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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,* DAVID A. TALLMON,† JULIE NIELSEN‡ and SANJAY PYARE‡

*Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA, †Biology and Marine Biology Program, University of Alaska Southeast, 11120 Glacier Highway, Juneau, AK 99801, USA, ‡Environmental Science Program, University of Alaska Southeast, 11120 Glacier Highway, Juneau, AK 99801, USA

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 south-east Alaska (Admiralty Island, ANM, and the Chilkat River Valley, CRV). We used two common 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. Our discordant results show the importance of examining multiple landscapes that differ in the variability of their features, 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 northern region.

Keywords: amphibians, boreal toads, circuit theory, geographic information systems, landscape genetics, least-cost path, south-east Alaska

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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 (i.e. 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 render the use of traditional ecological methods (e.g. radio telemetry, capture–mark–recapture) alone insufficient for understanding gene flow in amphibians.

Correspondence: Jennifer A. Moore, Fax: (517) 432-1699; E-mail: moore.jennifer@gmail.com

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 because of 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 south-east 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.* 2004), 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 chytrid 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.

South-east 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 for many terrestrial vertebrates inhabiting northern regions. Further, because south-east 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 gene flow among populations in a relatively natural system. Lastly, south-east 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 (south-east 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 3860 km^2 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 1800). The CRV and surrounding areas have a rugged topography and are heavily influenced by glaciers and coastal mountains (e.g. the Takshanuk Mountains, 1200 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 growing field, with a need for more evaluative 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 considers multiple paths depending on the dimensions of the underlying matrix between sampling points.

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 landscape genetic methodologies, we addressed three main questions:

1 To what degree are boreal toad populations in south-east 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

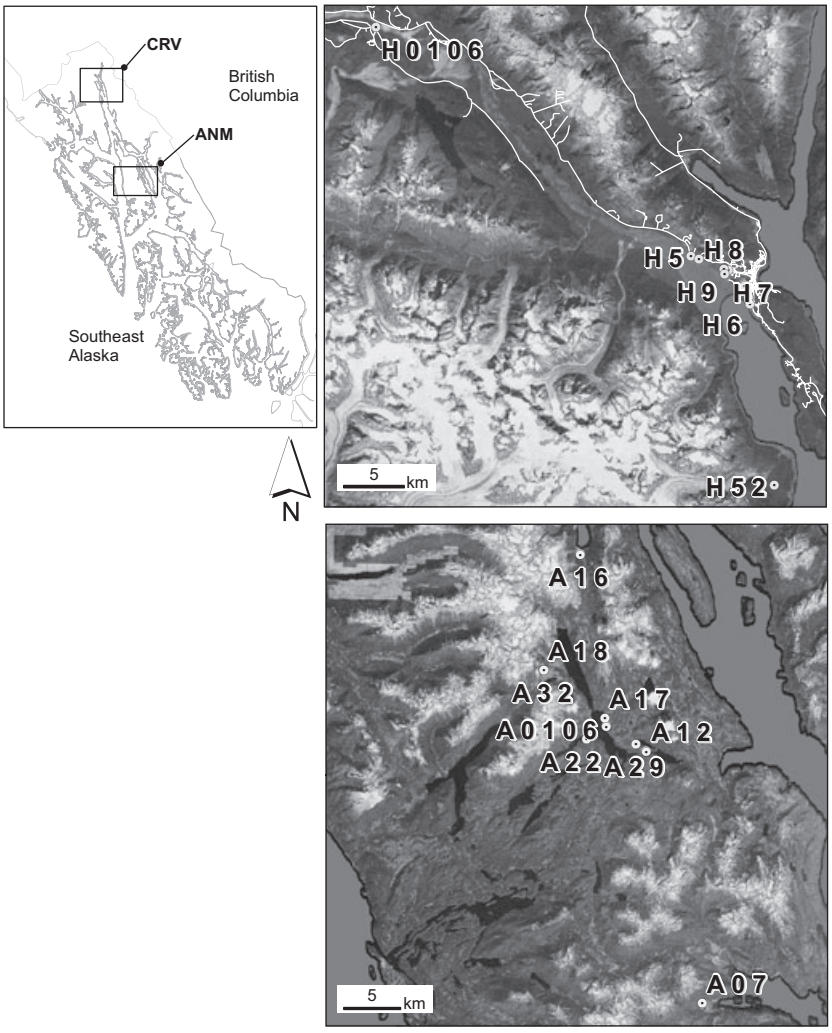

Fig. 1 Boreal toad sampling sites in the Chilkat River Valley (top) and Admiralty Island (bottom) in south-east Alaska (inset). White lines in CRV indicate roads. White areas are indicative of permanent snow/ice (at high elevation). Sampling sites are labelled as in Table 4, and for clarity, some overlapping labels have been omitted where sampling sites are most dense.

does the pattern–process relationship hold true for two distinct landscapes within the same region?

