

Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence

A. J. ZELLMER and L. L. KNOWLES

Department of Ecology and Evolutionary Biology, University of Michigan, Museum of Zoology, 1109 Geddes Avenue, Ann Arbor, MI 48109-1079, USA

Abstract

Increasing habitat fragmentation poses an immediate threat to population viability, as gene flow patterns are changed in these altered landscapes. Patterns of genetic divergence can potentially reveal the impact of these shifts in landscape connectivity. However, divergence patterns not only carry the signature of altered contemporary landscapes, but also historical ones. When considered separately, both recent and historical landscape structure appear to significantly affect connectivity among 51 wood frog (*Rana sylvatica*) populations. However, by controlling for correlations among landscape structure from multiple time periods, we show that patterns of genetic divergence reflect recent landscape structure as opposed to landscape structure prior to European settlement of the region (before 1850s). At the same time, within-population genetic diversities remain high and a genetic signature of population bottlenecks is lacking. Together, these results suggest that metapopulation processes – not drift-induced divergence associated with strong demographic bottlenecks following habitat loss – underlie the strikingly rapid consequences of temporally shifting landscape structure on these amphibians. We discuss the implications of these results in the context of understanding the role of population demography in the adaptive variation observed in wood frog populations.

Keywords: conservation genetics, habitat fragmentation, metapopulation dynamics, *Rana sylvatica*, rapid differentiation

Received 10 March 2009; revision received 9 June 2009; accepted 20 June 2009

Introduction

Landscape connectivity is not only an essential aspect of population dynamics for many species, but it can also have important evolutionary consequences. Heterogeneity in the landscape matrix separating populations can impede or facilitate dispersal (Ricketts 2001) and gene flow, shaping patterns of genetic variation (e.g. Funk *et al.* 2005; Spear *et al.* 2005; Cushman *et al.* 2006; Lowe *et al.* 2006). However, landscape structure can also vary across time, and relatively quickly, as with changes in human land-use practices (Skole & Tucker 1993). This temporal dynamic, in addition to the spatial landscape structure, is becoming increasingly important as anthropogenic impacts have the potential to outpace the ability of organisms to cope with altered landscapes.

Yet, the consequences of temporal shifts in landscape connectivity on patterns of gene flow have rarely been considered (except see Keyghobadi *et al.* 2005; Vandergast *et al.* 2007). The implications of these changes are especially important for amphibian populations, which are facing global declines (Stuart *et al.* 2004).

While there is increasing evidence that habitat fragmentation reduces genetic connectivity in disparate taxa (Epps *et al.* 2005; Proctor *et al.* 2005; Coulon *et al.* 2006; Cushman *et al.* 2006; Riley *et al.* 2006; Vandergast *et al.* 2007), the impact of contemporary landscape changes can be difficult to assess, because patterns of genetic differentiation reflect not only recent shifts in landscape structure, but also historic patterns. It may take tens of thousands of generations to reach equilibrium between genetic drift and gene flow following habitat fragmentation (Crow & Aoki 1984; Varvio *et al.* 1986), making recent landscape changes relatively more difficult to detect. Additionally, historic and contemporary

Correspondence: Amanda J. Zellmer, Fax: 734-763-4080; E-mail: azellmer@umich.edu

landscape structure may be correlated. By assessing only the effects of contemporary landscapes, we run the risk of incorrectly attributing contemporary genetic patterns to recent landscape changes when in fact the genetic structure reflects more historic processes.

To account for these difficulties, we assessed the impact of changes in landscape structure across time by comparing the contribution of landscape features from three time periods (Fig. 1), representing pre- and post-European settlement, to genetic connectivity of 51 wood frog (*Rana sylvatica*) populations (Fig. 2). Genetic structure among wood frog populations is expected to be correlated with landscape structure, because forested habitat is critical for dispersal and foraging of juveniles and adults (Regosin *et al.* 2003). Much older processes are unlikely to play a role in structuring contemporary populations because phylogeographic patterns across the wood frog range indicate that this region was only recolonized during the last 10 000 years following the most recent glacial period (Lee-Yaw *et al.* 2008). While amphibians, in general, are highly sensitive to the effects of habitat fragmentation because of their strict habitat requirements (Cushman 2006), based on the recency of the landscape changes across the study site, we expected the genetic structure of wood frog populations to reflect historic as opposed to contemporary landscape patterns.

Methods

Fifty-one ponds were sampled across southeastern Michigan (Fig. 2); approximately 20 *R. sylvatica* tadpoles were collected from each pond for a total of 1089

individuals. Each pond was sampled by multiple people spread out across the pond to ensure a thorough sample of each population. As wood frogs are explosive breeders and adults continue to breed in the pond in which they first bred (Berven & Grudzien 1990), we equate ponds with breeding populations and refer to them as populations throughout the text. The study area is located within a terminal moraine, and is a composite of forest and wetland fragments separated by agricultural and urban areas. The landscape has undergone dramatic transitions with shifting patterns of land-use following European settlement, as documented in county archives of vegetation surveys from 1816–1856 (Michigan Department of Natural Resources) and satellite images from the Michigan DNR for *c.* 1978 and the National Land Cover Dataset for 2001 (Homer *et al.* 2004). Most of the ponds used in this study are natural woodland ponds or wetlands; however, some wetlands have been created from small dams scattered throughout the region. Ponds ranged in size from approximately 100 to 3000 m². The extent to which each individual pond has remained stable since the mid-1800s is unknown, because wood frog populations from individual ponds frequently go extinct and are recolonized. However, over the period between the two recent time periods used in this study, the number of breeding sites within this region has remained constant (Skelly *et al.* 1999), whereas the number of breeding populations has likely declined since post-European settlement because of loss of both wetland and terrestrial habitat.

