

Do landscape processes predict phylogeographic patterns in the wood frog?

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Abstract

Understanding factors that influence population connectivity and the spatial distribution of genetic variation is a major goal in molecular ecology. Improvements in the availability of high-resolution geographic data have made it increasingly possible to quantify the effects of landscape features on dispersal and genetic structure. However, most studies examining such landscape effects have been conducted at very fine (e.g. landscape genetics) or broad (e.g. phylogeography) spatial scales. Thus, the extent to which processes operating at fine spatial scales are linked to patterns at larger scales remains unclear. Here, we test whether factors impacting wood frog dispersal at fine spatial scales are correlated with genetic structure at regional scales. Using recently developed methods borrowed from electrical circuit theory, we generated landscape resistance matrices among wood frog populations in eastern North America based on slope, a wetness index, land cover and absolute barriers to wood frog dispersal. We then determined whether these matrices are correlated with genetic structure based on six microsatellite markers and whether such correlations outperform a landscape-free model of isolation by resistance. We observed significant genetic structure at regional spatial scales. However, topography and landscape variables associated with the intervening habitat between sites provide little explanation for patterns of genetic structure. Instead, absolute dispersal barriers appear to be the best predictor of regional genetic structure in this species. Our results suggest that landscape variables that influence dispersal, microhabitat selection and population structure at fine spatial scales do not necessarily explain patterns of genetic structure at broader scales.

Keywords: amphibian, dispersal, gene flow, genetic structure, isolation by distance, isolation by resistance, landscape genetics, spatial scale

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Introduction

The spatial arrangement of populations and geographic range limits of species are thought to reflect the outcome of a balance between several ecological and evolutionary processes (Brown *et al.* 1996; Kirkpatrick & Barton 1997; Holt & Keitt 2000; Gaston 2003; Bridle & Vines 2006). Of these processes, dispersal and subsequent gene flow is a critical driver of range dynamics. In addition to governing colonization, dispersal and gene flow impact population fitness and persistence through their demographic (e.g.

Bahn *et al.* 2006) and evolutionary roles (reviewed by Garant *et al.* 2007). Examining the factors that influence dispersal and population connectivity is thus an important first step to understanding population dynamics and ultimately, species' geographic distributions.

Until relatively recently, investigations into the dispersal patterns of species have typically focused on understanding the spatial scale of dispersal (e.g. see references in Heywood 1991 and Parker *et al.* 1998). With improvements in the availability of high-resolution geographic information, investigators have become increasingly interested in quantifying the influence of landscape features on dispersal and subsequent population structure. As a result, two main areas of inquiry have taken hold. At broad spatial scales,

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often at the scale of species' geographic ranges, studies within the field of phylogeography (Avice *et al.* 1987) examine the geographic context of major evolutionary lineages with the goal of determining which landscape features have historically shaped diversification and the distributions of lineages. At the other end of the spectrum, studies within the nascent field of landscape genetics (Manel *et al.* 2003; Storfer *et al.* 2007) seek to quantify the effect of specific landscape variables on dispersal patterns and levels of genetic structure at fine spatial scales (e.g. over distances within the lifetime dispersal capabilities of individuals).

To date, most studies examining the influence of the landscape on dispersal and genetic structure have been conducted at either very broad or fine spatial scales. Consequently, the extent to which landscape processes operating at fine spatial scales are linked to patterns at broader spatial scales is largely unexplored. There are at least two possible patterns. On the one hand, factors influencing the movement of individuals at fine spatial scales may be important predictors of genetic structure at broader scales. For example, species that display reduced dispersal in relation to changes in elevation at local scales might be expected to exhibit major phylogenetic breaks in relation to mountain ranges. On the other hand, it is possible that processes operating at fine spatial scales have little bearing on patterns observed at broader spatial scales. For example, regional patterns of population connectivity may be influenced by multiple avenues of gene flow, diminishing the effects of local dispersal barriers on broader population structure. To distinguish between these alternative outcomes, studies that specifically link information on local landscape processes to the genetic patterns observed at broader spatial scales are needed.

Owing to their relatively limited mobility, sensitivity to dispersal barriers and strong microgeographic habitat associations, amphibians are regarded as highly appropriate species for examining landscape effects on genetic structure (Austin *et al.* 2002; Spear *et al.* 2005). The wood frog (*Rana sylvatica* or *Lithobates sylvaticus*) is a continentally distributed species whose range encompasses many different landscape features. Furthermore, being geographically widespread and common throughout much of its range, the wood frog has been the focus of several, fine-scale demographic and genetic studies (see Table S1, Supporting Information). Although the questions addressed, location and methodology of these studies vary, several landscape features are consistently implicated in influencing wood frog dispersal and population structure (Table S1). Specifically, aquatic features (Guttman *et al.* 1991; Rittenhouse & Semlitsch 2007; Zellmer & Knowles, University of Michigan, Ann Arbor, unpublished results), land cover (Gibbs 1998; Vasconcelos & Calhoun 2004; Meyer 2007; Crosby *et al.* 2009; Patrick *et al.* 2008), slope (Boone *et al.* 2006; Zellmer & Knowles, University of Michigan, Ann Arbor, unpublished results)

and moisture levels (Heatwole 1961; Regosin *et al.* 2005; Baldwin *et al.* 2006; Boone *et al.* 2006) have been shown to impact wood frog movement. The consistency of results among these fine-scale studies can be used to generate well-informed hypotheses regarding regional population structure of the wood frog.