3 Which method of calculating resistance distances (LCP vs. CT) performs best in our study system, 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 analysed and visualized using ABI Peak Scanner software (version 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 <0.75%.

Larvae samples can be problematic because of allele frequency bias from sampling siblings (Allendorf & Phelps 1981). Thus, we used a maximum likelihood approach in the program COLONY (Wang 2004) to identify full sibling clusters (Goldberg & Waits 2010). Samples were then randomly filtered to only include one individual from each full sibling family, to avoid biasing allele frequencies from sampling kin (Goldberg & 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 & Thompson (1992) and applied a Bonferroni correction for a table-wide significance level of 0.001. We calculated observed and expected heterozygosities, as well as the number of alleles per locus in GenAlEx version 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 version 6.3 (Wright 1931; Weir & Cockerham 1984; Peakall & Smouse 2006). We tested for significant deviations from zero based on 999 permutations and applied a Bonferroni correction for multiple tests (adjusted $\alpha = 0.001$). We also calculated pairwise Cavalli-Sforza and Edwards chord distance (D_c; Cavalli-Sforza & Edwards 1967) in Microsatellite Analyzer (MSA; Dieringer & Schlötterer 2002) for use in landscape analyses. D_c weighs mutation as insignificant compared with 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). 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 LD 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 2011). 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) vs. 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, nonvegetative 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, Supporting information for resistance values).

We also tested for the influence of the following five variables: (i) Insolation, which reflects the thermal properties of the landscape and is important for toad connectivity because of their high rates of evaporative water loss and temperature sensitivity (Bartelt *et al.* 2004; Bartelt & Peterson 2005); (ii) 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; (iii) Permanent snow or ice fields, as this affects breeding phenology (Corn 2003) and may be a thermal barrier to dispersal; (iv) Saltwater, which may be a physiological barrier (Taylor 1983) and (v) Roads (for CRV only), which are known to limit amphibian dispersal and movements (Carr & Fahrig 2001; Arens *et al.* 2007; Bull

Table 1 Model variables used to derive landscape resistance surfaces for boreal toad populations in south-east Alaska

Surface	Abbrev	Description	Spatial data source	Ecological rationale
Habitat structure	*str*	Structural complexity and permeability of the landscape	Derived from Terrestrial Ecosystems Classification (The Nature Conservancy), National Wetland Inventory (USGS), and data from Alaska Dept of Natural Resources, and US Forest Service	Different habitat types may impede or enhance dispersal because of high or low cover and/or moisture (Bartelt *et al.* 2004; Adams *et al.* 2005; Bartelt & Peterson 2005; Murphy *et al.* 2010a,b)
Insolation	*sol*	Amount of solar radiation received on a given surface area in a given time	Derived from SRTM digital elevation model and ESRI ArcGIS 9.3 Solar Analyst Tools	Hot areas can impede amphibian dispersal because of high rates of evaporative water loss (Bartelt *et al.* 2004; Bartelt & Peterson 2005)
Rugosity	*rug*	Ratio of the true surface area to the geometric surface area	Derived from SRTM digital elevation model	High cost of dispersing through more rugged areas, ridges may impede amphibian dispersal (Funk *et al.* 1999)
Permanent snow/ice	*ice*	Snow and ice that persists year round	Derived from Terrestrial Ecosystems Classification (The Nature Conservancy)	Snow cover affects breeding phenology and subsequent gene flow (Corn 2003); permanent ice pack limits dispersal
Saltwater	*salt*	Saltwater	Derived from bathymetry data (NOAA) and SRTM digital elevation model	Saltwater is a physiological impediment to dispersal (Taylor 1983)
Roads	*roads*	Roads	Data 3 from Alaska Dept of Natural Resources	High cost of crossing roads for amphibians because of increased mortality from vehicles and desiccation (Carr & Fahrig 2001; Arens *et al.* 2007; Bull 2009; Murphy *et al.* 2010a,b)