Deoxyribonucleic acid (DNA) was extracted from tail clips using the DNeasy tissue kit (QIAGEN). Nine microsatellite loci developed specifically for *R. sylvatica* were

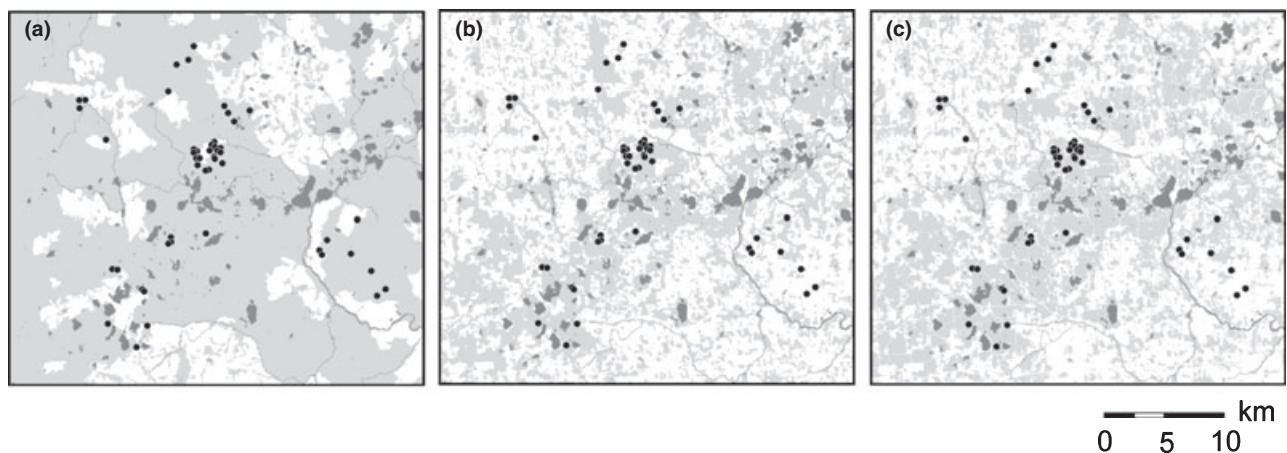


Fig. 1 Landscape structure of the study site from 1800s to 2001. Maps showing the landscape transitions that have accompanied shifting land-use practices over the last century: (a) reconstruction of the area from the 1800s, and aerial photographs of the area from (b) 1978 and (c) 2001. Areas identified as habitat (shown in light grey) vs. nonhabitat (shown in white) correspond to forested, shrubland and wetland areas vs. grassland, savannah, agricultural and urban areas, respectively (Regosin *et al.* 2003). Rivers and lakes are shown in dark grey, and the sampled populations are represented by white circles.

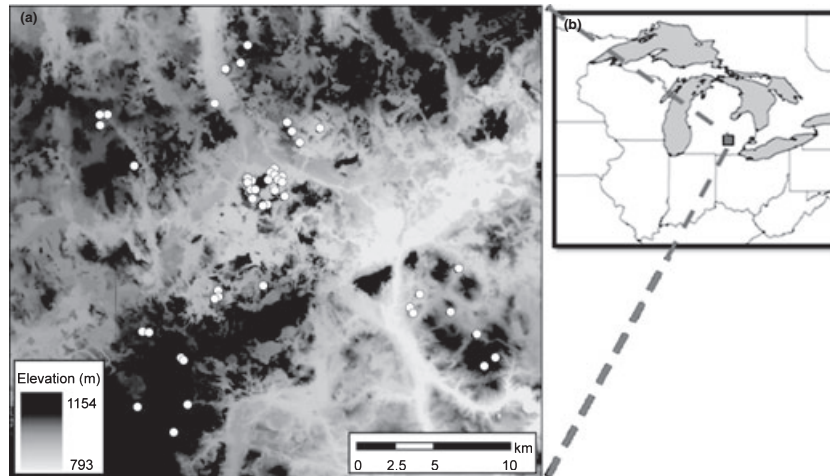


Fig. 2 Sampled populations. Topographic map of the study area (a) from southeastern Michigan, USA (b), where sampled ponds are marked with white circles.

analysed for each individual: loci AAT23 and AAT46 (Newman & Squire 2001), loci C23, C41, D33, D40, and D88 (Julian & King 2003), and loci 1A11 and 2B02 (Table 1) developed for this study following the protocol of Glenn & Schable (2005). Polymerase chain reaction (PCR) conditions corresponded to those from Newman & Squire (2001) and Julian & King (2003) for the two former sets of microsatellite markers, respectively. For loci 1A11 and 2B02, PCR reactions included 1.0 μ L of genomic DNA, 1.0 μ L of 10 \times PCR buffer (Invitrogen), 0.5 μ L of 10 μ M primer for both the fluorescently labelled forward primer and the reverse primer, 0.3 μ L of 50 mM MgCl₂, 0.6 μ L of 10 mM dNTPs, 0.4 μ L of 250 μ g/mL bovine serum albumin and 0.2 U of *Taq* DNA polymerase (Invitrogen). Reactions were run for 120 s at 94 $^{\circ}$ C, and then 35 cycles at 94 $^{\circ}$ C for 60 s, 60 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 30 s, followed by 240 s at 72 $^{\circ}$ C. Individuals were genotyped with ABI PRISM Genetic Analyzer (Applied Biosystems) and GENEMARKER software (Softgenetics).

Tests for genotyping errors and/or null alleles were conducted for each locus with MICROCHECKER v. 2.2.0 (Van Oosterhout *et al.* 2004), and tests for linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were assessed with GENEPOP v. 3.4 (Raymond & Rousset 1995), where a sequential Bonferroni correction

was applied to reduce type I errors (Rice 1989). Genetic diversity within ponds was assessed by calculating Nei's unbiased gene diversity (Nei 1987), the total number of alleles, and the allelic richness (FSTAT: Goudet 1995), as well as the private allelic richness of each population (HP-Rare: Kalinowski 2005). Populations were also assessed for evidence of population bottlenecks using the program BOTTLENECK (Piry *et al.* 1999) with 1000 replications and under the assumption of the Stepwise Mutation Model, because this model has been identified as appropriate for microsatellite loci, instead of the Infinite Alleles Model (Luikart & Cornuet 1998). Significance was assessed using the Wilcoxon's test after Bonferroni correction.

