

Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*)

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Abstract

The field of landscape genetics has great potential to identify habitat features that influence population genetic structure. To identify landscape correlates of genetic differentiation in a quantitative fashion, we developed a novel approach using geographical information systems analysis. We present data on blotched tiger salamanders (*Ambystoma tigrinum melanostictum*) from 10 sites across the northern range of Yellowstone National Park in Montana and Wyoming, USA. We used eight microsatellite loci to analyse population genetic structure. We tested whether landscape variables, including topographical distance, elevation, wetland likelihood, cover type and number of river and stream crossings, were correlated with genetic subdivision (F_{ST}). We then compared five hypothetical dispersal routes with a straight-line distance model using two approaches: (i) partial Mantel tests using Akaike's information criterion scores to evaluate model robustness and (ii) the BIOENV procedure, which uses a Spearman rank correlation to determine the combination of environmental variables that best fits the genetic data. Overall, gene flow appears highly restricted among sites, with a global F_{ST} of 0.24. While there is a significant isolation-by-distance pattern, incorporating landscape variables substantially improved the fit of the model (from an r^2 of 0.3 to 0.8) explaining genetic differentiation. It appears that gene flow follows a straight-line topographic route, with river crossings and open shrub habitat correlated with lower F_{ST} and thus, decreased differentiation, while distance and elevation difference appear to increase differentiation. This study demonstrates a general approach that can be used to determine the influence of landscape variables on population genetic structure.

Keywords: *Ambystoma tigrinum*, gene flow, GIS, landscape genetics, microsatellites, tiger salamander

Received 9 December 2004; revision received 15 February 2005; accepted 8 March 2005

Introduction

A central goal of evolutionary ecology is to understand processes that determine the geographical distribution of genetic diversity. Population geneticists have long recognized the importance of species-specific life history and dispersal characteristics in determining population genetic structure (Wright 1931, 1943, 1946). Although dispersal and gene flow are not synonymous, as dispersers may not successfully breed in new sites, dispersal and gene flow are generally correlated (Bohonak 1999). Because dispersal is largely determined by landscape and habitat

features, detailed consideration of landscape variables is critical to understanding the process of population differentiation (Keyghobadi *et al.* 1999; Arnaud 2003; Bockelmann *et al.* 2003; Manel *et al.* 2003; Geffen *et al.* 2004). Until recently, technological limitations restricted population genetic studies to using either simple linear distances among populations (Storfer 1999; Matocq *et al.* 2000) or measures of 'habitat distance' (King 1987; Arter 1990; Keyghobadi *et al.* 1999; Arnaud 2003) as correlates/determinants of genetic distance. However, geographical information systems (GIS) provide the framework in which high-resolution analyses of habitat variables can be performed. GIS-based analyses should add to the emerging field of landscape genetics, which combines landscape ecology with population genetics to examine the extent to which landscape

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features influence genetic structure via dispersal (Manel *et al.* 2003).

Despite this potential, GIS has not been extensively used in landscape genetic studies. Two exceptions are studies by Michels *et al.* (2001), who used GIS to model different dispersal scenarios for zooplankton based on environmental and landscape variables, and Coulon *et al.* (2004), who calculated dispersal scenarios for roe deer based on extent of forested patches. Both of these studies calculated an 'effective geographical distance' for each scenario. Dispersal scenarios were created using a 'least-cost' modelling approach, which calculates a modified distance between two areas based on detailed landscape information. Michels *et al.* (2001) found that least-cost distances based on dispersal rates of the zooplankton correlated much better with genetic distance than linear distance. Coulon *et al.* (2004) had similar results, finding that the least-cost distance based on movement through forested patches explained more of the variation in genetic differences between individuals than straight-line Euclidean distance. Both of these studies empirically demonstrate the value of GIS-based investigations of genetic population structure.

Herein, we used microsatellite markers to quantify the effect of multiple landscape characteristics, based on GIS data, on the genetic structure of an amphibian. Microsatellites are appropriate for a landscape genetic approach because they have a high mutation rate and can detect recent genetic differentiation (Jarne & Lagoda 1996). Amphibians are an excellent group for a landscape genetic analysis because of their life history characteristics. First, most amphibians have a biphasic life history, with an aquatic larval phase and a terrestrial adult phase (Duellman & Trueb 1994). Second, amphibians generally have limited vagility, and their terrestrial habitat use and movement patterns are poorly understood, due primarily to low probability of detection on land (MacKenzie *et al.* 2002; Semlitsch 2003). Thus, multiple landscape features related to both aquatic and terrestrial environments are likely important for explaining population genetic structure in amphibians, and a landscape genetic analysis should yield insight into the relative influence of specific habitat features on genetic structure. In addition, because amphibians are in global decline (Blaustein & Kiesecker 2002; Collins & Storfer 2003), information concerning landscape influence on amphibian genetic structure will assist in developing conservation and management plans (Semlitsch 2002). For example, the effect of anthropogenically created barriers to dispersal can be modelled quantitatively, allowing researchers to distinguish the relative influence of proposed management actions on population genetic structure (Costello *et al.* 2003). This approach could be used to model possible future habitat changes such as climate change to predict which populations are vulnerable to inbreeding and extinction processes (Keyghobadi *et al.* 1999).