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

Model	Basis	Hypothesis	Land cover ranks (no. classifications)
Str1	Habitat type	Wetland is least resistant, conifer forest/unvegetated is most, other forest/scrubby habitat is semi-resistant	Coarse (5)
Str2	Habitat type	Wetland is least resistant, conifer forest/unvegetated is most, other forest/scrubby habitat is semi-resistant	Fine (8)
Str3	Moisture	Wet habitats are least resistant, dry habitats are most resistant	Coarse (3)
Str4	Cover	Little to medium cover is least resistant, full cover is most resistant	Fine (6)
Str5	Cover	Medium cover is lowest resistance, no cover is highest resistance, high cover is medium resistance	Fine (7)

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, Supporting information). 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 (IBD) 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. Because of computational limits of the Circuitscape program and the extreme variation of south-eastern Alaska landscapes, we limited the extent of our analysis to within 50 km 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 more 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 IBD whereby genetic differentiation increases with geographic distance. IBD is expected under mutation-migration-drift equilibrium and requires a stepping-stone migration model (Rousset 1997). 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 data set to 426 individuals for all further analyses (mean = 20 full siblings per site, range = 9–40). No locus or population showed consistent deviations from HWE, or consistent LD 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 to 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 to 0.21 and 0.00 to 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 because of small sample sizes for some of the populations.

Table 3 Summary of genetic measures for boreal toad populations from ANM and the CRV in south-east Alaska

	ANM (n = 10)			CRV (n = 11)		
	Mean	Min	Max	Mean	Min	Max
Allelic Richness	3.44	2.36	3.78	3.30	2.68	3.73
Pairwise F_{ST}	0.060	0.004	0.21	0.068	0.00	0.21
Pairwise D_c	0.26	0.16	0.39	0.23	0.11	0.38
N_b (ONeSAMP)	26	9	56	40	13	91
N_b (LDNe)	46	1	214	30	2	130
Euclidean distance (km)	12.17	0.033	40.99	11.4	0.11	50.04

ANM, Admiralty Island; CRV, Chilkat River Valley; F_{ST}, genetic differentiation; D_c, 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 south-east Alaska

(A)

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

(B)

	H1	H2	H3	H5	H6	H7	H8	H9	H10	H52	H0106
H1	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
H2	0.089	–	0.002	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001
H3	0.069	0.042	–	0.255	0.001	0.171	0.034	0.417	0.005	0.001	0.001
H5	0.094	0.082	0.003	–	0.001	0.059	0.092	0.412	0.008	0.001	0.002
H6	0.104	0.090	0.034	0.046	–	0.017	0.004	0.001	0.001	0.001	0.001
H7	0.085	0.087	0.009	0.022	0.038	–	0.183	0.124	0.008	0.001	0.001
H8	0.061	0.054	0.011	0.009	0.032	0.010	–	0.022	0.001	0.001	0.001
H9	0.094	0.083	0.000	0.000	0.040	0.013	0.011	–	0.002	0.001	0.001
H10	0.105	0.087	0.015	0.020	0.081	0.036	0.039	0.016	–	0.001	0.001
H52	0.210	0.167	0.108	0.105	0.123	0.097	0.097	0.105	0.105	–	0.001
H0106	0.128	0.129	0.064	0.042	0.125	0.082	0.063	0.059	0.075	0.147	–

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 $r = 0.44$, $P = 0.13$). 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 saltwater explained 72% of the variation in the data set ($P = 0.001$). 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 nonoverlapping 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 south-east 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 & Storfer 2010; Short Bull *et al.* 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