Genetic and landscape distances

Pairwise F_{ST} values (Wright 1951; Weir & Cockerham 1984) were calculated among ponds using a weighted analysis of variance (Weir & Cockerham 1984) with GENEPOP v. 3.4 (Raymond & Rousset 1995). Significance of F_{ST} values was assessed after Bonferroni correction. F_{ST} was used as a measure of genetic distance rather than R_{ST} , because F_{ST} has a lower mean squared error than R_{ST} at the level of differentiation observed among ponds (Gaggiotti *et al.* 1999). Permutation tests were

Table 1 The forward and reverse primers, the repeat motifs, the fragment lengths and the GenBank accession numbers for the microsatellite loci developed for this study

| Locus | Primer sequence (5' to 3') | Repeat | PCR size (bp) | GenBank No. |
|-------|--|--------|---------------|-------------|
| 1A11 | F: AGCCACCTGGAGTAGGAGT R: TCCTGCCCTGGAAAAGTAAAA | GT | 173–275 | GQ422446 |
| 2B02 | F: GGAACAGTTGGCTTTGGAA R: TTCAAACCTGCAGTGCCTAA | GT | 121–189 | GQ422447 |

carried out using SPAGED1 v.1.2 (Hardy & Vekemans 2002) to confirm that R_{ST} and F_{ST} converge ($P = 0.3991$, based on 20 000 permutations) (Hardy *et al.* 2003).

Two geographic distances were calculated among each pair of ponds, including the Euclidean (straight-line) distance (ED) and the resistance distance (RD; McRae 2006), a distance weighted according to the permeability of the landscape separating populations. The ED between each pair of ponds was calculated using the PATHMATRIX extension (Ray 2005) in ARCVIEW GIS v 3.3 (ESRI 2006). The RD was calculated using CIRCUITSCAPE v 3 (McRae 2006) from 30 m resolution friction maps created in ArcGIS v 9.2 (ESRI 2006). Friction maps were generated by coding each pixel of the map as a cost to dispersal based on the type of landscape that it encompassed, with a cost of one assigned to the most permeable habitats and higher values representing less permeable habitats. This method results in correspondingly greater distances between ponds for landscape features incurring a high cost to traverse.

Friction maps were generated for two permanent landscape features – slope and rivers/lakes – and land cover for each of the three time periods (i.e. 1800s, 1978 and 2001; see Fig. 1), as well as composite friction maps for each of the time periods that included the permanent landscape features (generated using the Map Algebra tool in ArcGIS). Land cover was classified as either *R. sylvatica* habitat (forests, shrubland and wetlands) or nonhabitat (agriculture, urban areas, grasslands and savannahs) based on habitat use of *R. sylvatica* (Regosin *et al.* 2003) (e.g. Fig. 1); wood frog habitat was assigned a cost of one, whereas a range of cost values were examined for non-wood-frog habitat. Rivers and lakes were included because rivers and lakes do not likely constitute stepping stones to other wetland habitat (wood frogs primarily breed in habitats that lack fish: Hopey & Petranka 1994). Areas not covered by rivers or lakes were correspondingly assigned a cost of one. Slope was calculated based on a 30-m resolution digital elevation model (Michigan Department of Natural Resources; Fig. 2) using the slope function in the ArcGIS data management toolbox, and modelled as a linear function with a cost of one assigned to a slope of zero and a maximum cost assigned to the highest slope possible. As our ability to detect the effects of landscape distance on genetic differentiation depends on both the landscape features used and the relative costs of each feature, a range of costs were evaluated for each (Perez-Espona *et al.* 2008).

For each of the friction maps, the relationship between genetic distance and landscape distance was evaluated with Mantel tests (Mantel 1967) and partial Mantel tests (Smouse *et al.* 1986) to control for the effects of distance. All analyses were completed using

IBDWS v 3 with 10 000 randomizations (Jensen *et al.* 2005). P -values were calculated in IBDWS using a modified method (Legendre & Legendre 1998) to avoid issues with statistical bias and autocorrelation (Bohonak 2002). R -values were used to determine the friction map with the highest support for each time period. Although not all possible combinations of costs could be evaluated as a result of computational constraints, a sufficient range of costs was evaluated to reveal a peak in R -values for each time period (Table S1). As landscape variables were combined to create a single predictor variable (each friction map), there is no expected inflation of explained variance because of adding additional landscape variables (as in Cushman *et al.* 2006). The relative support of each friction map could thus be evaluated by ranking R -values. To test the validity of this approach, we assessed the extent to which adding additional landscape variables affected R -values using mirror images of each of the landscape features. Mirror images allowed us to maintain the same amount of information provided in each landscape variable while removing any correlations between genetics and landscape structure. The addition of multiple landscape variables in mirror image did not consistently lead to an inflation of explained variance (Table S2), demonstrating that model support can be assessed according to the rank of the model's respective R -values.

We additionally evaluated whether land cover from each time period remained significant after removing the effects of the other two time periods. Partial Mantel tests were used to control for the effects of time as opposed to distance. To test the robustness of our results, the partial Mantel tests were repeated for all joint friction maps that were significant for both historic and contemporary landscape.

Results

Genetic structure

There was no consistent evidence of deviations from HWE or LD within populations across all loci. Although there was some evidence of null alleles, there was no consistent pattern across loci or within populations. To test the robustness of our results, the data were reanalysed after removing the locus with the highest percentage of populations with evidence of null alleles (locus 1A11); the results from these analyses were qualitatively the same (results not shown).