There are several types of landscape features that should influence genetic population structure of amphibians. These include presence of wetlands, variation in slope, presence of fish-bearing water bodies, and cover type. Amphibians generally require wet areas to survive and reproduce (duellman & Trueb 1994), and therefore the presence of wetlands should be important for maintaining gene flow. Differences in slope among sites may explain variation in gene flow, as high levels of amphibian population subdivision exist among populations separated by mountains compared to low subdivision within basins in these populations (Tallmon *et al.* 2000; Funk *et al.* 2005), presumably due to reduced mobility as slope increases. In fact, the effect of slope and topography has been suggested as a major predictor of amphibian gene flow (Funk *et al.* 2005). Water bodies containing fish are also likely to be barriers to amphibian dispersal. It is well documented that predatory fish limit the distribution and abundance of amphibians (see reviews by Kats & Ferrer 2003; Dunham *et al.* 2004) and therefore sites with predatory fish may limit gene flow across the landscape (Storfer 1999). Finally, cover type may have a strong influence on landscape connectivity for amphibians. Several recent studies have indicated that amphibians will avoid open habitats, such as fields and road areas, and move more through forested areas (Madison & Farrand 1998; deMaynadier & Hunter 1999; Rothermel & Semlitsch 2002). These results are likely due to increased risks of desiccation and predation in open areas. There is some evidence that these trends in movement also translate to reduced gene flow in open areas (Hitchings & Beebee 1997; Gibbs 1998; Curtis & Taylor 2003).

In this study, we examine population genetic structure of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*) across the northern range of Yellowstone National Park using eight microsatellite loci. This study had two main objectives. The first was to estimate the amount of genetic differentiation among populations in breeding ponds. In general, we expected overall high subdivision due to the mountainous terrain and rivers with predatory trout that exist throughout Yellowstone National Park. Second, we wished to test the extent to which landscape variables, such as wetland presence, slope, rivers and cover type, would better explain tiger salamander population differentiation than a standard isolation-by-distance model. The combination of spatial analyses with modern molecular genetic techniques should provide unique insight into the landscape genetics of our study system, and our approach can be applied to a variety of organismal systems.

Materials and methods

Sampling and microsatellites

We surveyed 10 ponds across the northern range of Yellowstone National Park for tiger salamanders during

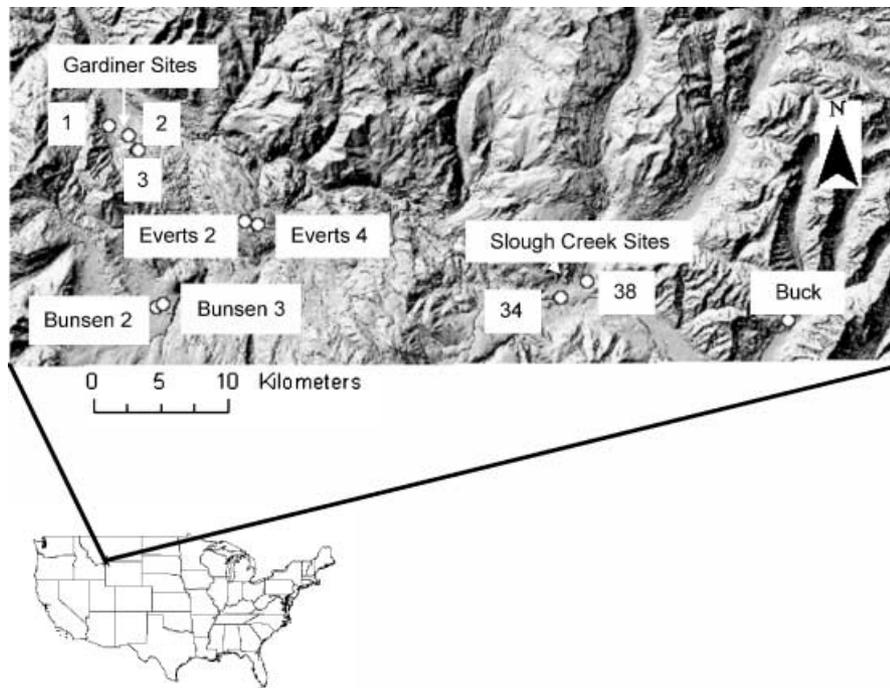


Fig. 1 Map of study area (northern range of Yellowstone National Park) with salamander sampling sites represented by white circles. Names of each site are in the white box nearest to each circle. The background is a shaded relief map. The inset is a map of the United States, with lines pointing to general location of study area.

May–July 2002 and 2003 (Fig. 1). The northern range of Yellowstone National Park is characterized by mountainous terrain and mostly open shrub habitat, with some sections of forested terrain. Three major rivers (the Yellowstone River, Gardner River and Lamar River) and many smaller tributaries run through the area. The majority of Yellowstone was completely covered by glaciers until 13 000–14 000 years BP (Pierce 1979). Currently, the study area is dry overall, with average annual precipitation of approximately 39 cm. In addition, the region is experiencing a prolonged drought, with continuous below average rainfall since 1998, and drought patches throughout the past century (Western Regional Climate Center data).

We captured salamanders using aquatic funnel traps and dipnetting (Adams *et al.* 1997), and recorded the life stage and snout–vent length of each individual. We clipped toes for tissue samples in 2002, but switched to tail clips (1 cm) in 2003 because they yielded more DNA. We used a standard phenol–chloroform extraction to isolate DNA (Sambrook *et al.* 1989). We amplified nine microsatellite loci developed by Mech *et al.* (2003) (Table 1). Polymerase chain reaction (PCR) conditions followed Mech *et al.* (2003) on a Bio-Rad programmable thermocycler, and we ran PCR products, with positive and negative controls, on agarose gels to verify amplification. Heavy-strand PCR primers were fluorescently labelled with either TET (1:10 dilution), FAM (1:20 dilution), or HEX (1:5 dilution) to allow for

multiplexing and run on an ABI 377 (Applied Biosystems Inc.) automated sequencer with TAMRA size standard. Each gel was analysed using GENESCAN version 3.1 (Applied Biosystems, Inc.). We scored each locus for each sample using GENOTYPER version 2.1 (Applied Biosystems, Inc.). One locus (*ATS 4-25*) was monomorphic, therefore analyses presented here include only eight loci.