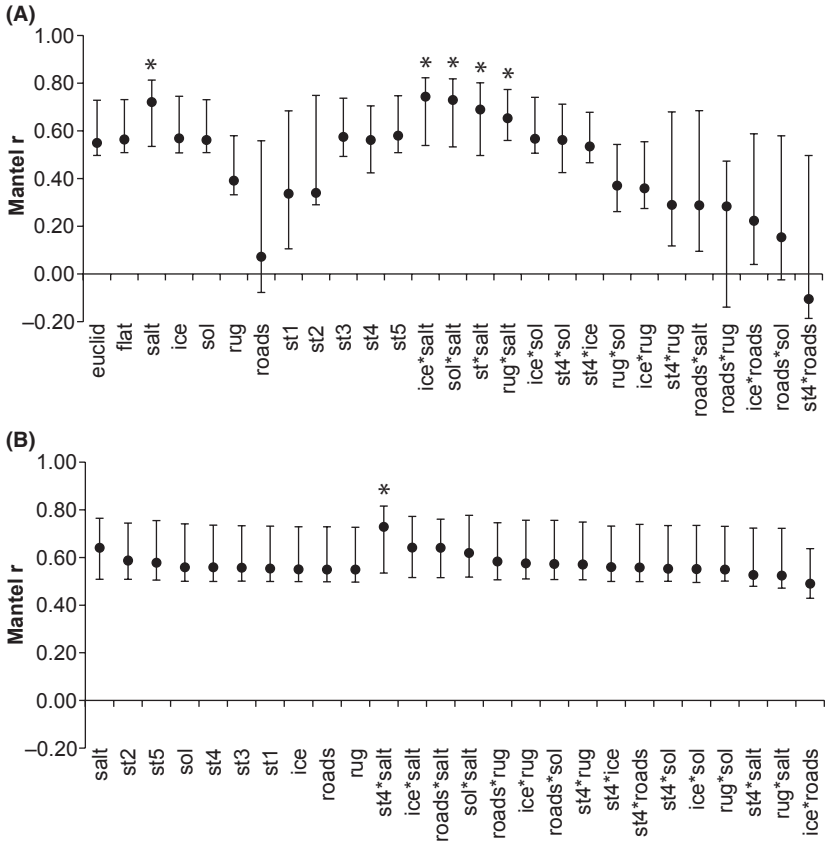

Fig. 2 Plot of Mantel *r*-values for landscape models and landscape-free models ('flat' and 'euclid') for boreal toad populations in the Chilkat River Valley (CRV) site in south-east Alaska based on (A) circuit theory and (B) least-cost path methods (st, structure; sol, insolation; salt, saltwater; rug, rugosity; ice, permanent snow/ice). Bars represent 95% confidence intervals. Asterisks indicate significant models based on a Bonferroni-corrected alpha.

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 probably 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 because of 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 *et al.* 2010a). 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 the CRV landscape may encompass more extreme variation that is driving our ability to detect the effect of these 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 that are interconnected by numerous freshwater lakes (Fig. 1). Chan & Zamudio (2009) showed that genetic differentiation is lower in amphibian species in habitats that are more homogeneous vs. those that are more variable (e.g. arid-adapted desert toads vs. pond-breeding temperate amphibians). Our results suggest that this pattern holds true for

intraspecific comparisons of populations from different landscapes as well, particularly when the predominant landscape feature promotes connectivity.

The lack of a good fitting IBD or landscape resistance model for ANM populations could be due to (i) populations not having reached migration-drift equilibrium (MDE) or (ii) 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 south-east Alaska, deglaciation provides new breeding habitats that could easily be colonized by toads. However, once colonized, populations are likely to reach MDE rather quickly because of their small effective population sizes (Allendorf & Phelps 1981). Although boreal toads are at the edge of their geographic range in south-east 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 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 often 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 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 south-east 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. If possible, landscape analyses should be replicated across a range of landscapes to capture any differences in variance of landscape features (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 south-east 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 south-east 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). South-east Alaska is a large coastal temperate rainforest that averages 3–4 m 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 south-east 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 south-east Alaska where timber harvesting is more common (S. Pyare, personal observation).

Most studies of amphibian connectivity base hypotheses on what is known about dispersal and movement of adults, as there is very little information available on the ecology of earlier life stages. 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 postmetamorphic 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, 2006).

In our study system, CT methods were more strongly supported than least-cost methods for calculating resistance distances (McRae & Beier 2007). Previous studies comparing these two methods have found conflicting results. McRae & Beier (2007) found stronger support for CT than LCP methods for population-based studies of mahogany and wolverines across their broad geographic ranges. On the other hand, Schwartz *et al.* (2009) found stronger support for LCP methods based on 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 should be the preferred method 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 methods may be preferable to CT methods where dispersal occurs primarily in narrow waterways (e.g. streams; Schmetterling & Young 2008). Further comparison of these, and other emerging methods (e.g. gravity models, Murphy *et al.* 2010b), would be useful for providing guidelines for use in future landscape genetics studies depending on animal life history and ecology (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 south-east 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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J.M. is currently a research associate at Michigan State University and is broadly interested in molecular and spatial ecology and conservation of vertebrates. S.P. and J.N. are interested in landscape and spatial ecology, and GIS, and D.T. is interested in molecular ecology, evolution and conservation.

Data accessibility

Microsatellite data deposited in the Dryad repository: doi:10.5061/dryad.k7v4811k.

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.

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