

Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape

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Abstract

Dispersal is a fundamental attribute of species in nature and shapes population dynamics, evolutionary trajectories and genetic variation across spatial and temporal scales. It is increasingly clear that landscape features have large impacts on dispersal patterns. Thus, understanding how individuals and species move through landscapes is essential for predicting impacts of landscape alterations. Information on dispersal patterns, however, is lacking for many taxa, particularly reptiles. Eastern foxsnakes (*Mintonius gloydi*) are marsh and prairie specialists that avoid agricultural fields, but they have persisted across a fragmented region in southwestern Ontario and northern Ohio. Here, we combined habitat suitability modelling with population genetic analyses to infer how foxsnakes disperse through a habitat mosaic of natural and altered landscape features. Boundary regions between the eight genetic clusters, identified through assignment tests, were comprised of low suitability habitat (e.g. agricultural fields). Island populations were grouped into a single genetic cluster, and comparatively low F_{ST} values between island and mainland populations suggest open water presents less of a barrier than unsuitable terrestrial habitat. Isolation by resistance and least-cost path analysis produced similar results with matrices of pairwise individual genetic distance significantly more correlated to matrices of resistance values derived from habitat suitability than models with an undifferentiated landscape. Spatial autocorrelation results matched better with assignment results when incorporating resistance values rather than straight-line distances. All analyses used in our study produced similar results suggesting that habitat degradation limits dispersal for foxsnakes, which has had a strong effect on the genetic population structure across this region.

Keywords: conservation genetics, habitat degradation, population genetics, reptiles

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Introduction

Both evolutionary theory (e.g. Wright 1948; Slatkin 1987) and empirical data (e.g. Postma & van Noordwijk 2005) show that dispersal has large impacts on how genetic variation is distributed among populations. Indeed, estimating dispersal and gene flow is key to understanding local adaptation (Postma & van Noordwijk 2005), population genetic models of diversifica-

tion (Slatkin 1987), population connectivity and persistence for species of conservation concern (e.g. Cegelski *et al.* 2003). Thus, studying factors that promote or impede dispersal has long been a central theme in evolutionary ecology (Greenwood & Harvey 1982) and conservation biology (Frankham *et al.* 2002).

Recent studies show that species' habitat preferences coupled with landscape features modulate dispersal patterns influencing genetic population structure (e.g. Pierny *et al.* 1998; Castric *et al.* 2001). Thus, understanding how individuals disperse through complex landscapes is essential for predicting the impact that

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landscape alterations (e.g. habitat fragmentation) have on populations and for devising effective schemes to mitigate their effects (e.g. habitat corridors) (Fahrig 2007). Information on dispersal patterns, however, is lacking for many taxa, and this is particularly true for terrestrial reptiles (Bowne & Bowers 2004), despite their importance as top predators in many ecosystems (Schwaner & Sarre 1988; Tzika *et al.* 2008).

Methods for spatially quantifying genetic population structure and landscape effects have been developing rapidly (Manel *et al.* 2003; Balkenhol *et al.* 2009; Guillot *et al.* 2009). A popular technique for quantifying landscape effects is the assignment test (reviewed in Manel *et al.* 2005), which allows researchers to identify boundaries between populations and to move away from arbitrary delineations of populations based on geographical location alone (Manel *et al.* 2005; Zalewski *et al.* 2009). Combining assignment tests with surface interpolation of posterior probabilities (Guillot *et al.* 2005; Murphy *et al.* 2008; Pierson *et al.* 2010) and admixture coefficients (Durand *et al.* 2009) can diagnose population boundaries and regions of admixture on the landscape, but is underutilized in the literature (but see Murphy *et al.* 2008), particularly with three or more clusters.