There was a significant amount of genetic structure across the 51 populations (pairwise F_{ST} -values ranged from -0.008 to 0.087), with 392 of 1275 (30.7%) significant pairwise comparisons of F_{ST} after Bonferroni correction. Genetic diversities within populations were

Table 2 Genetic diversity within populations. Nei's unbiased genetic diversity (GD; Nei 1987), number of alleles, allelic richness (AR) and private allelic richness (PAR) for each population. Both AR and PAR were rarified based on the smallest sample size in any population ($n = 10$). Also shown is the population number (Pop No.) as well as the sample size in each population (SS)

| Pop No. | SS | GD | | No. of alleles | | AR | | PAR | |
|---------|----|------|------|----------------|------|------|------|------|------|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 1 | 21 | 0.73 | 0.26 | 8.67 | 5.32 | 6.91 | 3.59 | 0.00 | 0.00 |
| 3 | 21 | 0.71 | 0.28 | 9.33 | 5.83 | 6.97 | 3.84 | 0.03 | 0.06 |
| 4 | 24 | 0.75 | 0.24 | 9.78 | 5.45 | 7.26 | 3.50 | 0.05 | 0.11 |
| 5 | 21 | 0.75 | 0.26 | 9.44 | 6.11 | 7.36 | 3.82 | 0.03 | 0.06 |
| 6 | 22 | 0.69 | 0.24 | 8.78 | 6.08 | 6.40 | 3.64 | 0.01 | 0.02 |
| 7 | 20 | 0.73 | 0.27 | 9.00 | 5.12 | 7.05 | 3.65 | 0.02 | 0.04 |
| 8 | 25 | 0.73 | 0.28 | 9.44 | 5.39 | 7.12 | 3.59 | 0.04 | 0.09 |
| 9 | 23 | 0.68 | 0.21 | 6.44 | 3.47 | 5.59 | 2.69 | 0.00 | 0.01 |
| 10 | 20 | 0.75 | 0.25 | 8.33 | 4.21 | 6.84 | 3.02 | 0.12 | 0.27 |
| 11 | 21 | 0.73 | 0.24 | 8.78 | 5.26 | 6.84 | 3.60 | 0.00 | 0.01 |
| 12 | 20 | 0.71 | 0.30 | 8.56 | 4.72 | 6.82 | 3.45 | 0.05 | 0.13 |
| 14 | 20 | 0.72 | 0.27 | 9.11 | 5.49 | 6.93 | 3.47 | 0.03 | 0.07 |
| 15 | 21 | 0.75 | 0.23 | 9.33 | 5.12 | 7.20 | 3.58 | 0.00 | 0.01 |
| 16 | 20 | 0.75 | 0.31 | 10.11 | 6.17 | 7.88 | 4.15 | 0.05 | 0.13 |
| 17 | 20 | 0.69 | 0.22 | 8.44 | 4.67 | 6.54 | 3.30 | 0.00 | 0.00 |
| 18 | 10 | 0.70 | 0.29 | 5.78 | 2.86 | 5.78 | 2.86 | 0.00 | 0.00 |
| 22 | 20 | 0.73 | 0.20 | 7.89 | 4.14 | 6.46 | 2.89 | 0.04 | 0.12 |
| 23 | 21 | 0.73 | 0.29 | 8.56 | 4.75 | 6.91 | 3.56 | 0.00 | 0.00 |
| 24 | 21 | 0.71 | 0.26 | 9.11 | 5.40 | 6.79 | 3.50 | 0.02 | 0.05 |
| 25 | 21 | 0.72 | 0.28 | 8.89 | 4.96 | 6.83 | 3.32 | 0.01 | 0.04 |
| 26 | 21 | 0.71 | 0.30 | 8.78 | 5.09 | 6.80 | 3.62 | 0.00 | 0.01 |
| 27 | 20 | 0.70 | 0.25 | 8.33 | 4.18 | 6.50 | 3.05 | 0.03 | 0.07 |
| 28 | 20 | 0.73 | 0.30 | 9.44 | 5.68 | 7.30 | 3.81 | 0.02 | 0.05 |
| 29 | 21 | 0.68 | 0.29 | 7.78 | 4.24 | 6.26 | 2.97 | 0.02 | 0.06 |
| 30 | 23 | 0.74 | 0.27 | 9.33 | 6.00 | 7.10 | 3.82 | 0.01 | 0.02 |
| 31 | 21 | 0.72 | 0.26 | 9.33 | 5.52 | 7.04 | 3.65 | 0.02 | 0.03 |
| 32 | 22 | 0.73 | 0.27 | 9.67 | 5.66 | 7.25 | 3.68 | 0.06 | 0.08 |
| 33 | 22 | 0.69 | 0.28 | 5.78 | 2.73 | 5.32 | 2.43 | 0.00 | 0.00 |
| 35 | 20 | 0.72 | 0.28 | 8.33 | 4.50 | 6.57 | 3.06 | 0.02 | 0.04 |
| 36 | 21 | 0.74 | 0.26 | 8.67 | 4.47 | 6.98 | 3.26 | 0.03 | 0.07 |
| 37 | 22 | 0.77 | 0.24 | 9.67 | 5.68 | 7.39 | 3.46 | 0.08 | 0.16 |
| 38 | 21 | 0.72 | 0.24 | 9.11 | 5.93 | 7.07 | 3.98 | 0.02 | 0.04 |
| 39 | 20 | 0.76 | 0.25 | 8.89 | 4.31 | 7.42 | 3.38 | 0.03 | 0.04 |
| 40 | 21 | 0.76 | 0.25 | 8.56 | 4.25 | 7.08 | 3.09 | 0.04 | 0.07 |
| 41 | 21 | 0.74 | 0.22 | 8.11 | 3.92 | 6.53 | 2.88 | 0.02 | 0.04 |
| 42 | 21 | 0.76 | 0.24 | 8.78 | 4.52 | 7.21 | 3.28 | 0.06 | 0.12 |
| 43 | 22 | 0.73 | 0.27 | 10.44 | 6.25 | 7.67 | 4.03 | 0.08 | 0.09 |
| 44 | 21 | 0.76 | 0.20 | 9.22 | 4.60 | 7.01 | 2.83 | 0.10 | 0.16 |
| 45 | 24 | 0.73 | 0.27 | 9.89 | 5.73 | 7.29 | 3.61 | 0.02 | 0.06 |
| 46 | 21 | 0.72 | 0.28 | 9.89 | 6.13 | 7.62 | 4.20 | 0.05 | 0.07 |
| 47 | 25 | 0.73 | 0.25 | 10.22 | 6.59 | 7.51 | 4.13 | 0.09 | 0.10 |
| 48 | 22 | 0.76 | 0.23 | 9.11 | 4.83 | 7.24 | 3.38 | 0.05 | 0.09 |
| 49 | 28 | 0.77 | 0.23 | 10.22 | 6.14 | 7.23 | 3.14 | 0.06 | 0.11 |
| 50 | 24 | 0.73 | 0.27 | 10.44 | 6.67 | 7.65 | 4.07 | 0.03 | 0.06 |
| 51 | 23 | 0.76 | 0.28 | 10.89 | 6.90 | 7.96 | 4.16 | 0.07 | 0.08 |
| 52 | 24 | 0.76 | 0.25 | 9.00 | 4.44 | 7.00 | 3.01 | 0.08 | 0.14 |
| 53 | 23 | 0.73 | 0.30 | 10.00 | 5.55 | 7.41 | 3.79 | 0.01 | 0.03 |
| 54 | 20 | 0.73 | 0.29 | 9.67 | 5.79 | 7.57 | 3.96 | 0.07 | 0.13 |
| 55 | 21 | 0.74 | 0.25 | 9.89 | 6.15 | 7.28 | 3.91 | 0.04 | 0.11 |
| 56 | 21 | 0.74 | 0.26 | 8.89 | 5.21 | 7.02 | 3.63 | 0.04 | 0.10 |
| 57 | 22 | 0.71 | 0.28 | 10.00 | 5.57 | 7.46 | 3.93 | 0.09 | 0.15 |
| Overall | | | | 9.02 | 0.92 | 7.00 | 0.42 | | |