In the present study, we ask whether landscape features that impact wood frog dispersal and population structure at fine spatial scales (generally less than 10 km) are important predictors of genetic structure at regional spatial scales (tens to hundreds of kilometres). We use the recently introduced method of isolation by resistance (IBR: McRae 2006) to determine whether genetic structure estimated from microsatellite data is correlated with increasingly complex models of landscape resistance to gene flow. We specifically test whether IBR models incorporating the landscape features listed above outperform a landscape-free model that only accounts for the Euclidean distance between sampling localities. If our landscape models are better correlated with genetic structure than our landscape-free model, it can be inferred that the factors influencing wood frog dispersal, microhabitat selection and population structure at fine spatial scales are also important predictors of genetic structure at broader spatial scales. Alternatively, lack of significant genetic structure in relation to the landscape at regional spatial scales would suggest that landscape effects do not transcend in scale. Finally, because the signature of historical events may be prominent in regional patterns of genetic structure, we address the potential confounding effects of history on our conclusions. Specifically, we use palaeogeophysical- and genetic-based measures of historical population connectivity to test for a correlation between observed genetic structure and historical events associated with patterns of postglacial colonization.

Methods

Sampling and genetic data

Sampling was conducted during the periods of March–July 2005. In total, 16 sites from Pennsylvania, New York, New Hampshire and Quebec were surveyed (Fig. 1; Table S2, Supporting Information). These sites belong to the eastern mitochondrial lineage identified by Lee-Yaw *et al.* (2008). Individuals within sampling sites were collected from the same or adjacent ponds (< 20 m), with Euclidean distances between sites ranging from 26 to 1654 km. Sampling involved taking toe clips from adult frogs or taking eggs and tadpoles when adults could not be found. To avoid sampling closely related kin when sampling larvae, only one egg per clutch was used in the genetic analyses and tadpoles were collected from different sections of the pond. Eggs were allowed to hatch before individuals were euthanized and subsets of all larval samples were raised to

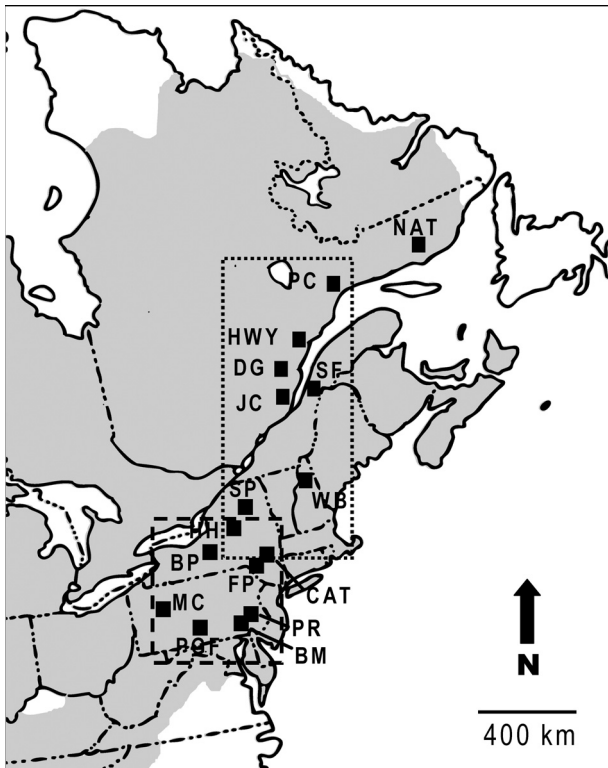


Fig. 1 Wood frog localities sampled in the present study. Site abbreviations correspond to those in Table S2. Grey shading denotes the species' geographic range in the east. Dashed lines indicate subsets of populations that were analysed in addition to the full data set (Subset 1, thick dashed line; Subset 2, thin dashed line).

metamorphosis in the laboratory to verify species' identity. In total, 288 individuals were sampled.

DNA was extracted from toes using a QIAGEN DNeasy tissue kit. DNA was extracted from larvae using the protocol outlined by Fetzner (1999). Individuals were genotyped for six of the microsatellite loci (D40, D20, D70, D88, C11, C52) described by Julian & King (2003). Polymerase chain reaction (PCR) and genotyping were done by the Genome Quebec and McGill University Innovation Centre (Montreal, Quebec, Canada). Briefly, PCRs contained 10 ng of genomic DNA in a total volume of 8 μ L. Amplification involved 40 cycles of denaturation at 95 $^{\circ}$ C for 30 s, annealing at 58 $^{\circ}$ C for 40 s and extension at 72 $^{\circ}$ C for 40 s followed by a final extension at 72 $^{\circ}$ C for 10 min. A mixture containing 2 μ L of PCR products, 0.15 μ L of GeneScan 500 Liz size standard (Applied Biosystems) and 8.5 μ L of Hi-Di Formamide (Applied Biosystems) was then run on Applied Biosystems 3730xl DNA Analyzer. Genotypes were analysed with Applied Biosystems GeneMapper 3.7 analysis software.