When populations are continuously distributed, spatial genetic structure has traditionally been quantified using isolation by distance (IBD) models (individuals or populations) (Wright 1943; Rousset 2000; Frantz *et al.* 2009). Landscape effects can be incorporated into IBD models by using Mantel's nonparametric permutation tests (Mantel 1967; Slatkin 1993) to compare the fit of the relationship between matrices of genetic distinctiveness and straight-line geographical distance or matrices of resistance values based on landscape features. Traditionally, resistance values have been calculated using a 'least cost' path (LCP) model (Adriaensen *et al.* 2003) based on the estimated propensity for organisms to travel through different habitat types. A related and potentially more powerful method, isolation by resistance (IBR) (McRae 2006), uses circuit theory to quantify the amount of potential connectivity between populations and accommodates larger and/or more habitat corridors between populations. IBR approaches thus far appear to produce better results than least-cost paths (McRae & Beier 2007), but have not been thoroughly evaluated with multiple empirical data sets, especially those at fine scales.

Spatial autocorrelation analysis (Slatkin & Arter 1991) is another potentially powerful approach in the landscape genetics tool kit that compares the relatedness of individuals within spatial categories of increasing magnitude to the relatedness of randomly distributed pairs of individuals. Researchers often equate the scale of spatial genetic structure in continuous populations as

the geographical distance of positive spatial autocorrelation (Epperson & Li 1997). Populations, species or sexes that show positive spatial autocorrelation across greater spatial extents are viewed as having greater dispersal ability (Beck *et al.* 2008; Hardy *et al.* 2008). As with IBD models, incorporating pairwise least-cost paths or resistance values into spatial autocorrelation analysis instead of straight-line distances would seem more biologically realistic. Although this is easily accomplished using most popular spatial autocorrelation software, this is rarely tested in natural populations.

Eastern foxsnakes (*Mintonius gloydi*) are marsh and prairie specialists, but have persisted across southwestern Ontario and northern Ohio where most of these habitat types have been converted to agricultural land (Whitaker 1938). Here, we evaluate the effects that habitat conversion, loss and fragmentation have had on this marshland-prairie specialist and infer how foxsnakes disperse through a complex habitat mosaic of natural and altered landscape features. Specifically, we combine the results of habitat suitability modelling and genetic patterns inferred using assignment tests with spatial interpolation, IBD (with IBR and LCP models) and spatial autocorrelation analysis to address the following questions:

- 1 Do the number and extent of genetic populations identified using Bayesian assignment methods correlate with current habitat distribution patterns and landscape features (e.g. road and urban barriers; lake barriers)?
- 2 Does the predictive ability of isolation models and spatial autocorrelation analysis significantly improve when using landscape resistance values derived from habitat suitability modelling?

Although studies increasingly deploy these methods to incorporate landscape structure into population genetic analysis, few fully combine ecological, genetic and spatial analysis. For example, to our knowledge, ours is one of the first studies to combine the results of habitat suitability modelling with genetic analysis (but see Wang *et al.* 2008). Thus, as a secondary goal, we compare results among the three methods and also determine whether IBR outperforms LCP analysis for a relatively fine-scale individual data set.

Methods

Genetic sampling and microsatellite screening

Over the active seasons of 2006–2009, we hand captured foxsnakes from across southwestern Ontario, took a small blood sample (~200 µL stored in 95% ethanol)

from the caudal vein and visually determined the sex. We also took tissues from individuals killed on roads and acquired samples from researchers working in other regions (Ohio and Michigan) leading to a total of 589 samples (Fig. 1). This sampling range represents the majority of the distribution of two geographically disjunct regions (large distribution gap between Norfolk county and other populations) of eastern foxsnakes and comprises close to 60% of the current range of eastern foxsnakes (Fig. A1.1 in Appendix S1, Supporting information).

We extracted DNA from blood and tissue using QIAGEN (Venlo, Netherlands) DNeasy blood and tissue kit following the manufacturer's protocols. All samples were genotyped for 11 microsatellite loci (FS24, FS50, FS33, FS52, FS67, FS82, FS77, FS63, FS09B, FS42B, FSV16B, accession # EU294198–EU294208) developed specifically for this species (Row *et al.* 2008) and one additional locus (EOB10, accession # AF544655) developed for eastern ratsnakes (Blouin-Demers & Gibbs 2003). PCR reaction mixes were made up of 10 ng of genomic DNA, 1× Taq buffer with $(\text{NH}_4)_2\text{SO}_4$ (Fermentas), 0.2 μM forward and reverse primer, 0.1 mM of each nucleotide, 0.03 μM of Well RED fluorescent-labelled M13 primer (Boutin-Ganache *et al.* 2001), 0.25 U of DNA polymerase Taq (Fermentas) and concentrations of MgCl_2 specific to the microsatellite (Row *et al.* 2008). PCRs were performed in a GeneAmp 9700 or 2700 (Applied Biosystems) using the cycling profile: 5-min denaturation at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C; and a final extension of 72 °C for 5 min. PCR products were run on a Beckman Coulter CEQ 8000 capillary automated sequencer, and microsatellite sizes were scored using CEQ 8000 Genetic Analysis System.