high (Table 2), and none of the populations showed significant evidence of a bottleneck after Bonferroni correction. A significant correlation between ED and genetic differentiation indicated a pattern of isolation by distance (Mantel test: $R^2 = 0.187$; $P < 0.0001$).

Effects of land cover

For each time period, R -values peaked at the same relative costs for each of the landscape features (Rivers/Lakes = 500, Slope = 200, Land Cover = 5; Table S1). Friction maps containing all three landscape features provided higher R -values than cost maps containing either one or two landscape factors (Table S1). For each of the three time periods, we detected a significant effect of spatial landscape structure on population connectivity among 51 *R. sylvatica* populations, as landscape distances (based on a joint friction map with optimal costs for each landscape feature; Table S1) explained a significant amount of the variation in patterns of genetic differentiation among populations, beyond the effects of straight-line geographic distance (partial Mantel tests, controlling for distance; Table 3; Fig. 3).

As there was support for land cover from each of the three time periods, the effects of each time period independent of the other time periods were also assessed. The results were consistent for all friction maps where both historic and contemporary landscape structure were initially supported (Table 4). Historic landscape structure was not significantly correlated with genetic differentiation after removing the effects of land cover from either contemporary land-cover map (Table 4), whereas both contemporary time periods were either significant (1978, 2001) or marginally significant (2001) after removing the effects of historic land cover (Table 4). Together, these results suggest that contemporary landscape structure explains more of the vari-

Table 3 Landscape structure from each time period explains a significant amount of the variation in contemporary genetic structure (F_{ST}) after controlling for the effects of Euclidean distance (partial Mantel tests). Results are based on landscape distances from a joint friction map that includes the optimal cost for each landscape feature, including: rivers/lakes (R/L), slope (S) and land cover (LC) significance of $P < 0.05$ is denoted with an asterisk.

| Time period | Costs | | | Controlling for distance | |
|-------------|-------|-----|----|--------------------------|---------|
| | R/L | S | LC | R | P |
| 2001 | 500 | 200 | 5 | 0.277 | <0.005* |
| 1978 | 500 | 200 | 5 | 0.276 | <0.012* |
| 1800 | 500 | 200 | 5 | 0.283 | <0.005* |

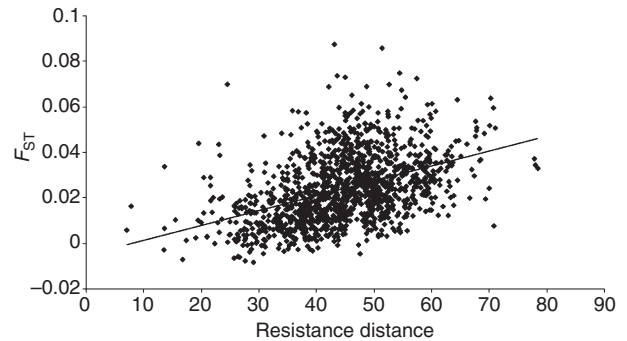


Fig. 3 Isolation by landscape distance. Pairwise comparisons of genetic differentiation (F_{ST}) as a function of landscape distance (resistance distance) (partial Mantel: $R^2 = 0.077$, $P < 0.005$; Table 3), based on a model for recent (1978) land cover that also includes rivers/lakes and slope.

ance in contemporary genetic structure than does historic landscape structure. There is slightly more support for land cover circa 1978 explaining contemporary genetic structure than circa 2001. However, the lack of significant support for 2001 land cover after removing the effects of 1978, and the marginal support for 1978 after removing the effects of 2001 (Table 4), suggest that land cover from both contemporary periods are highly correlated (Fig. 1).

