useful for determining any patterns based on animal life history (e.g., endo- vs. ectotherms, vagility, movement patterns, and range size).

Conclusions

Our examination of two distinct landscapes showed that landscape impacts on a species’ gene flow can differ within an ecological region. The importance of features across different landscapes depends upon the spatial scale and extent of sampling and the underlying landscape heterogeneity and variability. We found that boreal toads are robust to habitat heterogeneity, yet can be genetically differentiated on small geographic scales, which is probably due to breeding-site philopatry and small effective population sizes. In intact landscapes with high moisture
levels (like southeast Alaska), there appear to be few real barriers to boreal toad dispersal. The
ability to easily permeate a variety of habitats, and overcome any potential barriers, has
important implications for range-margin populations under future climate regimes. Climate
change will likely force geographic range shifts or expansions, particularly for species that are
closely tied to environmental conditions (e.g., ectotherms). With few real barriers to dispersal in
the northern-most populations, boreal toads may be well suited to a northward range expansion.
Their ability to tolerate and disperse across variable habitats may enable them to colonize newly
available habitats and be resilient in the face of a warming trend.

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Supporting information

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

**Appendix S1** Habitat types and resistance values for structure models

**Appendix S2** All landscape models and results of Mantel tests

Jennifer Moore is currently a research associate at Michigan State University, and is broadly interested in molecular ecology, conservation, and population and landscape genetics of vertebrates. Sanjay Pyare and Julie Nielsen are interested in landscape and spatial ecology, and GIS, and David Tallmon is interested in molecular ecology, evolution and conservation.
Table 1. Model variables used to derive landscape resistance surfaces for boreal toad populations in Southeast Alaska

<table>
<thead>
<tr>
<th>Surface</th>
<th>Abbrev</th>
<th>Description</th>
<th>Spatial Data Source</th>
<th>Ecological Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>str</td>
<td>Structural</td>
<td>Derived from Terrestrial Ecosystems Classification, National Wetland Inventory, and</td>
<td>Different habitat types may impede dispersal due to high or low cover and/or moisture (Adams et al., 2005;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>complexity</td>
<td>data from Alaska Dept of Natural Resources, and U.S. Forest Service</td>
<td>Bartelt et al. 2004; Murphy et al. 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>permeability of the landscape</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insolation</td>
<td>sol</td>
<td>Amount of</td>
<td>Derived from SRTM and ESRI ArcGIS 9.3 Solar Analyst Tools</td>
<td>Hot areas can impede amphibian dispersal due to high rates of evaporative water loss (Bartelt et al. 2004;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>solar radiation</td>
<td></td>
<td>Bartelt &amp; Peterson 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>received on a given surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rugosity</td>
<td>rug</td>
<td>Ratio of the true surface to the geometric surface area</td>
<td>Derived from SRTM and ESRI ArcGIS 9.3 Solar Analyst Tools</td>
<td>High cost of dispersing through more rugged areas, ridges may impede amphibian dispersal (Funk 1999)</td>
</tr>
<tr>
<td>Permanent</td>
<td>ice</td>
<td>Snow and ice</td>
<td>Derived from Terrestrial Ecosystems Classification</td>
<td>Snow cover affects breeding</td>
</tr>
</tbody>
</table>

28
snow/ice that persists year round Ecosystems Classification phenology and subsequent gene flow (Corn 2003); permanent ice pack limits dispersal

Saltwater *salt* Saltwater Derived from bathymetry Saltwater is a physiological impediment to dispersal (Taylor 1983)

digital elevation model

Roads *roads* Roads Data 3 from Alaska Dept of Natural Resources High cost of crossing roads for amphibians due to increased mortality from vehicles and dessication (Carr & Fahrig 2001; Arens *et al.* 2007; Bull 2009; Murphy *et al.* 2010)
Table 2. Hypotheses for five models of habitat structural complexity and permeability including the basis of the hypothesis and the number of land cover classifications in each model