Genetic data analysis

We used MICROSATELLITE ANALYSER version 3.12 (Dieringer & Schlötterer 2003) to calculate number of alleles at each locus per site and expected heterozygosities. To test for linkage disequilibrium (LD) and departures from Hardy–Weinberg equilibrium (HWE) among the loci and samples, we used GENEPOP version 3.4 (Raymond & Rousset 1995). ARLEQUIN version 2.0.1.1 (Schneider *et al.* 2000) was used to estimate population subdivision using a modification of F_{ST} proposed by Weir & Cockerham (1984) to correct for finite population sizes. The Mantel test (Mantel 1967) in ARLEQUIN was used to test for a significant isolation-by-distance correlation. Finally, we used FSTAT version 2.9.3 (Goudet 2001) to perform exact tests for pairwise site differentiation based on multilocus genotypes of individuals in each site pair.

To analyse the influence of landscape variables on among-site gene flow (measured by F_{ST}), we quantified the extent

Table 1 Expected heterozygosity and number of alleles for populations of *Ambystoma tigrinum melanostictum* at 10 sites sampled across the northern range of Yellowstone National Park. Sample sizes are in parentheses beneath site names (Fig. 1; SC signifies Slough Creek). Individual locus names are as in Mech *et al.* (2003)

Locus		Site									
		Bunsen 2	Bunsen 3	Buck	Everts 2	Everts 4	SC 34	SC 38	Gardiner 1	Gardiner 2	Gardiner 3
		(20)	(20)	(16)	(18)	(17)	(18)	(23)	(20)	(31)	(16)
ATS 10-7	H_E	0.516	0.358	0	0.056	0	0.458	0.371	0.05	0	0.063
	Alleles	2	2	1	2	1	2	2	2	1	2
ATS 12-3	H_E	0	0	0	0.382	0.219	0.206	0	0.5	0.631	0.654
	Alleles	1	1	1	3	3	2	1	2	3	3
ATS 14-3	H_E	0.497	0.489	0.279	0.412	0.61	0.435	0.489	0.142	0.204	0.4
	Alleles	2	2	2	3	3	2	3	2	2	2
ATS 4-11	H_E	0.587	0.47	0.4	0.368	0.271	0.438	0.464	0.533	0.125	0
	Alleles	3	3	3	3	2	2	2	3	3	1
ATS 4-20	H_E	0.142	0.224	0	0.056	0.059	0.382	0.045	0	0	0.125
	Alleles	2	2	1	2	2	2	2	1	1	2
ATS 5-6	H_E	0.224	0.05	0.225	0.213	0.114	0.353	0.437	0.516	0.152	0.513
	Alleles	2	2	2	2	2	2	3	2	2	2
ATS 5-7	H_E	0.508	0.421	0.613	0.504	0.309	0.575	0.62	0.662	0.481	0.654
	Alleles	2	2	3	3	3	3	5	4	3	4
ATS 5-8	H_E	0.511	0.513	0.181	0.516	0.525	0	0.543	0.463	0.508	0.365
	Alleles	2	2	4	2	2	1	3	3	2	3
Mean	H_E	0.373	0.316	0.212	0.313	0.263	0.356	0.371	0.358	0.263	0.347
	Alleles	2	2	2.125	2.5	2.25	2	2.625	2.375	2.125	2.375

to which six possible movement path models explained genetic substructuring among populations (Fig. 2). The first route was a straight-line distance path between sites as a null model, because straight-line distance is the traditional model for analyses of genetic population structure (Slatkin 1987). We did not incorporate any landscape variables into the null model, consistent with an isolation-by-distance model. The second route was a modification of the first in which we calculated topographically corrected straight-line distance among all site pairs using a digital elevation model in GIS. Funk *et al.* (2005) showed that gene flow was highly restricted over areas of topographic relief in Columbia spotted frogs, *Rana luteiventris*, from Montana. For the third route, we chose a 'stepping-stone' topographic route using salamander localities identified through surveys by the Idaho State University Herpetology Laboratory (Peterson & Patla, unpublished). In this model, salamanders disperse via a pathway moving through proximate wetlands that have historical records of salamander occurrence. A stepping-stone pattern often results from postglacial colonization of an area (McCauley 1993), and therefore might be expected in Yellowstone. We used a strict stepping-stone model (Kimura & Weiss 1964) whereby individuals had to move to the nearest wet site before advancing through the landscape, with the eventual destination being one of the 10 sites sampled herein. To create both straight-line and stepping-stone paths, we digitized each route using ARCGIS 8.2 (ESRI).

The final three routes (separate from the straight-line and stepping-stone models) used the least-cost approach to model movement (Michels *et al.* 2001; Adriaensen *et al.* 2003; Coulon *et al.* 2004) to test some of our hypotheses. Using this approach, we tested the extent to which salamander movement was influenced directly by slope, wetland presence, and a combination of these two factors. We did not develop least-cost paths based on cover type and rivers because we had no data that would allow us to quantify the specific numerical cost of moving through different cover types or across rivers. Slope was calculated for each pixel (100 m²) based on a digital elevation model. We used a wetland likelihood GIS layer created for Yellowstone National Park (Wright 2004) that assigned a percentage likelihood of wetland presence to each 900 m² pixel. We created least-cost paths using ARCINFO by developing cost-distance grids for each sampling site. Cost values to each pixel surrounding the sampling site of interest were assigned based on slope and the reverse of wetland likelihood. The higher the value, the greater the cost it is to move through that pixel. We then used the cost-distance surface for each variable to generate least-cost paths between all sites.