Prescreening of microsatellite loci and populations

The program GenAlEx 6.0 (Peakall & Smouse 2006) was used to calculate number of alleles, effective number of

alleles (corrected for expected heterozygosity), number of private alleles and expected and observed heterozygosities (Table S3, Supporting Information). The Markov chain estimation of P values from Fisher's exact test as implemented in GenePop 3.4 (Raymond & Rousset 1995) was used to test sites for deviations from Hardy–Weinberg equilibrium (HWE; 100 batches, 1000 iterations per batch) and to test loci for evidence of linkage disequilibrium (LD; 100 batches, 1000 iterations). Sequential Bonferroni correction (Rice 1989) was applied to the P values to correct for multiple comparisons. We used Micro-Checker 2.2.3 (Van Oosterhout *et al.* 2004) to check the data for putative null alleles and scoring errors.

Repeatability of allele scores was high (94%, based on genotyping 4.2% of the samples twice). Thirteen of the 16 sampling localities harboured private microsatellite alleles (Table S3). However, in only three cases did a private allele represent more than 10% of the total allelic variation at a given locus within a site, and the highest private allele frequency at a locus (at locus D20 in Natashquan) was 31.6%. Most populations met HWE expectations and there were no loci that consistently exhibited departures from HWE (Table S3). The locus with the most deviations from HWE was D20, which exhibited a deficiency of heterozygotes in seven (out of 17) populations after sequential Bonferroni correction. Four pairs of loci showed evidence for linkage disequilibrium, which is more than is likely to occur by chance alone ($L \sim 0.005$ following the method of Chapman *et al.* 1999). However, no loci were consistently linked within all or even a majority of populations, indicating that the loci in question are not strongly linked. Results from Micro-Checker indicate that one of the loci (D70) involved in two cases of putative LD may have null alleles in eight populations. D20 also showed evidence for null alleles in multiple populations. Although the results from these screens do not unambiguously call for the exclusion of any of our loci, we conducted subsequent analyses both with and without loci D20 and D70 to mitigate concern over potential departures from HWE, LD and null alleles. The exclusion of these loci did not affect the nature of our results. Finally, the low number of loci in general prompted us to explore the effects of each locus in turn on our conclusions. To do so, we jackknifed across all loci and reran all analyses. Results were consistent across all iterations of the data, indicating that no locus has a disproportionate effect on our results (Table S4, Supporting Information). Thus, we report the results from all loci combined.

We used GenAlEx 6.0 (Peakall & Smouse 2006) to test whether relatedness among individuals within samples where only larvae or larvae and adults were taken was higher than within samples comprised only of adults – a potential problem highlighted by Zamudio & Wiczorek (2007). The relatedness estimator X of Queller & Goodnight (1989) was used to estimate relatedness of individuals

within sites. We calculated the 95% CI for these values using 1000 bootstrap resamplings. Genotypes were permuted over sites 999 times and X was recalculated to generate a distribution of X expected if reproduction were random across sites.

Most sites did not exhibit higher relatedness than expected under a null distribution generated by 999 permutations of data from all sites (Fig. S1, Supporting Information). More importantly, there were no substantial differences in levels of relatedness among sites. Only three sites had elevated levels of relatedness: Natashquan, Hwy 385, and Port Cartier. In no case can these values be attributed to the sampling of the larval population. First, only adults were sampled from Natashquan. Second, only eggs were sampled from Hwy 385; as all eggs were collected from different clutches, the relatedness of this site reflects the extent to which the breeding adult population is related. Finally, only two larvae were sampled from Port Cartier. Thus, sampling strategy does not appear to introduce bias into our analyses and all populations and individuals were consequently included in our analyses.

Landscape data and landscape resistance models

We used the program Circuitscape 2.2 (McRae 2006) to model landscape resistance to gene flow among sampling sites based on those landscape features that consistently influence wood frog dispersal, microhabitat selection and gene flow at fine spatial scales: absolute dispersal barriers (such as the ocean), land cover, slope and wetness (Table S1). The algorithm in Circuitscape, borrowing from circuit theory, evaluates total landscape resistance between sampling sites based on multiple paths (McRae 2006). A multiple-path approach was chosen over the widely used least-cost path approach taken in other landscape genetic studies because, at the spatial scales considered here, it is unlikely that a single pathway will explain the movement of genes among widely separated regions over many generations.

The input for Circuitscape is a raster data set (map) in which each cell is assigned a conductance value corresponding to the relative probability of the study organism moving through the habitat type encoded by the cell. To generate the absolute dispersal barriers and land cover data sets, we acquired a 1-km-resolution digital land cover map of North America (Latifovic *et al.* 2004). This data set contains 110 detailed land classes. We aggregated these classes into six general categories representing basic land cover types that are hypothesized to influence wood frog dispersal: water (includes water bodies > 2 km in diameter and ice and snow), urban and built-up areas, grassland and agriculture, agriculture–woodland transition, forest and wetland. To assess landscape resistance based on absolute dispersal barriers alone, we assigned each of the land cover types an equal conductance value except for large water bodies

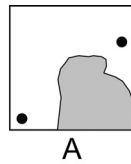
(including the ocean), which were given a conductance of 0, effectively masking them out.