Previous studies using these same loci found neither deviations from Hardy–Weinberg Equilibrium (HWE) nor linkage disequilibrium (Row *et al.* 2008), nor were null alleles prevalent (DiLeo *et al.* 2010). Because we use additional populations and loci (EOB 10), we again tested for HWE (100 batches, 1000 iterations per batch) and linkage disequilibrium (100 batches, 1000 iterations) using Fisher's exact tests as implemented in Genepop 4.0.1 (Raymond & Rousset 1995) and used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to test for scoring errors and null alleles. We split our samples into our 16 geographically defined 'populations' (Fig. 1; excluding the Chatham population) where we had samples with >10 individuals. In the MICRO-CHECKER analysis, only samples from the eight populations identified by clustering analysis were used.

Landscape quantification and habitat suitability

Across southwestern Ontario (Fig. 1), we used Ontario digital topographic maps (Ontario Base Map, Ontario Ministry of Natural Resources, scale of 1:10 000) as base maps for the major habitat types. These maps were generally out of date (collected from 1977 to 2000) and missing some important features (e.g. open semi-natural habitat). We therefore overlaid a grid ($\sim 5 \text{ km}^2$), and using 30 cm² resolution aerial photography taken in 2006 (SWOOP, Ontario Ministry of Natural Resources), we confirmed existing habitat features and added new features (>15 m² in size) resulting in a map with open water, semi-natural open, marsh, forest, residential/urban, agriculture, roads and small creeks/drains. Using these maps and 722 occurrence records spread across this region (see Fig. A1.1 in Appendix S1, Supporting information), we used Ecological Niche Factor

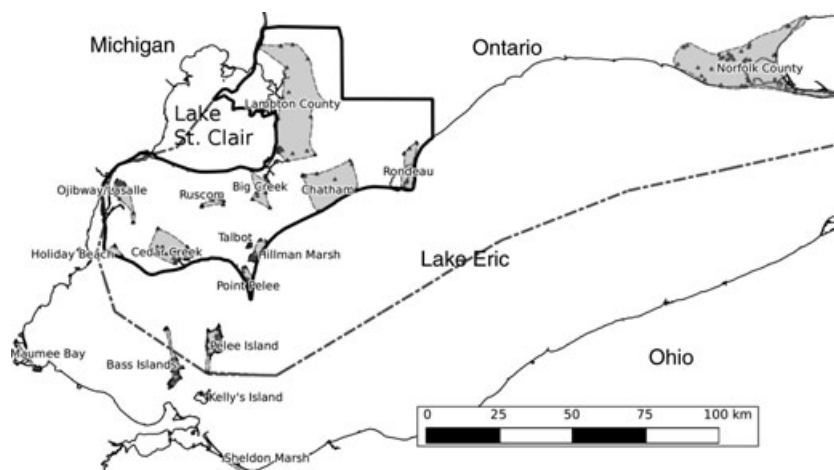


Fig. 1 Map of study area with black triangles representing sample locations of eastern foxsnakes and grey transparent polygon outlining populations for display purposes (see Fig. 2). Dark black line outlines region where detailed habitat modeling was completed.