For each of the routes (excluding the null model), we estimated percent variation in F_{ST} explained by (i) mean wetland likelihood, (ii) percent of each cover type along the route, (iii) elevation, and (iv) number of streams and rivers crossed by each route in addition to topographical distance.

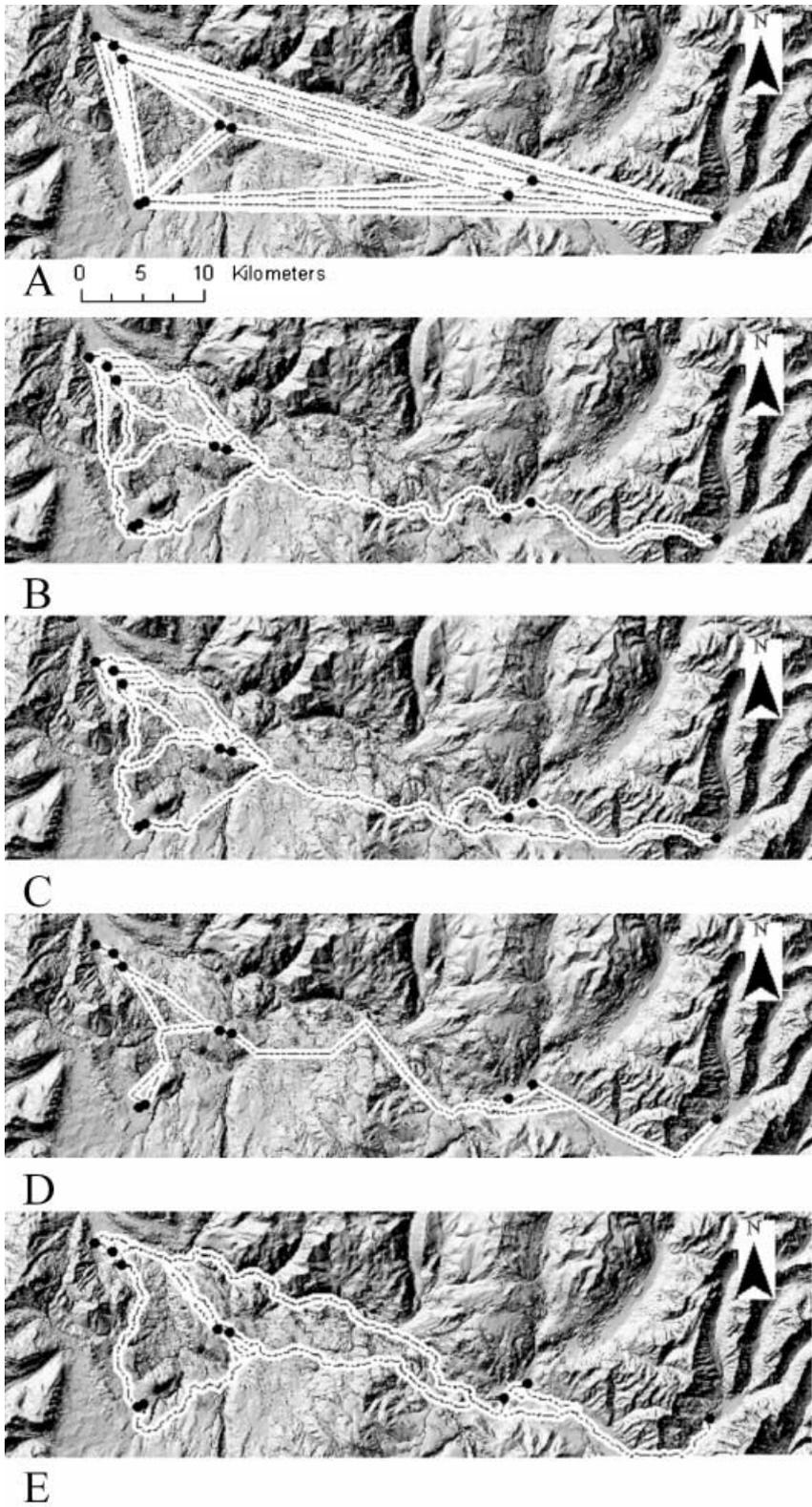


Fig. 2 Maps representing model routes for salamander gene flow across the northern range. Background is a shaded relief map of the study area. (A) straight-line route; (B) wetland likelihood route; (C) combination least slope/wetland likelihood; (D) stepping-stone route; (E) least slope route.

However, we did not use wetland likelihood as an independent variable for analyses with the least-cost routes because this variable was already incorporated in the least-cost paths. Otherwise, all other landscape variables were initially included in each model.

Mean wetland likelihood was calculated by first multiplying each individual likelihood value by percent of the overall route that passed through pixels with that value, and then adding individual calculations together to produce a weighted average. Cover type was divided into 10 discrete cover types (Bartelt, unpublished), including closed forest, open forest, open shrub/fire regeneration, sagebrush, dry meadow, moist meadow, wet meadow/bog, talus, mudflow, and water. We calculated percentage of the route that comprised each cover type. To incorporate elevation, we used the elevational difference between the two sites. For stream and river crossings, the number of times the route crossed a stream or river was calculated, based on a stream shape file developed by the Yellowstone Spatial Analysis Center.