To generate our land cover input file, we assigned different conductance values to each land cover type while maintaining the absolute dispersal barrier mask. Thus, the following assignments were made (where a low conductance value indicates a high resistance to dispersal): water = 0; urban and built-up = 1; grassland and agriculture = 4; agriculture–woodland transition = 6; forest = 8; wetland = 8. These ranks were chosen to reflect the relative order of conductance of these landscape features based on empirical studies of wood frog dispersal and habitat requirements (e.g. Gibbs 1998; Gibbs *et al.* 2005; Grant 2005; Patrick *et al.* 2006). In the absence of a study specifically comparing the magnitude of differences in the relative permeability of land cover types (e.g. akin to those of Stevens *et al.* 2004, 2006), the magnitude of these ranks, although reflecting expert opinion, are arbitrary. Subsequently, two sensitivity analyses were conducted to evaluate the extent to which the results are influenced by the magnitudes of these rankings. The results of the sensitivity analyses suggest that our overall conclusions are robust to the relative land cover resistance values used (Table S5, Supporting Information).

Data sets for slope and a wetness index were acquired from the USGS HYDRO 1k Elevation Derivative Database of North America (USGS 2006). In the slope data set, the value for each grid cell corresponds to the maximum change in the elevations between each cell and its eight neighbours (USGS 2006). The wetness index, also known as a compound topographic index, is a raster data set where the value for each grid cell is a function of the cell's upstream contributing area (e.g. the area draining through it from upslope) and the local slope angle of the landscape (Moore *et al.* 1991). Again cells corresponding to absolute dispersal barriers associated with large bodies of water were given a conductance of 0 in both of these data sets. All input data sets were reprojected to the Lambert Azimuthal Equal Area projection with common latitude and longitude bounds to ensure consistency in the spatial resolution of the geographic data and resampled to a 2-km spatial resolution to facilitate computation in Circuitscape.

Analyses

To evaluate the relative importance of various landscape features in predicting levels of genetic structure across the study region, we conducted a series of Mantel tests (Mantel 1967) examining correlations between pairwise genetic structure and increasingly more complex models of pairwise geographic distance (Fig. 2). First, we generated a matrix of landscape resistance in Circuitscape 2.2 (McRae 2006) based on an entirely 'flat' landscape; that is, a landscape in which all cells have equal resistance. We then tested for a significant pattern of isolation by landscape resistance



Model	Landscape Features (s) Incorporated	Example Input for CIRCUITSCAPE
IBR-Flat	Landscape-free model; Equivalent to isolation by (log) linear distance	<pre> 1 </pre>
IBR-Barriers (single variable)	Absolute Dispersal Barriers (Ocean and large bodies of water)	<pre> 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 0 0 0 0 1 1 0 0 0 0 1 1 0 0 0 0 </pre>
IBR-Barriers +Other (two variable)	1) Absolute Dispersal Barriers 2) Landcover OR Slope OR Wetness	<pre> 1 1 1 1 2 2 1 1 1 2 2 1 1 3 0 0 1 1 3 3 0 0 0 0 1 1 0 0 0 0 </pre> Variable 1 <pre> 1 3 2 2 1 1 1 3 1 1 1 1 1 1 0 0 1 1 2 1 0 0 0 0 1 1 0 0 0 0 </pre> Variable 2
IBR-Combined (three or four variable)	1) Absolute Dispersal Barriers 2) Landcover and Slope OR Landcover and Wetness OR Slope and Wetness OR Land cover, Slope and Wetness	<pre> 2 4 3 4 3 2 4 3 3 1 2 4 0 0 2 5 4 0 0 0 2 0 0 0 0 </pre> Variable 1 + Variable 2

B

Fig. 2 Strategy for testing the effects of increasingly complex models of geographic distance on the genetic structure of eastern wood frog populations. A simple example of a landscape (A) and schematic diagrams of the input files for Circuitscape corresponding to various tests of landscape resistance (B). Arrows point in the direction of increasing model complexity. Black dots denote sampling localities. Higher cell values indicate greater conductivity to gene flow. A conductance value of 0 indicates that gene flow is not permitted through that cell. Total landscape resistance is calculated based on multiple pathways through the input landscape as outlined in McRae (2006).

(IBR). This model serves as a landscape-free model of expected levels of genetic structure in relation to the physical distance between sampling sites alone and is expected to yield similar results as isolation by (log) Euclidean distance (IBD). However, unlike IBD which assumes an unbounded (infinite) landscape, this model accounts for the finite size of the input landscape being analysed and is therefore more appropriate for comparison with our other models than IBD.

Next, to evaluate the influence of absolute barriers to wood frog dispersal on genetic structure, we used Circuitscape to generate pairwise landscape resistance based on the landscape in which the ocean and other large bodies of water were given a conductance of 0 (see above). We then repeated the Mantel tests with this landscape resistance matrix and looked for a significant pattern of isolation by landscape resistance. To investigate additional effects of each of our chosen landscape features (slope, wetness and land cover), we tested for significant patterns of IBR based on each of the resistance matrices incorporating both absolute dispersal barriers and an additional landscape variable. Finally, we tested for the combined effects of multiple landscape features. To do so, we generated input maps for Circuitscape in which individual cells were given a value corresponding to the sum of the standardized resistances to gene flow of two or more landscape variables. We then used these input landscapes in Circuitscape to generate matrices of pairwise landscape resistance based on multiple landscape features and tested for significant IBR based on these matrices.