Discussion

Although the effects of land cover from all three time periods on genetic differentiation were initially supported when considered individually (Table 3), after controlling for landscape structure from each time period, our results suggest that contemporary patterns of genetic differentiation among wood frog populations reflect recent as opposed to historic landscape structure (Table 4). These results demonstrate how the use of multiple time periods can be used to understand the processes contributing to patterns of genetic variation. Even though the substantial human-induced changes to the landscape have been quite recent, the genetic structure nonetheless reflects current landscape structure (after controlling for the influence of the historic landscape configuration on genetic structure). The comparison of multiple time periods thus not only allows for a determination of how genetic structure is affected by the contemporary landscape, but also an assessment of the rate of differentiation following landscape alteration. The small temporal and spatial scales at which the effects of temporally shifting landscape structure are seen highlight the importance of connectivity for amphibian populations.

The differentiation of wood frog populations associated with recent habitat fragmentation (Fig. 1) has been

Table 4 Contemporary land cover is consistently related to genetic differentiation (F_{ST}) after controlling for effects of historical land cover. Partial Mantel results for only the joint friction maps that supported both historic and contemporary landscape structure, with various costs for rivers/lakes (R/L), slope (S) and land cover (LC)

| Time period | Costs | | | Controlling for time | | | | | |
|-------------|-------|-----|----|----------------------|--------|--------|--------|-------|---------|
| | | | | 2001 | | 1978 | | 1800 | |
| | R/L | S | LC | R | P | R | P | R | P |
| 2001 | 500 | 200 | 5 | — | — | -0.156 | <0.907 | 0.172 | <0.053 |
| 1978 | 500 | 200 | 5 | 0.187 | <0.058 | — | — | 0.218 | <0.018* |
| 1800 | 500 | 200 | 5 | -0.082 | <0.782 | -0.120 | <0.862 | — | — |
| 2001 | 500 | 200 | 50 | — | — | 0.016 | <0.446 | 0.337 | <0.001* |
| 1978 | 500 | 200 | 50 | 0.180 | <0.064 | — | — | 0.379 | <0.001* |
| 1800 | 500 | 200 | 50 | 0.174 | <0.085 | 0.178 | <0.077 | — | — |
| 2001 | 50 | 50 | 5 | — | — | -0.100 | <0.804 | 0.183 | <0.044* |
| 1978 | 50 | 50 | 5 | 0.187 | <0.059 | — | — | 0.233 | <0.012* |
| 1800 | 50 | 50 | 5 | 0.087 | <0.244 | 0.062 | <0.317 | — | — |
| 2001 | 200 | 50 | 5 | — | — | -0.103 | <0.808 | 0.170 | <0.055 |
| 1978 | 200 | 50 | 5 | 0.183 | <0.062 | — | — | 0.218 | <0.019* |
| 1800 | 200 | 50 | 5 | 0.089 | <0.239 | 0.063 | <0.311 | — | — |
| 2001 | 500 | 500 | 5 | — | — | -0.162 | <0.918 | 0.169 | <0.055 |
| 1978 | 500 | 500 | 5 | 0.175 | <0.070 | — | — | 0.212 | <0.019* |
| 1800 | 500 | 500 | 5 | -0.134 | <0.893 | -0.175 | <0.945 | — | — |

much more rapid than expected – the genetic consequences having manifested in less than 50 generations. Why would these landscape changes become evident in patterns of neutral genetic divergence so quickly in this species? Two likely demographic scenarios could have enhanced genetic drift, and thereby led to rapid differentiation, among the wood frog populations. Habitat loss and fragmentation might have caused strong bottlenecks, promoting population differentiation. Alternatively, demographic processes, such as metapopulation dynamics, could have enhanced drift-induced divergence through recurrent extinction and recolonization. Although metapopulation dynamics theoretically can either increase or decrease genetic differentiation (depending on the specific modes of colonization, dispersal and population growth: Pannell & Charlesworth 2000; Slatkin 1977), metapopulation processes tend to increase the variance in reproductive success among populations, thereby enhancing the impact of genetic drift across a wide range of conditions (Giles & Goudet 1997; Whitlock & Barton 1997).

There are several reasons why metapopulation dynamics most likely explain why we observed a significant effect of recent shifts in land-use practices over such a short evolutionary timescale. Genetic diversities remain high within populations (Table 2) and there is no evidence for bottlenecks within any of the populations. Moreover, pond-breeding amphibians are often thought to exhibit aspects of metapopulation structure because of their reliance upon discrete aquatic environments for breeding, their high degree of philopatry and

high rates of population turnover (Alford & Richards 1999; Cushman 2006). Although few amphibian populations likely exhibit classic (*sensu* Levins 1969) metapopulation structure (Smith & Green 2005), many amphibian populations, including the wood frog, show high rates of population turnover (Hecnar & M'Closkey 1996; Skelly *et al.* 1999; Trenham *et al.* 2003; Werner *et al.* 2007), providing the opportunity for extinction and recolonization dynamics to play an important role in the genetic structure of these populations.