We used partial Mantel tests in *FSTAT* with 10 000 randomizations to test the percentage of variation in F_{ST} explained by each landscape variable. This test is a modification of multiple regression and accounts for nonindependence of points by creating a random distribution to use as a null distribution and test for significance (Mantel 1967; Smouse *et al.* 1986). The partial Mantel test calculates partial correlations for each variable by controlling for any variables already entered into the model. Furthermore, to select the 'best approximating model' (Burnham & Anderson 1998) based on the Mantel results, we used Akaike's information criterion (AIC). This method, originally described by Akaike (1973), uses the Kullback–Liebler (K–L) distance to select the best model. This distance represents how far a given model is from the true model (Kullback & Leibler 1951). It is not necessary to know the true model because it mathematically cancels out in the calculation of the K–L distance. The lower the K–L distance (and AIC value), the closer a candidate model is to the true model. In general, models that have AIC values greater than 10 compared to the model with the lowest AIC score are not supported (Burnham & Anderson 1998). Because we had relatively low sample size of pairwise comparisons, we adjusted AIC values to AIC_c (Hurvich & Tsai 1989). We first used AIC to narrow down all possible models to five candidate models (the best model for each of the hypothetical routes). Therefore, landscape variables that were not included in the candidate models were excluded from further analysis. We then compared each candidate model, based on the AIC scores, and determined both which route and which variables were most strongly correlated with the genetic data.

There has been recent controversy regarding the validity of the partial Mantel test (Raufaste & Rousset

2001; Castellano & Balletto 2002; Rousset 2002). The major question is whether the test produces biased P values due to the introduction of 'nuisance parameters' that are not controlled for in the partial Mantel test (Raufaste & Rousset 2001). Therefore, we used a second approach, the BIOENV procedure described by Clarke & Ainsworth (1993), to compare with the partial Mantel results. We ran BIOENV analyses using the program *PRIMER* version 5.2.4 (Clarke & Gorley 2001) to evaluate the relationship between the genetic distance matrix based on F_{ST} and the landscape variables described above. The procedure calculates a weighted Spearman rank correlation coefficient (ρ_w) between the genetic distance matrix and all possible sets of variables. The combination of variables that has the highest value of ρ_w is considered to provide the best support for the genetic data. The drawback of this analysis is that it is best thought of as an exploratory analysis (Clarke & Ainsworth 1993) because it does not currently allow for significance testing, due to nonindependent calculations in the procedure. In addition, because the test does not calculate partial correlation coefficients, it is not possible to conclude whether a variable has a positive or negative relationship with the genetic data, although a separate scatterplot could give an indication of the direction. Nonetheless, the addition of this test allows an independent analysis of consistency of data with the partial Mantel test.

Results

Genetic structure among sites

Overall, genetic diversity was consistently low across all sites, with an average heterozygosity of 0.32 and 2.2 alleles per locus at each site (Table 1). None of the eight loci showed evidence of significant linkage disequilibrium, after Bonferroni correction (Sokal & Rohlf 1995). Furthermore, all loci and sites did not show significant departures from Hardy–Weinberg proportions, with the exception of Gardiner 3, which was heterozygote deficient at locus ATS 5–7 ($P = 0.001$).

There was high genetic differentiation among sites across the study area ($F_{ST} = 0.24$; Table 2). There were only two population pairs that did not show significant pairwise differentiation based on the exact test: the two Bunsen sites and the two Everts sites (Table 2). These were the only two pairs of sites less than a kilometre apart. Thus, based on this test, gene flow apparently diminishes at distances greater than 1 km. This conclusion is supported by the F_{ST} values (Table 2). For example, the Gardiner sites had F_{ST} values of 0.15 and 0.17 for distances between 1 and 2 kilometres. There was a significant isolation-by-distance pattern across the study area, as evidenced by a Mantel test ($P = 0.001$).

Table 2 Pairwise F_{ST} (below the diagonal) and probability that the genotypes are identical between sampling sites when all loci are combined (above the diagonal) for tiger salamander populations across northern Yellowstone. Significant values are indicated by bold type

Pop	Bunsen 2	Bunsen 3	Buck	Everts 2	Everts 4	SC 34	SC 38	Gardiner 1	Gardiner 2	Gardiner 3
Bunsen 2	—	0.057	0.001							
Bunsen 3	0.026	—	0.001							
Buck	0.278	0.310	—	0.001						
Everts 2	0.106	0.040	0.287	—	0.609	0.001	0.001	0.001	0.001	0.001
Everts 4	0.105	0.011	0.359	0.010	—	0.001	0.001	0.001	0.001	0.001
SC 34	0.102	0.170	0.269	0.227	0.250	—	0.001	0.001	0.001	0.001
SC 38	0.061	0.089	0.221	0.141	0.124	0.114	—	0.001	0.001	0.001
Gardiner 1	0.254	0.282	0.447	0.256	0.268	0.369	0.194	—	0.001	0.001
Gardiner 2	0.313	0.319	0.479	0.298	0.313	0.382	0.245	0.176	—	0.001
Gardiner 3	0.250	0.266	0.453	0.204	0.252	0.359	0.218	0.093	0.152	—

Route	Variable	r	Overall model r^2	Distance AIC_c	Model AIC_c
Null straight line	distance***	0.581	0.337	-84.82	-84.82
Topo straight line	distance***	0.587	0.828	-85.05	-103.53
	elevation**	0.422			
	open shrub**	-0.397			
	ridges**	-0.384			
Topo stepping stone	distance***	0.495	0.618	-82.27	-90.63
	dry meadow***	-0.517			
	wetland likelihood*	-0.325			
Topo least slope path	distance***	0.527	0.422	-83.02	-84.97
	dry meadow**	0.381			
Topo wetland likelihood path	distance***	0.542	0.675	-83.59	-93.8
	ridges***	-0.558			
	open shrub*	-0.265			
Topo least slope + wetland	Distance***	0.530	0.632	-83.08	-91.37
	Rivers***	-0.546			
	open shrub*	-0.231			