For the Mantel tests outlined above, multilocus estimates of F_{ST} (Weir & Cockerham 1984) between all population pairs were generated from the microsatellite data using FSTAT 2.9.3 (Goudet 2001). Significance was determined using the permutation test implemented in FSTAT with 2400 permutations. Pairwise F_{ST} values were linearized using the transformation, $F_{ST}/(1 - F_{ST})$ (Rousset 1997). All Mantel tests were conducted in the program IBDWS 3.15 (Jensen *et al.* 2005) with 10 000 matrix permutations to assess significance. All analyses were bootstrapped over population pairs in IBDWS (10 000 replicates) to generate 95% confidence intervals for the r^2 values. Comparison of the r^2 values allowed for assessment of relative model performance.

In addition, because the model incorporating absolute dispersal barriers alone explained a large proportion of the variation in gene flow (see Results) making it statistically difficult to detect the influence of additional landscape features on genetic structure, we recalculated landscape resistance based on all landscape variables and repeated the IBR analyses for two, smaller subsets of our total data set (Fig. 1). The first subset included sampling sites interior to the continent. These sites are not separated by large bodies of water for the most part, yet are located in a topographically diverse region. In this subset, we expect the effects of absolute dispersal barriers to be limited, potentially allowing for observation of the independent effects of other landscape features. The second subset included some populations separated by the St Lawrence River (Fig. 1), but was such that absolute barriers were no longer as

prominent as they are across the whole region due to the large continent–ocean boundary. For this subset, we expect absolute dispersal barriers to be a significant predictor of genetic structure. However, the relative importance of dispersal barriers should be lower than it is for the full data set, such that there is room for model improvement with the addition of other landscape features.

Finally, because historical patterns of colonization and population connectivity may be important predictors of genetic structure at broad spatial scales, we examined relationships between genetic structure and two different proxies of historical population connectivity. First, we determined the earliest time when each sampling locality was free of ice and glacial meltwater. Dates were based on the Geological Survey of Canada's Deglaciation of North America database (Open File 1574; Dyke *et al.* 2003) which codes information about glacial margins (based on C^{14} dating). We used the subset of maps depicting ice and meltwater at time intervals of 500 years for the period of 5000 to 18 000 years ago. Based on these maps, we generated a matrix of pairwise dates representing the earliest time since the last glacial maximum when pairs of sites were

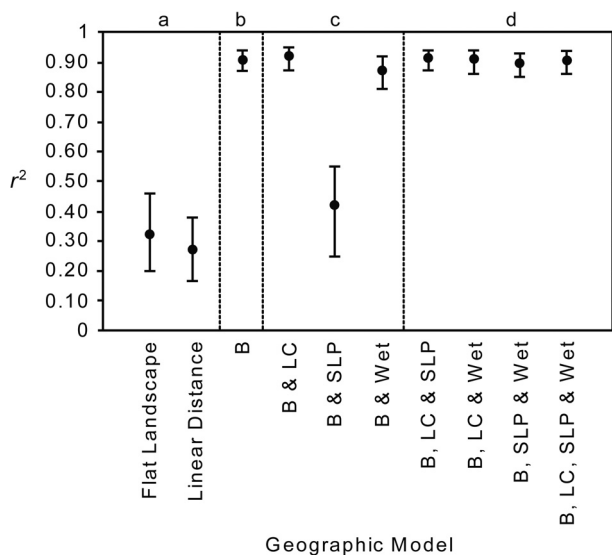


Fig. 3 Comparison of the r^2 values from Mantel tests of the relationships between genetic structure and increasingly complex models of geographic distance in eastern populations of the wood frog. All models were significant based on 10 000 permutations of the data. Ninety-five per cent confidence intervals were calculated by bootstrapping over population pairs. Lower-case letters above the graph denote the class of model as per Fig. 2 (a, landscape-free model; b, single landscape variable model; c, two-variable models; d, three- and four-variable models). Landscape variables are B, absolute dispersal barriers; LC, land cover; SLP, slope; Wet, wetness index. Isolation by linear distance is shown next to IBR based on a flat landscape for comparison. These two models are expected to yield identical results except for the edge effects incorporated by IBR.

potentially occupied and able to exchange migrants. For example, for a given pair of sites, if one site was deglaciated 12 000 years ago and the other 10 000 years ago, the earliest time for potential gene flow between the sites is 10 000 years ago. Previous studies have suggested that the wood frog was a primary colonizer that quickly moved north following glacial retreat (Holman 1992; Lee-Yaw *et al.* 2008). Thus we make the assumption that this matrix represents an early, but conservative estimate, of the possible ages of the various population connections.

As a second measure of historical population connectivity, we used Arlequin 3.01 (Excoffier *et al.* 2005) to estimate the average number of nucleotide differences between populations, π (Nei & Li 1979), from the mitochondrial sequence data (650-bp region corresponding to ND2/tRNA^{TRP}) provided for these localities ($n = 5$ –11 individuals/population, mean = 9.4) by Lee-Yaw *et al.* (2008). Significance was determined by 1000 permutations of the data. π is expected to increase linearly with divergence time and is thus related to the colonization history of populations. We tested the relationship between pairwise genetic structure based on microsatellites and these estimates of historical population connectivity with Mantel tests. We also used Mantel tests to determine the extent to which these two measures of historical population connectivity are correlated and thus the extent to which they provide separate information. As before, Mantel tests were conducted in IBDWS with 10 000 matrix permutations to assess significance.