The rapid drift-induced differentiation of populations, as measured by the neutral microsatellite markers (*i.e.* it is highly improbable that the nine markers are linked with selected loci), is especially intriguing in the context of the adaptive phenotypic differences seen among *R. sylvatica* populations (Relyea 2002; Skelly 2004). Wood frog populations show evidence of local adaptation of behavioural, morphological and life history traits to opposing selective forces in ponds with varying predator regimes (Relyea 2002). These adaptive differences occur over very small spatial scales (*i.e.* within the dispersal capabilities of wood frogs: Berven & Grudzien 1990), and it is yet unclear what maintains these phenotypic differences in the face of potentially high levels of gene flow. Our results suggest that metapopulation dynamics may play an important role in contributing to the striking adaptive differences observed over such small spatial scales (*e.g.* Relyea 2002). Population turnover that increases differentiation of populations over short evolutionary timescales (as opposed to rapid divergence associated with population bottlenecks)

could maintain a source of standing genetic variation relevant to adaptive responses among the wood frog populations. Standing genetic variation provides a unique opportunity for selection to operate, as adaptation from standing genetic variation can proceed faster than adaptation from new mutations (Barrett & Schluter 2008). As a result, gene flow because of extinction and recolonization dynamics may instead facilitate the local adaptation of populations (e.g. Morjan & Rieseberg 2004). Future research should focus on comparing species with alternative demographic substructure to fully understand the extent to which metapopulation dynamics contributes to population differentiation.

Although numerous studies have shown an effect of population bottlenecks on rates of genetic differentiation (e.g. Baker & Moeed 1987; Bouzat *et al.* 1998; Rowe *et al.* 1998), very few studies have empirically demonstrated that high rates of extinction and recolonization can result in rapid differentiation among populations (e.g. Clegg *et al.* 2002; Knowles & Richards 2005). Furthermore, while metapopulation dynamics have been implicated in cases where genetic differentiation appears to have taken place over very short timescales (Orsini *et al.* 2008), without an assessment of historic landscape structure, past processes may confound interpretations based on the contemporary landscape. This study provides an important empirical example (see also Giles & Goudet 1997) that complements a growing body of theoretical research (e.g. Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990; Pannell & Charlesworth 2000) on the evolutionary consequences of metapopulation dynamics.

Conclusions

Our results highlight the importance of not only considering spatial heterogeneity in landscape structure, but also temporal landscape changes. Although initially the effects of land cover on contemporary genetic structure were supported for all three time periods, analyses controlling for correlations across time suggest that genetic differentiation reflects recent as opposed to historic land cover. We thus revealed an effect of recent human-induced shifts in landscape structure on patterns of genetic differentiation among wood frog populations, with differentiation having manifest in less than 50 generations. Moreover, the pattern of genetic diversity maintained within populations, suggests a role of metapopulation dynamics in the observed population genetic differentiation. As such, this study provides empirical evidence of the evolutionary consequences of ecological demographic processes, highlighting that such connections are not limited to organisms with short generations (e.g. viruses), but also apply to

longer-lived species. Without similar analyses, conservation decisions may be misled by failing to control for the confounding factors caused by correlations in landscape from different temporal periods, let alone, whether species-specific demographic structures will need to be taken into account in devising conservation strategies.

Acknowledgements

The authors thank C. Davis, M. Fraker, J. Handy, J. Middlemis Maher, C. Richards and E. Werner for assistance in collecting samples for this study, as well as K. Gibbons, J. McCormack, L. Tran, and E. Werner for helpful comments on the manuscript. The authors also thank B. Kovach, C. Kovach, C. Ketzner, G. Ketzner, S. Walker, C. Walker, S. Wellman and Earth Art Tree Farm for access to ponds on their properties. Funding for this research was provided by the University of Michigan's Helen Olsen Brower Scholarship and Block Grant to AJZ.

References

- Alford RA, Richards SJ (1999) Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics*, **30**, 133–165.
- Baker AJ, Moeed A (1987) Rapid genetic differentiation and founder effect in colonizing populations of common mynas (*Acridotheres tristis*). *Evolution*, **41**, 525–538.
- Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, **23**, 38–44.
- Berven KA, Grudzien TA (1990) Dispersal in the wood frog (*Rana sylvatica*) – implications for genetic population structure. *Evolution*, **44**, 2047–2056.
- Bohonak AJ (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.
- Bouzat JL, Cheng HH, Lewin HA *et al.* (1998) Genetic evaluation of a demographic bottleneck in the Greater Prairie Chicken. *Conservation Biology*, **12**, 836–843.
- Clegg SM, Degnan SM, Kikkawa J *et al.* (2002) Genetic consequences of sequential founder events by an island-colonizing bird. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8127–8132.
- Coulon a, Guillot G, Cosson JF *et al.* (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology*, **15**, 1669–1679.
- Crow JF, Aoki K (1984) Group selection for a polygenic behavioral trait – estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences*, **81**, 6073–6077.
- Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation*, **128**, 231–240.
- Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *American Naturalist*, **168**, 486–499.
- Epps CW, Palsboll PJ, Wehausen JD *et al.* (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters*, **8**, 1029–1038.