Table 3 Partial Mantel test results for the best model (based on AIC) for each route simulating tiger salamander movement across the northern range of Yellowstone. The statistic r represents the partial correlation coefficient for each variable and the model r^2 is the total variance explained by the model. Topo indicates that routes were adjusted to represent true topographical distance. Asterisks beside each variable represent the significance level (* indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$). AIC_c values represent a Mantel test comparing F_{ST} with only distance (distance AIC_c) and a partial Mantel incorporating all model variables (model AIC_c)

Landscape effects: partial Mantel test

Overall, topographic distance was the most consistent predictor variable of F_{ST} and the only variable included in every candidate model (Table 3). However, based on AIC_c values, in four out of five models, distance explained much less of the variation in F_{ST} than the total model that included landscape variables, with the exception of the least slope model. In addition, although we primarily evaluated model variables based on AIC_c score, all variables included in the models for each route did have statistically significant P values after Bonferroni correction (Table 3).

The null (line-of-sight) model (Fig. 2A) without landscape variables explained the least amount of variation among all the models ($r^2 = 0.337$; $AIC_c = -84.82$; Table 3). For the straight-line topographic model (Fig. 2A), topographical distance, elevational difference, open shrub habitat, and number of river and stream crossings explained

a significant proportion of the variation, with distance explaining the most variation (Table 3). While topographic distance alone was not a better model than the null model, the full model had the highest r^2 (0.828), and lowest AIC_c (-103.53) score of any model (Table 3). Distance and elevation were positively correlated with F_{ST} , whereas open shrub habitats and river crossings were negatively correlated with F_{ST} and thus positively correlated with reduced genetic differentiation.

None of the remaining candidate models had significant support as compared to the straight-line model. The least-cost path based on wetland likelihood (Fig. 2B) generated the model with the second highest level of support, based on both r^2 (0.675) and AIC_c (-93.8) score. River crossings and open shrub were negatively correlated with F_{ST} , while distance was positively correlated with F_{ST} (Table 3). The candidate model based on the least cost path of both slope and wetland likelihood (Fig. 2C) explained slightly less of the variance ($r^2 = 0.632$; $AIC_c = -91.37$; Table 3) in F_{ST} than

that of the wetland likelihood model. It contained the same variables as the model based on wetland likelihood. The model based on a stepping-stone route (Fig. 2D) explained about 60% of the overall variance ($r^2 = 0.609$; $AIC = -90.21$) in F_{ST} . Included in the stepping-stone model were three significant variables: topographical distance, presence of dry meadow cover type, and wetland likelihood (Table 3). Distance explained the highest proportion of variation in this model, and dry meadow and wetland likelihood were both negatively correlated with F_{ST} . Finally, the least-cost path based on slope (Fig. 2E) had the least support of any candidate model that included landscape variables (Table 3). This model also had distance as the most significant explanatory variable of population subdivision ($r = 0.527$), with dry meadows also being positively correlated with F_{ST} (Table 3).

Landscape effects: BIOENV test

Similar to the partial Mantel results, the null model of straight-line distance without landscape variables had the least support of all models evaluated ($\rho_w = 0.178$; Table 4). The straight-line route with distance, elevation difference and open shrub/fire regeneration had the highest ρ_w of any model (0.327; Table 4). This is the same result as the partial Mantel, the only difference being that rivers were excluded. The route with the second-highest support was the least slope model, which included distance, closed forest, open forest, and wet meadow ($\rho_w = 0.302$). However, the data for wet meadow includes one large outlier. If this outlier is removed, wet meadow is no longer included in the model, and the ρ_w is reduced to 0.278 (Table 4). The final three route models, stepping-stone ($\rho_w = 0.208$), wetland likelihood ($\rho_w = 0.223$), and the combined slope/

wetland likelihood model ($\rho_w = 0.213$), all had similar support and each included the same two variables: distance and elevation difference (Table 4).

Discussion

Genetic structure

Overall, it appears that tiger salamanders across the northern range of Yellowstone are characterized by low genetic diversity and high genetic differentiation among sites. Low genetic diversity is often characteristic of founder effects due to postglacial colonization (McCauley 1993), and this is a likely explanation because Yellowstone was covered by glaciers throughout the Pleistocene. The high degree of population differentiation is consistent with an amphibian species that has low vagility and a specific breeding centre (Shaffer *et al.* 2000). In this study, strong genetic differentiation may occur at a distance as small as 1 km, although additional site comparisons less than a kilometre apart are needed to further resolve the threshold gene flow distance.

The level of genetic differentiation (based on F_{ST}) found in this study (0.24) is about equal or higher than that typically found in studies of salamanders that use both aquatic and terrestrial habitats (Routman 1993; Storfer 1999; Tallmon *et al.* 2000; Curtis & Taylor 2003). Storfer (1999), using nine allozymes, found high subdivision in the streamside salamander (*Ambystoma barbouri*) at greater than 5 km ($F_{ST} = 0.270$), but less so among sites within 5 km of each other ($F_{ST} = 0.162$). In the long-toed salamander (*Ambystoma macrodactylum*), differentiation was low within mountain basins ($F_{ST} = 0.026$), but was much higher among mountain basins a few kilometres apart ($F_{ST} = 0.124$), based on 18 allozyme markers (Tallmon *et al.* 2000). The lowest degree of population structure was within populations of the Pacific giant salamander, *Dicamptodon tenebrosus*, which had F_{ST} values of 0.084 for three microsatellite loci and 0.117 for 38 AFLP markers (Curtis & Taylor 2003). Finally, the only study that reported much higher F_{ST} values than our results was Routman's (1993) investigation of *Ambystoma tigrinum mavortium* populations within 36 km of each other in western Nebraska (allozyme $F_{ST} = 0.372$, mitochondrial $F_{ST} = 0.443$). While it is difficult to directly compare the differentiation measures in these studies due to use of different molecular markers, it appears that salamanders generally have restricted gene flow, and our genetic results are consistent with previous findings.