Results

Pairwise genetic distances between sampling localities based on six microsatellites (Table S5), ranged from zero (Catskills-Frick Parking; Catskills-Herkimer/Hamilton; Frick Parking-Herkimer/Hamilton) to 0.254 (Natashquan-St Francois). Out of 120 pairwise comparisons, 116 were significant after sequential Bonferroni correction (Table S5). We observed a significant pattern of IBR based on the flat landscape at this spatial scale, although the proportion of variation explained was low ($r^2 = 0.32$, $P < 0.0001$). For comparison, we note that this model was not statistically significant from IBR based on Euclidean distances between sites ($r^2 = 0.27$, $P < 0.0001$).

Landscape resistance values that incorporated absolute dispersal barriers resulted in a significant and stronger relationship between landscape resistance and genetic structure than those based on a flat landscape (Fig. 3). Similarly, several models incorporating additional landscape features were much more highly correlated with genetic structure than the landscape-free model (Fig. 3). Based on 95% confidence intervals, only the model based on slope did not outperform the landscape-free model (Fig. 3). Notably, the incorporation of dispersal barriers alone was sufficient to achieve the marked improvement in the

Table 1 Results of Mantel tests testing the association between pairwise microsatellite [$F_{ST}/(1 - F_{ST})$] genetic structure and increasingly complex models of geographic distance among eastern wood frog populations in two subsets of the total data set. Subsets are as per Fig. 1

Geographic variable(s)	Subset 1			Subset 2		
	r^2	95% CI	P	r^2	95% CI	P
'Flat' landscape	0.17	0.002 to 0.57	0.04	0.16	0.005 to 0.48	0.0164
Barriers	0.20	0.003 to 0.58	0.03	0.69	0.41 to 0.87	< 0.0001
Land cover	0.27	0.012 to 0.67	0.005	0.63	0.34 to 0.85	< 0.0001
Slope	0.20	0.036 to 0.44	0.93	0.066	0 to 0.32	0.2
Wetness	0.06	0 to 0.37	0.16	0.58	0.27 to 0.81	< 0.0001
Land cover and slope	0.17	0.002 to 0.98	0.039	0.65	0.38 to 0.86	< 0.0001
Land cover and wetness	0.20	0.003 to 0.59	0.019	0.64	0.35 to 0.84	< 0.0001
Slope and wetness	0.076	0 to 0.408	0.13	0.63	0.34 to 0.84	0.0002
All variables combined	0.15	0.001 to 0.53	0.047	0.64	0.36 to 0.85	< 0.0001

relationship between genetic structure and geography. Landscape resistance calculated with oceans and large bodies of water coded as absolute barriers to movement explained 91% of the total variation in genetic structure ($P < 0.0001$). The incorporation of other landscape variables or combinations of other landscape variables did not further improve the relationship between genetic structure and geographic distance (Fig. 3).

The strong effects of the ocean–continent boundary (the largest landscape feature in the region we studied) on genetic structure may limit our ability to detect the effects of other landscape features on genetic structure (i.e. with limited sampling, it is difficult to statistically improve on a model with an r^2 value of 0.91). As such, we also report analyses of two subsets of the total landscape in which absolute dispersal barriers are not as prominent. As expected, for the first subset (which was chosen to minimize the degree to which sampling localities are separated by ocean or large bodies of water) the landscape resistance model incorporating absolute dispersal barriers did not outperform the landscape-free model ($r_{\text{flat}}^2 = 0.17$, $P = 0.04$; $r_{\text{barriers}}^2 = 0.20$, $P = 0.03$). However, none of the other isolation-by-resistance models incorporating other landscape variables or combinations of landscape variables outperformed the landscape-free model (Table 1).

For the second subset, which was chosen to incorporate the St Lawrence River but to reduce the total proportion of ocean in the landscape, there was a significant improvement in the relationship between genetic structure and geographic distance when absolute dispersal barriers were incorporated ($r_{\text{flat}}^2 = 0.16$, $P = 0.0164$; $r_{\text{barriers}}^2 = 0.69$, $P < 0.0001$); although by the criterion of non-overlapping confidence intervals, the difference in the r^2 values was just outside the limits of statistical significance (Table 1). Similar to what was observed for the entire region, none of the other landscape variables outperformed the model incorporating absolute dispersal barriers for subset 2, and, for this subset, did not statistically outperform the landscape-free model

(Table 1). The results for both subsets held regardless of whether the resistances between the selected sampling locations were the same as those used in the initial analyses for the entire region (not shown) or were recalculated in Circuitscape using a cropped input landscape of just the region occupied by the subset.

The significance of the effects of historical population connectivity on microsatellite-based genetic structure varied depending on the measure of historical population connectivity used. The putative age of population connections estimated from the timing of deglaciation was negatively correlated with microsatellite-based genetic structure ($r^2 = 0.24$, $P < 0.0001$) as well as with landscape resistance based on absolute dispersal barriers ($r^2 = 0.37$, $P < 0.0001$). In contrast, historical population connectivity estimated as π was not significantly correlated with microsatellite-based genetic structure ($r^2 = 0.02$, $P = 0.37$) nor with landscape resistance based on absolute dispersal barriers ($r^2 \sim 0$, $P = 0.37$). Finally, we note that the age of putative population connections is weakly associated with π ($r^2 = 0.13$, $P = 0.037$).