<table>
<thead>
<tr>
<th>Model</th>
<th>Basis</th>
<th>Hypothesis</th>
<th>Land cover ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Str1</td>
<td>Habitat</td>
<td>Wetland is least resistant, conifer type forest/unvegetated is most, other forest/scrubby habitat is semi-resistant</td>
<td>coarse (5)</td>
</tr>
<tr>
<td>Str2</td>
<td>Habitat</td>
<td>Wetland is least resistant, conifer type forest/unvegetated is most, other forest/scrubby habitat is semi-resistant</td>
<td>fine (8)</td>
</tr>
<tr>
<td>Str3</td>
<td>Moisture</td>
<td>Wet habitats are least resistant, dry habitats are most resistant</td>
<td>coarse (3)</td>
</tr>
<tr>
<td>Str4</td>
<td>Cover</td>
<td>Little to medium cover is least resistant, full cover is most resistant</td>
<td>fine (6)</td>
</tr>
<tr>
<td>Str5</td>
<td>Cover</td>
<td>Medium cover is lowest resistance, no cover is highest resistance, high cover is medium resistance</td>
<td>fine (7)</td>
</tr>
</tbody>
</table>
Table 3. Summary of genetic measures for boreal toad populations from Admiralty Island (ANM) and the Chilkat River Valley (CRV) in Southeast Alaska

<table>
<thead>
<tr>
<th>Measure</th>
<th>ANM (n = 10)</th>
<th>CRV (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>min</td>
</tr>
<tr>
<td>Allelic Richness</td>
<td>3.44</td>
<td>2.36</td>
</tr>
<tr>
<td>Pairwise $F_{ST}$</td>
<td>0.060</td>
<td>0.004</td>
</tr>
<tr>
<td>Pairwise $D_e$</td>
<td>0.26</td>
<td>0.16</td>
</tr>
<tr>
<td>$N_b$ (ONeSAMP)</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>$N_b$ (LDNe)</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>Euclidean distance (km)</td>
<td>12.17</td>
<td>0.033</td>
</tr>
</tbody>
</table>

ANM, Admiralty Island; CRV, Chilkat River Valley; $F_{ST}$, genetic differentiation; $D_e$, Cavalli-Sforza and Edwards chord distance; $N_b$, effective number of breeders.
Table 4. Pairwise $F_{ST}$ values (below diagonal) and p-values based on permutation tests (above diagonal) for populations of boreal toads from (A) Admiralty Island and the (B) Chilkat River Valley in southeast Alaska. Values in bold are significant at $p<0.05$.

A.

<table>
<thead>
<tr>
<th></th>
<th>A12</th>
<th>A16</th>
<th>A17</th>
<th>A18</th>
<th>A22</th>
<th>A25</th>
<th>A29</th>
<th>A32</th>
<th>A0106</th>
<th>A07</th>
</tr>
</thead>
<tbody>
<tr>
<td>A12</td>
<td>-</td>
<td>0.001</td>
<td>0.078</td>
<td>0.213</td>
<td>0.011</td>
<td>0.001</td>
<td>0.033</td>
<td>0.003</td>
<td>0.001</td>
<td>0.213</td>
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<tr>
<td>A16</td>
<td>0.125</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>A17</td>
<td>0.012</td>
<td>0.142</td>
<td>-</td>
<td>0.014</td>
<td>0.004</td>
<td>0.001</td>
<td>0.109</td>
<td>0.003</td>
<td>0.004</td>
<td>0.017</td>
</tr>
<tr>
<td>A18</td>
<td>0.004</td>
<td>0.142</td>
<td>0.024</td>
<td>-</td>
<td>0.022</td>
<td>0.001</td>
<td>0.274</td>
<td>0.073</td>
<td>0.002</td>
<td>0.311</td>
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<tr>
<td>A22</td>
<td>0.030</td>
<td>0.125</td>
<td>0.065</td>
<td>0.027</td>
<td>-</td>
<td>0.001</td>
<td>0.043</td>
<td>0.167</td>
<td>0.003</td>
<td>0.038</td>
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<tr>
<td>A25</td>
<td>0.051</td>
<td>0.207</td>
<td>0.096</td>
<td>0.067</td>
<td>0.122</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>A29</td>
<td>0.023</td>
<td>0.150</td>
<td>0.020</td>
<td>0.006</td>
<td>0.033</td>
<td>0.089</td>
<td>-</td>
<td>0.169</td>
<td>0.091</td>
<td>0.180</td>
</tr>
<tr>
<td>A32</td>
<td>0.031</td>
<td>0.148</td>
<td>0.053</td>
<td>0.016</td>
<td>0.012</td>
<td>0.096</td>
<td>0.014</td>
<td>-</td>
<td>0.002</td>
<td>0.042</td>
</tr>
<tr>
<td>A0106</td>
<td>0.029</td>
<td>0.105</td>
<td>0.035</td>
<td>0.032</td>
<td>0.044</td>
<td>0.100</td>
<td>0.017</td>
<td>0.046</td>
<td>-</td>
<td>0.068</td>
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<tr>
<td>A07</td>
<td>0.008</td>
<td>0.130</td>
<td>0.037</td>
<td>0.004</td>
<td>0.030</td>
<td>0.090</td>
<td>0.012</td>
<td>0.026</td>
<td>0.018</td>
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</table>