Landscape variables as correlates to gene flow

One limitation to using an indirect estimate of gene flow such as F_{ST} is that it is based on a number of unrealistic assumptions, such as equilibrium, and therefore, these estimates are unreliable as exact estimations of gene flow

Table 4 BIOENV results for the best model for each route. The statistic ρ_w represents the weighted Spearman rank correlation. Topo indicates that routes were adjusted to represent true topographical distance

Route	Variable	ρ_w
Null straight line	distance	0.178
Topo straight line	distance	0.327
	elevation	
	open shrub	
Topo stepping stone	distance	0.208
	elevation	
Topo least slope path	distance	0.278
	closed forest	
	open forest	
Topo wetland likelihood path	distance	0.223
	elevation	
Topo least slope + wetland	distance	0.213
	elevation	

or dispersal (Bossart & Prowell 1998; Whitlock & McCauley 1999). However, a measure such as F_{ST} is effective for estimating genetic differentiation (Whitlock & McCauley 1999), and therefore our results are best described as the effect of landscape on differentiation. However, because we chose focal landscape variables that are expected to impact amphibian genetic structure due to their effect on movement, we cautiously interpret our results as the effect of the landscape on dispersal, which would ultimately affect differentiation through the process of gene flow.

Our landscape genetic analysis identified landscape variables in addition to distance that significantly influenced genetic differentiation. As compared to the null model of straight-line distance, all alternative models that incorporated landscape variables explained a higher proportion of variation in F_{ST} . While distance appears an important factor limiting gene flow in this landscape, distance alone explained only 30–40% of the variation in F_{ST} . Thus, our knowledge of the system has been greatly enhanced with the addition of other factors, such as rivers, elevation and cover type. It is interesting that topographical distance had the same correlation with genetic distance as did the simpler null model. This is probably due to the fact that the entire study area is mountainous, and therefore topographical relief is likely similar among most of the site pairs. Nevertheless, we recommend the use of true topographical distances in GIS-based landscape genetics studies, because topographic relief can significantly influence gene flow (Funk *et al.* 2005). Finally, this study demonstrates the importance of considering various routes for modelling gene flow, as routes varied in their correlation with genetic distance. While the straight-line was the best path for this system, this is not usually the case for studies that have used multiple routes (Michels *et al.* 2001; Roach *et al.* 2001; Arnaud 2003; Coulon *et al.* 2004).

In fact, the strong overall performance of the straight-line path model was unexpected. Based on the presence of the pineal gland that attracts amphibians to water sources (Adler 1976) and relatively recent historical colonization of the area, a stepping-stone model might be expected. Similarly, wetland likelihood should affect amphibian gene flow, and it has also been shown that ridgelines can limit gene flow (Tallmon *et al.* 2000; Funk *et al.* 2005). While the stepping-stone and wetland likelihood models still explained a great deal of the variation in the genetic data (60–70%; $\rho_w = 0.208$ – 0.223), they did not perform as well as the straight-line model (83%; $\rho_w = 0.327$). Contrary to our expectation, slope itself was a poor predictor of differentiation because the least slope model performed relatively poorly (42%) in the partial Mantel test, although the least slope model did have the second highest Spearman correlation value based on BIOENV ($\rho_w = 0.278$).

There are several possible explanations for the high relative performance of the straight-line model. First,

salamanders may be moving in a relatively straight line. We have located tiger salamanders on steep slopes when a more gradual path from the pond was not far away (Spear, unpublished). Second, the stepping-stone model may have been limited in its utility because there may have been unsampled sites that salamanders use. Another potential problem is that many sites used in the stepping-stone model may be unsuitable for salamander use due to drought. Several of the sites where salamanders have been observed previously were dry during the study period (2002–2003), and may have been in previous years as well. Additionally, two sites included as stepping-stones have had recent disease outbreaks (Patla & Peterson 2004). Therefore, diseased sites might create population sinks as opposed to stepping-stones. Finally, it is possible that salamanders follow a least-cost route, but that we did not model the correct one. For example, we did not create alternative paths for cover type or rivers. If an accurate least-cost path could be developed based on these variables, it might provide a better correlation than straight-line distance.

There were three variables that were included in at least one route in both the partial mantel and BIOENV analyses. These included distance, elevation difference and open shrub cover type. In addition, river/stream crossings and dry meadow cover type were only in the partial Mantel test, and closed forest and open forest cover types were only included using BIOENV. In the partial Mantel, the direction of the correlation of the variables was the same across the different models, with the exception of dry meadows, which was positively correlated with gene flow in the stepping-stone model and negatively correlated in the least slope model. Contradictory results associated with dry meadow suggest that it is probably not a good predictor variable for gene flow, probably because it was only present in a few small areas across the study site, increasing the chance of a spurious result. One of the two variables unique to the BIOENV analysis – open forest cover type (included in the slope model) was rare across the study area, and is thus also probably not biologically meaningful. The other significant variable resulting from BIOENV – closed forest – is relatively common across the study area, and a scatterplot indicates that the presence of closed forest is positively associated with F_{ST} .

Elevation difference appeared to increase differentiation in both analyses. This finding was consistent with our hypothesis and provides support for the conclusion by Funk *et al.* (2005) that elevation is important for structuring populations. However, the route based on least slope was not the route with the greatest support, so the low gene flow between high and low elevation sites may be due to some other factor.