Discussion

Regional genetic structure of the wood frog and landscape effects

Our results suggest that wood frogs exhibit significant population structure at regional spatial scales (hundreds of kilometres). Pairwise genetic structure based on microsatellites was significant in 97% of our comparisons and we detected a significant pattern of isolation by resistance based on a flat landscape (equivalent to isolation by linear distance). These results indicate that the spatial scale considered is appropriate for studying the factors that influence population structure in this species. Following indications that slope (Boone *et al.* 2006; Zellmer & Knowles, University of Michigan, Ann Arbor, unpublished results),

moisture levels (Heatwole 1961; Regosin *et al.* 2005; Baldwin *et al.* 2006; Boone *et al.* 2006), land cover (Vasconcelos & Calhoun 2004; Meyer 2007; Crosby *et al.* 2008) and lakes (Zellmer & Knowles, University of Michigan, Ann Arbor, unpublished results) influence wood frog dispersal, microhabitat selection and population structure at fine spatial scales, we asked whether these landscape features are important predictors of genetic structure at larger spatial scales. In general, models of geographic distance incorporating the landscape outperformed the landscape-free model. However, our overall results suggest that the incorporation of absolute barriers to dispersal alone is sufficient for explaining over 90% of the variation in genetic structure observed.

Which landscape features are important at regional scales?

Our strategy of comparing the performance of increasingly complex landscape resistance models in analyses of IBR allowed us to deduce which landscape features have the largest impact on genetic structure. Our simplest landscape resistance model incorporated continent shape and the biological implausibility of wood frog movement through large bodies of water, such as the Great Lakes. Landscape resistance calculated with these restrictions was significantly better correlated with genetic structure than was the landscape-free model of geographic distance and explained 91% of the variation in genetic structure. Thus, absolute barriers to dispersal are highly useful predictors of genetic structure at regional spatial scales in this species. The inclusion of additional landscape features in the model of landscape resistance did not improve model performance over the incorporation of dispersal barriers alone (Fig. 3). None of the three or four landscape variable models outperformed the isolation-by-resistance model based on dispersal barriers alone (Fig. 3) and the model incorporating slope actually reduced model performance. Apart from a few main corridors, landscape resistance based on slope was very high (and thus connectivity low) across most of this topographically diverse region. Thus, the particularly poor fit of the landscape resistance model incorporating slope to the genetic data suggests that the connectivity of wood frog populations is independent of this landscape feature. Overall, our results suggest that, of the landscape variables tested, absolute dispersal barriers associated with the ocean–continent boundary and large bodies of water have the largest impact on the genetic structure observed among eastern wood frog populations.

Recognizing that the strong performance of the landscape resistance model incorporating absolute dispersal barriers may limit our ability to statistically detect the effects of incorporating additional landscape variables into the model, we repeated our analyses on two subsets of the total data set. Results from the first subset, chosen to minimize

absolute dispersal barriers in the landscape, suggest that land cover, slope and wetness provide little explanation for the observed patterns of genetic structure. Specifically, these models do not outperform the landscape-free model in this subset or the model incorporating dispersal barriers (Table 1). Results from the second subset, chosen to incorporate a major dispersal barrier (the St Lawrence River), mirror those observed for the full data set: incorporating dispersal barriers in the IBR analyses results in a larger r^2 value than is observed for the landscape-free model. This r^2 value is closely matched but not surpassed when other landscape variables are included in the model. However, in this subset, although the incorporation of absolute dispersal barriers results in some improvement in the r^2 value over that obtained from IBR, the difference is neither statistically significant nor as substantial as it is for the full data set. Thus, we argue that if other landscape variables were actually significant predictors of regional patterns of genetic structure in the wood frog, analysing this subset should have resulted in marked improvements in the performance of the landscape models as these other landscape variables were incorporated. That we did not detect such improvements suggests that land cover, slope and wetness, as modelled presently, are not significant predictors of the observed genetic structure. Overall then, the results of our analyses on both subsets provide additional support for the conclusion that landscape effects on wood frog genetic structure at regional spatial scales are limited. Instead, apart from the effects of geographic distance, genetic structure across the entire region appears to be best explained by the effects of absolute barriers to wood frog dispersal.

Our results are largely consistent with the genetic patterns observed at a continental scale. The wood frog is a continentally distributed species, yet it demonstrates very few phylogenetic breaks across its range (Lee-Yaw *et al.* 2008). In line with the results from our current analysis, those phylogenetic breaks that do exist do not align with any obvious landscape features, suggesting that colonization was largely independent of many landscape features. For instance, unlike other amphibians (see Austin *et al.* 2002; Zamudio & Savage 2003), northern range expansion of the wood frog was not influenced by the Appalachian Mountains, a prominent landscape feature in the region studied herein. Instead, phylogeographic patterns point to rapid colonization of all of Canada in the 10 000 years since the Late Pleistocene, limited only by the rate at which the landscape became free from ice and meltwater (Lee-Yaw *et al.* 2008). This result is consistent with our finding that only absolute dispersal barriers impact broad-scale patterns of genetic structure in this species.

The role of history

Patterns of genetic structure may result from both contemporary and historical processes. A lack of equilibrium

between contemporary gene flow and genetic drift can lead to error in the interpretation of genetic structure (Hutchison & Templeton 1999; Whitlock & McCauley 1999), especially at spatial scales beyond the single-generation dispersal capabilities of individuals. This problem may be particularly pervasive in studies of northern taxa that have only recently advanced their ranges following the last glacial retreat (Green *et al.* 1996; Rafiński & Babik 2000; Castric *et al.* 2001; but see Crispo & Hendry 2005) and whose population structure may more readily reflect historical patterns of colonization.