Analysis (ENFA) (Hirzel *et al.* 2002) to determine landscape scale habitat preference patterns and developed two (40 × 40 m resolution) habitat suitability maps: (i) a ranked habitat suitability map with suitability scores between 0 and 100 and (ii) a grouped habitat suitability map with four habitat suitability classes: unsuitable, marginal, suitable and optimal, based on a plot of the predicted frequency of evaluation points in each habitat class to the expected frequency based on a random model (Fig. A1.2a in Appendix S1, Supporting information) (Hirzel *et al.* 2006). Both of these ecologically derived habitat suitability maps were used to develop landscape conductance and resistance scores (see IBR and least-cost path analysis). Ninety-nine per cent of individuals were found in habitat with a suitability ranking >2 suggesting that they rarely travel in low-quality habitat. We therefore added a fifth 'barrier' habitat class (habitat suitability = ranking between 0–2) for some of the fine-scale genetic analysis. A detailed account of the methods and results of the ENFA analysis can be found in Appendix S1 (Supporting information).

Assignment tests

Because of their superiority at detecting fine-scale population structure when genetic clusters are spatially distinct (Chen *et al.* 2007) (which is expected for a relatively low motility species such as snakes), we chose clustering programs that explicitly incorporate spatial information. There are now multiple techniques for individual clustering with spatial options, each of which make different assumptions about the data (Guillot *et al.* 2009). We therefore used two approaches to test whether our results were consistent. We used the program Bayesian Analysis of Population Structure (BAPS 5.1) (Corander *et al.* 2008) because of the low subjectivity involved in the methods for choosing the number of clusters (k). BAPS outputs a probability for the number of clusters, which may or may not match the maximum number of clusters (max k). Because the probability for the number of clusters can vary with max k , we ran BAPS using individual spatial clustering with 20 replicates for each of max k set to 10, 15, 20 and 25. We conducted admixture analysis using the number of clusters chosen in the nonadmixture analysis with 200 iterations, 200 reference individuals and 20 iterations for each reference individual (Corander & Marttinen 2006).

We also used TESS 1.3 (Chen *et al.* 2007), which has a spatial option and allows for a detailed admixture analysis (Durand *et al.* 2009). Using TESS, we ran 70 000 (20 000 burn-in) MCMC iterations 10 times from $k = 3$ to $k = 12$ using nonadmixture analysis. The ideal cluster

number was chosen based on when the deviance information criterion (DIC) values reached a plateau and/or the Q-matrix of individual posterior probabilities stabilized (no additional clusters became apparent). Following the choice of the number of clusters, we ran an additional 60 replicates for that number of clusters and averaged the top models (based on DIC) in clumpp 1.2 (Jakobsson & Rosenberg 2007) and displayed clusters using DISTRUCT 1.1 (Rosenberg 2004). We chose the number of models to average based on the distribution of DIC values. We estimated admixture proportions, using the number of clusters established with the nonadmixture analysis, with a conditional autoregressive (CAR) Gaussian model with a trend degree of two (Besag 1975; Durand *et al.* 2009). We again conducted 60 runs with 70 000 (20 000 burn-in) MCMC iterations, and averaged the top runs in CLUMPP 1.2 and displayed using DISTRUCT 1.1.

For the nonadmixture analysis, we considered an individual as a migrant if its genotype implied that it originated from a population other than where it was captured ($P > 80\%$ of nonmembership) and an individual not assigned to any population with $P > 80\%$ probability as having unknown ancestry. In the admixture analysis, BAPS tests for individuals showing significant levels of admixture ($\alpha = 0.05$) (Corander & Marttinen 2006) and, comparing DIC values in TESS, can establish if using admixture provides a better fit for the data (Durand *et al.* 2009). For comparative purposes, we also ran a nonspatial, admixture analysis using Structure 2.3.3 (Pritchard *et al.* 2000) (see Appendix S2, Supporting information).

We subsequently determined the extent of differentiation between, and patterns of genetic diversity within, identified genetic clusters by calculating pairwise F_{ST} (Weir & Cockerham 1984) and Jost's D (Jost 2008) between all clusters and expected heterozygosity [H_e – corrected for sample size; (Nei 1978)], mean number of alleles, standardized allelic richness (Hurlbert 1971) and mean F_{IS} within clusters using Microsatellite analyzer 4.05 (Dieringer & Schlotterer 2003) and SMOGD 1.2.5 (Crawford 2010).