Open areas were expected to be barriers to movement based on previous studies (Madison & Farrand 1998; deMaynadier & Hunter 1999; Rothermel & Semlitsch

2002). However, in this study, open shrub habitat was positively correlated with gene flow and closed forest cover appeared to decrease gene flow. Although this result is counterintuitive to the majority of amphibian studies, it is consistent with two studies of western tiger salamanders: a study of *Ambystoma tigrinum melanostictum* in British Columbia in which salamanders moved away from forested areas and used open habitats (Richardson *et al.* 2000) and a study of *Ambystoma californiense* (California tiger salamander) which found that they primarily used open habitat, although individuals also were located near scattered oak trees (Trenham 2001). The open shrub cover type across Yellowstone excluded sagebrush-dominated areas and featured areas that had been previously burned (primarily in the widespread 1988 fires), but had some vegetation regrowth. Therefore, burned areas may not be a hindrance to movement as long as some plant regeneration is taking place. Possible downfall from previous fires might also provide ground cover and protection, further facilitating movement. There is also some evidence that recent fires can increase dispersal in amphibians. Western toads, *Bufo boreas*, in Glacier National Park in Montana have colonized breeding sites that have been burned within the past 5 years (Corn & Hossack, unpublished). If a similar pattern exists for tiger salamanders, this would be an explanation for our results.

Rivers and streams were also expected to be barriers because most Yellowstone rivers and streams contain fish (Varley 1981), and ambystomatid salamanders generally do not coexist with fish, likely due to poor antipredator abilities (Kats *et al.* 1988; Tyler *et al.* 1998; Sih *et al.* 2003). The apparent positive effect of rivers and streams on gene flow could be due to the ongoing drought in the area. The loss of pond sites might result in movement along riverine systems to avoid desiccation, as has been seen in western toads in Idaho (P. Bartelt, personal communication). Riverine travel would expose salamanders to fish predation, and it is unlikely that salamanders use moving water for extended periods. However, tiger salamanders have used streams as breeding habitat (Collins 1981), although this has never been documented in Yellowstone. Small, fishless pools or beaver ponds that are often adjacent to streams could also provide habitat or breeding sites. Finally, periodic flooding events might facilitate salamander dispersal by carrying individuals to other areas.

Conclusions

This study demonstrates the utility of GIS-based landscape genetic approaches in population genetic analyses. This method allowed for insights into tiger salamander ecology that would not have been possible with only a distance-based approach. While distance is a restrictive factor for salamander movement at the spatial scale of this study, it

appears that the presence of open shrub, postburn habitat, and possibly rivers, increases genetic connectivity, while elevation difference decreases connectivity. Furthermore, presence of burned open shrub cover in this system is a result of relatively recent landscape processes, which suggests that the positive correlation between open shrubs and genetic connectivity reflects contemporary gene flow.

Our landscape genetic analysis raises important questions relative to salamander conservation and management. For example, how will climate change influence patterns of gene flow? Could riverine dispersal cause increased spread of disease? Could prescribed burns be a technique for increasing movement among populations? These questions would not have emerged without the inclusion of GIS landscape analyses in our evaluation of genetic structure. Thus, the GIS-based landscape genetic approach generates questions and hypotheses for further research that may not have emerged from a traditional isolation-by-distance study.

Finally, landscape genetic methodology developed herein can be applied to a broad range of organisms. The types of GIS data used in this study are increasingly available for many geographical areas. Clearly, the next step in the field is the development of statistical analyses that are able to rigorously evaluate several landscape variables simultaneously. While the incorporation of both partial Mantel and BIOENV produced the same general conclusions, the individual results varied, and it is difficult to compare the relative importance of elevation and cover type in structuring populations. Landscape genetics is an exciting new development for population genetics and evolutionary biology because it provides researchers a way to model quantitatively the extent to which landscape features explain variation in gene flow, a distinct advantage over traditional straight-line distance models. The ever-increasing landscape alteration by humans will likely affect the future evolution and ecology of many organisms. As a result, incorporating landscape variables in studies of population genetic structure and developing new techniques to test those correlations will become increasingly important for understanding ongoing evolutionary processes.

Acknowledgements

This project was supported by the University of Wyoming-National Park Service Research Station, the Idaho State University Graduate Student Research and Scholarship Committee, the Idaho State University Department of Biological Sciences and NSF DEB-9977163 and IBN-0213851 to A.S. G. Elrod, T. Elrod, D. Jochimsen, A.A. Spear, A.M. Spear, B. Spear and K. Spear all assisted with field collection of samples. K. Lew provided assistance with the laboratory work. We thank P. Bartelt, B. Klaver, and J. Shive for help with the GIS techniques. We thank C. Hendrix and the National Park Service for granting permission to work in Yellowstone National Park. M. Pfrender and two anonymous reviewers

provided helpful comments that improved this manuscript. This research was approved by the Animal Welfare Committee at Idaho State University (#02-10-463).

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This study was conducted as part of Stephen Spear's master's research at Idaho State University. Stephen Spear is currently a PhD student at Washington State University examining the conservation and landscape genetics of tailed frogs. Charles Peterson's research focuses on describing the spatial distribution patterns of individuals and populations of amphibians and reptiles, mechanistically analyzing those patterns, and applying the information to conservation problems. Marjorie Matocq's research combines molecular genetics with field studies to elucidate historic and ongoing processes that determine the geographic distribution of genetic diversity within and among wild populations. Andrew Storfer studies limits to species' ranges and uses population genetics as a tool to understand the factors that shape distributions of species. He is also interested in host-pathogen coevolution and conservation of amphibians.