B.

<table>
<thead>
<tr>
<th></th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H5</th>
<th>H6</th>
<th>H7</th>
<th>H8</th>
<th>H9</th>
<th>H10</th>
<th>H52</th>
<th>H0106</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>0.089</td>
<td>-</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>0.069</td>
<td>0.042</td>
<td>-</td>
<td>0.255</td>
<td>0.001</td>
<td>0.171</td>
<td>0.034</td>
<td>0.417</td>
<td>0.005</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>0.094</td>
<td>0.082</td>
<td>0.003</td>
<td>-</td>
<td>0.001</td>
<td>0.059</td>
<td>0.092</td>
<td>0.412</td>
<td>0.008</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>0.104</td>
<td>0.090</td>
<td>0.034</td>
<td>0.046</td>
<td>-</td>
<td>0.017</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H7</td>
<td>0.085</td>
<td>0.087</td>
<td>0.009</td>
<td>0.022</td>
<td>0.038</td>
<td>-</td>
<td>0.183</td>
<td>0.124</td>
<td>0.008</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H8</td>
<td>0.061</td>
<td>0.054</td>
<td>0.011</td>
<td>0.009</td>
<td>0.032</td>
<td>0.010</td>
<td>-</td>
<td>0.022</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H9</td>
<td>0.094</td>
<td>0.083</td>
<td>0.000</td>
<td>0.000</td>
<td>0.040</td>
<td>0.013</td>
<td>0.011</td>
<td>-</td>
<td>0.002</td>
<td>0.001</td>
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</tr>
<tr>
<td>H10</td>
<td>0.105</td>
<td>0.087</td>
<td>0.015</td>
<td>0.020</td>
<td>0.081</td>
<td>0.036</td>
<td>0.039</td>
<td>0.016</td>
<td>-</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>H52</td>
<td>0.210</td>
<td>0.167</td>
<td>0.108</td>
<td>0.105</td>
<td>0.123</td>
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<td>0.097</td>
<td>0.105</td>
<td>0.105</td>
<td>-</td>
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</tr>
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<td>H0106</td>
<td>0.128</td>
<td>0.129</td>
<td>0.064</td>
<td>0.042</td>
<td>0.125</td>
<td>0.082</td>
<td>0.063</td>
<td>0.059</td>
<td>0.075</td>
<td>0.147</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig 1. Boreal toad sampling sites in the Chilkat River Valley (top) and Admiralty Island (bottom) in southeast Alaska (inset). White lines in CRV indicate roads. White areas are indicative of permanent snow/ice (at high elevation). Sampling sites are labelled as per Table 4.
Fig 2. Plot of Mantel r-values for landscape models and landscape-free models (‘flat’ and ‘euclid’) for boreal toad populations in the Chilkat River Valley (CRV) site in southeast Alaska based on (A) circuit theory, and (B) least-cost path methods (st=structure, sol=insolation, salt=saltwater, rug=rugosity, ice=permanent snow/ice). Bars represent 95% confidence intervals. Asterisks indicate significant models based on a Bonferroni-corrected alpha.