Spatial kriging

Using the R (The R Core Development Team 2009) package *spatial* 7.2 and *gstat* 0.9, we mapped the extent of genetic clusters and identified barriers between clusters using ordinary kriging surface interpolation (Ripley 1981) of admixture proportions. For all clusters, we fit a zero polynomial (constant) trend surface regression with an exponential covariance function to the admixture proportions (psill = 1, nugget = 0) for each cluster, with a range parameter of 100 000 (100 km), and

extrapolated the trend over the study area at a resolution of 500 m. This resulted in eight maps, equal to the number of genetic clusters, with each map extrapolating the admixture proportions (proportion of genotype belonging to that particular cluster) across the study area. We identified common barriers by combining kriging maps of all the clusters and taking the maximum values. Therefore, we considered areas with low values in the combined map to be boundary regions between genetic clusters. The surface interpolation extrapolates trends beyond areas with samples, so patterns in zones with large sampling gaps and in nonusable habitat (e.g. lakes) must be interpreted with caution.

We determined whether genetic boundaries were spatially related to regions of low-habitat suitability by overlaying the habitat suitability map on top of the kriging surface maps across southwestern Ontario where detailed habitat suitability maps were developed (Fig. 1). Subsequently, we tested whether mean admixture proportions were lower (i.e. boundary regions between genetic clusters) within lower habitat suitability classes using a one-way ANOVA.

IBR and least-cost path analysis

Across southwestern Ontario, where detailed habitat maps were available (Fig. 1), we conducted IBR and LCP analysis. Resistance values for the analysis are often not derived from ecological data and most studies test a series of models with a variety of costs assigned to landscape features (e.g. Schweiger *et al.*

2004; Cushman *et al.* 2006; Quéméré *et al.* 2010). A rarely used alternative approach is to use the results of habitat suitability modelling (Wang *et al.* 2008). We used a method similar to Wang *et al.* (2008) and derived landscape costs using habitat suitability scores derived from the ENFA analysis (see Appendix S1, Supporting information). In the IBR analysis, we used the conductance settings, with higher values (i.e. higher suitability) having a greater conductance (i.e. lower landscape resistance). Using the habitat suitability scores, we derived six models based on: (i) the habitat suitability values produced from the ENFA analysis, (ii) the five grouped habitat suitability classes (barrier, unsuitable, marginal, suitable and optimal – see Landscape quantification and habitat suitability) and (iii) suspected barriers on the landscape (e.g. major highways and urban centres) (Table 1). Values for grouped models (Cond2, Cond3, Cond5, Cond6) were the average value of the predicted/expected score of the ENFA analysis (Fig. A1.2a in Appendix S1, Supporting information) within that habitat suitability class. These models were compared to a model with all landscape values equal to a conductance of 1, which is analogous to a straight-line distance model, but bounded by the study area and therefore a more direct comparison between models (Lee-Yaw *et al.* 2009). Pairwise resistance scores between individuals were calculated using CIRCUITSCAPE 3.5 (McRae 2006). CIRCUITSCAPE uses electrical theory to measure electrical resistance (measured in ohms) between sampling locations based on the assigned resistance or conductance values (in our case

Table 1 Conductance and resistance models used for isolation by resistance (IBR) and least-cost path analysis

Model	Unsuitable	Marginal	Suitable	Optimal	Barriers
IBR					
Condeq	1	1	1	1	None
Cond1	1–30	31–45	46–80	81–101	None
Cond2*	1	4	6	10	None
Cond3*	1	4	6	10	0–2 HS score†
Cond4	1–30	31–45	46–80	81–101	Major 4 lane highway*; urban centres
Cond5*	1	4	6	10	4 lane highway; urban centres
Cond6*	1	4	6	10	0–2 HS score†; 4 lane highway; urban centres
Least-cost path analysis					
Costeq	1	1	1	1	None
Cost1	101–81	80–46	45–31	30–1	None
Cost2*	10	6	4	1	None
Cost3*	10	6	4	1	0–2 HS score†
Cost4	101–81	80–46	45–31	30–1	Major 4 lane highway*; urban centres
Cost5*	10	6	4	1	4 lane highway; urban centres
Cost6*	10	6	4	1	0–2 HS score†; 4 lane highway; urban centres

Models were derived from habitat suitability (HS) scores derived from ecological niche factor analysis. See text for additional details.