In the present study, we used two different estimates of historical population connectivity to determine the extent to which microsatellite-based genetic structure reflects historical processes. Our results were mixed but suggest that the effects of historical processes on microsatellite-based genetic structure are negligible relative to the effects of absolute dispersal barriers. On the one hand, the putative age of population connections as estimated from the timing of deglaciation is negatively correlated with genetic structure based on the microsatellite data, suggesting that a relatively short history of genetic exchange may contribute to high F_{ST} values between some sites. On the other hand, the correlation between this measure of history and F_{ST} ($r^2 = 0.24$) is weak relative to that between our best landscape resistance model and F_{ST} ($r^2 = 0.91$), indicating that landscape resistance is a much better predictor of genetic structure than the putative age of population connections.

Our second measure of historical population connectivity, π , further weakens the evidence of historical effects. This measure showed no correlation with pairwise F_{ST} based on the microsatellite data or with our landscape resistance model. This result suggests that the genetic structure we observe at the microsatellite loci and the corresponding associations with geography are relatively independent of historical population structure arising from the shared ancestry of these populations.

Taken together, these results suggest that population history is, at best, a weak predictor of current genetic structure, and that contemporary dispersal barriers play the strongest role in structuring populations. In particular, π should reflect not only the wood frog's colonization history but also the species' response to a broad suite of historical events, including historical patterns of land cover and climate change. That π is not correlated with microsatellite-based genetic structure suggests that the signatures of other historical events are not evident in the microsatellite data and do not confound conclusions from our isolation-by-resistance analyses.

Broader implications

The role of dispersal barriers in influencing broad-scale patterns of genetic structure has been widely appreciated. However, few studies have explicitly tested the relative

importance of absolute dispersal barriers vs. other aspects of the landscape in explaining regional patterns of genetic structure. The results of our study suggest that dispersal barriers may play a disproportionate role in driving patterns of diversity at broad spatial scales. This conclusion is consistent with the results from other studies conducted at regional spatial scales. For instance, Dionne *et al.* (2008) found that coastal distance is a strong predictor of regional levels of genetic structure in Atlantic salmon (*Salmo salar*), a species whose breeding range is restricted to adjacent shallow waters. Similarly, impenetrable dispersal barriers associated with dams are correlated with regional levels of genetic structure in yellow perch (*Perca flavescens*) (Leclerc *et al.* 2008). In contrast, several other landscape variables were not strong predictors of regional levels of genetic structure in either of these species (Dionne *et al.* 2008; Leclerc *et al.* 2008).

More generally, our results do not support the hypothesis that local landscape processes are important predictors of genetic structure at broader spatial scales (see also Castric *et al.* 2001). One possible explanation for the limited scale at which landscape effects are observed is that multiple avenues of gene flow serve to connect populations at broader spatial scales, reducing the overall impact of areas with high resistance to gene flow on levels of genetic structure. In the most extreme case, the only restrictions to population connectivity at broad scales would be those imposed by the continent for terrestrial species or the ocean for marine species. The high performance of models incorporating both ocean–continent barriers and multiple dispersal pathways in explaining continental patterns of genetic structure in big-leaf mahogany (*Swietenia macrophylla*) and wolverines (*Gulo gulo*) documented by McRae & Beier (2007) lends some support to this hypothesis. Overall, the results of several studies suggest that landscape effects on population structure may be limited in spatial scale, with only those landscape features that serve as absolute dispersal barriers for a given species impacting patterns of genetic structure at broader spatial scales.

Nevertheless, we caution against extrapolating our results to other species and systems. The wood frog has fairly high dispersal capabilities and can overcome many of the barriers that serve as obstacles to dispersal for other amphibians (e.g. Baldwin *et al.* 2006). Indeed, compared to what has been observed for the wood frog (Newman & Squire 2001; Squire & Newman 2002; Crosby *et al.* 2009), other amphibian species demonstrate much stronger genetic structure at fine spatial scales (Funk *et al.* 2005; Spear *et al.* 2005; Stevens *et al.* 2006; Giordano *et al.* 2007). In turn, regional genetic structure may be strongly correlated with landscape features in these species and in other types of organisms for which there are strong dispersal limitations. Ultimately then, to gain further understanding of the connection between fine-scale processes and large-scale patterns, additional

studies that integrate information across multiple spatial scales in species with a range of dispersal capabilities are needed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Mean within-population relatedness for eastern wood frog populations.

Table S1 Studies examining wood frog movement, dispersal, microhabitat selection and population structure at spatial scales < 30 km and implications for landscape variable selection in the present analysis of regional landscape genetics

Table S2 Collection sites and summary of samples

Table S3 Genetic diversity indices for populations and loci

Table S4 Results for the correlation between landscape resistance and wood frog genetic structure based on each landscape model after jack-knifing across all loci

Table S5 Sensitivity analyses evaluating the extent to which the relative land cover values chosen for input into CIRCUITSCAPE influence the final outcome of the isolation-by-resistance analyses

Table S6 Pairwise genetic structure among northeastern wood frog populations

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