- ESRI (2006) ArcGIS 9.2. Environmental Systems Research Institute, Inc., Redlands, CA.
- Funk WC, Blouin MS, Corn PS *et al.* (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, **14**, 483–496.
- Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513–1520.
- Giles BE, Goudet J (1997) A case study of genetic structure in a plant metapopulation. In: *Metapopulation biology: ecology, genetics, and evolution* (eds Hanski I, Gilpin ME), Academic Press, San Diego, CA. pp. 429–454.
- Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.
- Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.
- Hardy OJ, Vekemans X (2002) SPAGED: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.
- Hardy OJ, Charbonnel N, Freville H, Heuertz M (2003) Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics*, **163**, 1467–1482.
- Hecnar SJ, M'Closkey RT (1996) Regional dynamics and the status of amphibians. *Ecology*, **77**, 2091–2097.
- Homer C, Huang CQ, Yang LM, Wylie B, Coan M (2004) Development of a 2001 national land-cover database for the United States. *Photogrammetric Engineering And Remote Sensing*, **70**, 829–840.
- Hopey ME, Petranksa JW (1994) Restriction of wood frogs to fish-free habitats – how important is adult choice. *Copeia*, **1994**, 1023–1025.
- Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *Bmc Genetics*, **6**, 13–18.
- Julian SE, King TL (2003) Novel tetranucleotide microsatellite DNA markers for the wood frog, *Rana sylvatica*. *Molecular Ecology Notes*, **3**, 256–258.
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, **5**, 187–189.
- Keyghobadi N, Roland J, Matter SF, Strobeck C (2005) Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 553–560.
- Knowles LL, Richards CL (2005) Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology*, **14**, 4023–4032.
- Lee-Yaw JA, Irwin JT, Green DM (2008) Postglacial range expansion from northern refugia by the wood frog, *Rana sylvatica*. *Molecular Ecology*, **17**, 867–884.
- Legendre P, Legendre L (1998) *Numerical Ecology*, 2nd edn. Elsevier, New York.
- Levins R (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America*, **15**, 237–240.
- Lowe WH, Likens GE, McPeck MA, Buso DC (2006) Linking direct and indirect data on dispersal: Isolation by slope in a headwater stream salamander. *Ecology*, **87**, 334–339.
- Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, **12**, 228–237.
- Mantel N (1967) Detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- McRae BH (2006) Isolation by resistance. *Evolution*, **60**, 1551–1561.
- Morjan CL, Rieseberg LH (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology*, **13**, 1341–1356.
- Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087–1100.
- Orsini L, Corander J, Alasentie A, Hanski I (2008) Genetic spatial structure in a butterfly metapopulation correlates better with past than present demographic structure. *Molecular Ecology*, **17**, 2629–2642.
- Pannell JR, Charlesworth B (2000) Effects of metapopulation processes on measures of genetic diversity. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **355**, 1851–1864.
- Perez-España S, Perez-Barberia FJ, McLeod JE *et al.* (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology*, **17**, 981–996.
- Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.
- Proctor MF, McLellan BN, Strobeck C, Barclay RMR (2005) Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2409–2416.
- Ray N (2005) PATHMATRIX: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes*, **5**, 177–180.
- Raymond M, Rousset F (1995) GENEPOP (version-1.2) – population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Regosin JV, Windmiller BS, Reed JM (2003) Terrestrial habitat use and winter densities of the wood frog (*Rana sylvatica*). *Journal of Herpetology*, **37**, 390–394.
- Relyea RA (2002) Local population differences in phenotypic plasticity: Predator-induced changes in wood frog tadpoles. *Ecological Monographs*, **72**, 77–93.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Ricketts TH (2001) The matrix matters: effective isolation in fragmented landscapes. *American Naturalist*, **158**, 87–99.
- Riley SPD, Pollinger JP, Sauvajot RM *et al.* (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, **15**, 1733–1741.
- Rowe G, Beebe TJC, Burke T (1998) Phylogeography of the natterjack toad *Bufo calamita* in Britain: genetic differentiation of native and translocated populations. *Molecular Ecology*, **7**, 751–760.
- Skelly DK (2004) Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. *Evolution*, **58**, 160–165.

- Skelly DK, Werner EE, Cortwright SA (1999) Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology*, **80**, 2326–2337.
- Skole D, Tucker C (1993) Tropical deforestation and habitat fragmentation in the Amazon – satellite data from 1978 to 1988. *Science*, **260**, 1905–1910.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.
- Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, **28**, 110–128.
- Smouse PE, Long JC, Sokal RR (1986) Multiple-regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology*, **14**, 2553–2564.
- Stuart SN, Chanson JS, Cox NA *et al.* (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306**, 1783–1786.
- Trenham PC, Koenig WD, Mossman MJ, Stark SL, Jagger LA (2003) Regional dynamics of wetland-breeding frogs and toads: Turnover and synchrony. *Ecological Applications*, **13**, 1522–1532.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Vandergast AG, Bohonak AJ, Weissman DB, Fisher RN (2007) Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera : Stenopelmatae : Stenopelmatus). *Molecular Ecology*, **16**, 977–992.
- Varvio SL, Chakraborty R, Nei M (1986) Genetic-variation in subdivided populations and conservation genetics. *Heredity*, **57**, 189–198.
- Wade MJ, McCauley DE (1988) Extinction and recolonization – their effects on the genetic differentiation of local-populations. *Evolution*, **42**, 995–1005.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Werner EE, Yurewicz KL, Skelly DK, Relyea RA (2007) Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos*, **116**, 1713–1725.
- Whitlock MC, Barton NH (1997) The effective size of a subdivided population. *Genetics*, **146**, 427–441.
- Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction – genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.
- Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Amanda J. Zellmer is a graduate student whose research focuses on the effects of landscape structure, demography, and selection on fine-scale population genetic structure as well as the role of genetic factors in population declines. Dr L. Lacey Knowles is an Associate Professor whose research focuses on the demographic and selective processes driving species divergence. Her research also addresses the methodological and statistical issues involved in studying species diversification, phylogeography, and phylogenetic relationships of recently diverged and rapidly speciating taxa.

Supporting information

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

Table S1 Comparison of support for each time period when varying the costs for rivers/lakes (R/L), slope (S) and land cover (LC) on the joint friction maps. Results are from partial Mantel tests that assess the correlation between genetic (F_{ST}) and landscape distance, while controlling for the effects of Euclidean distance. Significant partial Mantel tests are denoted with an asterisk. The results demonstrate support for all three time periods (before controlling for correlations among time periods) because the friction maps with the highest *R*-values are significant for each time period

Table S2 Correlations between genetic distance (F_{ST}) and landscape distance from mirror image friction maps, controlling for Euclidean distance. Friction maps were created using the mirror image of each landscape variable [including rivers/lakes (R/L), slope (S) and land cover (LC)] to assess whether or not the addition of multiple landscape variables leads to an inherent inflation of explained variance. Mirror images allowed us to maintain the same amount of information provided in each landscape variable while removing any correlations between genetics and landscape structure. Friction maps that include multiple landscape features (i.e. models with nonzero cost values applied to multiple features) do not consistently have higher *R*-values than individual friction maps, demonstrating that the rank order of the *R*-values can be used to evaluate model support

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.