*Gaps in highway barrier were left at major drains and rivers underpasses.

†99% of locations were in habitat with a score >2.

conductance) provided for the landscape (McRae *et al.* 2008).

We used a similar method to develop landscape resistance values for the LCP analysis. We needed to develop resistance and not conductance scores, however, and so we used values opposite to the conductance values (Table 1). As with the IBR analysis, we compared cost models to a model with all landscape values equal to 1. All pairwise least-cost distances were derived using the *PATHMATRIX* 1.1 (Ray 2005) extension in ArcView 3.2 (ESRI, Redlands, CA, USA).

For both the IBR and LCP analysis, we used Mantel's tests (Mantel 1967) to determine whether matrices of pairwise individual genetic distances were more highly correlated to landscape-derived resistance values or resistance values based on an equal landscape. Subsequently, we used partial Mantel's tests (Smouse *et al.* 1986) to determine whether there was a significant correlation between genetic distance and landscape-derived resistance values when controlling for straight-line distance (resistance values based on an equal landscape) and *vice-versa*. Using SPAGeDi 1.3 (Hardy & Vekemans 2002), we calculated pairwise genetic differentiation between individuals using both Loiselle's kinship coefficient (Loiselle *et al.* 1995), as it has been shown to be the best estimator in comparative tests (Vekemans & Hardy 2004), and Rousset's 'a' genetic distance (Rousset 2000) because it does not rely on a reference population and is analogous to $F_{ST}/(1 - F_{ST})$ (Rousset 2000) and so more appropriate for larger scales (Calderon *et al.* 2007).

We calculated Mantel's and partial Mantel's correlation coefficients (r) using the *ecodist* 1.2.2 package (Goslee & Urban 2007) in R (R Core Development Team 2009). Significance was determined with 9999 permutations, and 95% bootstrap confidence intervals were determined with 1000 iterations.

Spatial autocorrelation analysis

Across southwestern Ontario where we had detailed habitat maps (Fig. 1), we also compared the results of spatial autocorrelation analysis using straight-line distances and resistance values. Because there are no direct tests available to compare these two approaches, we compared the scale of spatial genetic structure (i.e. geographical distance or resistance where the autocorrelation function crosses the x -axis) using both straight-line and resistance values. We expected that the scale of spatial genetic structure would closely match genetic populations (e.g. individuals spaced $>$ the scale of positive autocorrelation would be assigned to separate genetic clusters). We determined the scale of spatial genetic structure using both straight-line distances and

resistance scores (using the best model from the Mantel's analysis) by calculating Loiselle's kinship coefficient (Loiselle *et al.* 1995) for all pairwise comparisons within increasing spatial distances and resistance categories. Spatial categories were based on an even distribution of the number of pairwise comparisons within 25 categories, and calculations were made using SPAGeDi 1.3 (Hardy & Vekemans 2002). We estimated the scale of autocorrelation by determining the distance and resistance values where the kinship coefficient dropped to or below zero (Sokal 1979; Epperson & Li 1997). We subsequently compared the results with assignment tests by mapping connections between individuals that were spaced less than the scale of the spatial genetic structure using straight-line and resistance values. If using resistance values provided a more 'biologically realistic' measure of the scale of genetic structure, then we expected fewer lines to cross between genetic clusters identified through assignment tests.

Results

Microsatellite screening

After sequential Bonferroni correction (Rice 1989), we found no evidence for deviations from HWE for any loci in any of the populations. Using the 16 geographically defined populations, we found two pairs of loci to be in linkage disequilibrium (FS82 & FS77; FS33 & FS67) for two populations (Peele Island and Bass Island). Because these trends were not seen in any other population, both loci were retained.

MICRO-CHECKER found no evidence of scoring errors, but did imply null alleles for three loci (FS77, FS67, FS52) for three different populations. Again, because there was no consistent trend across populations for any of the loci and because no individual sample ever failed to amplify for a particular locus, null alleles are not a pervasive problem and we retained all loci for analysis.

Assignment tests

Results of our *BAPS* analysis indicated that the most likely number of clusters was 8. The probabilities for eight genetic clusters were 1, 1, 0.76 and 0.91 when setting max k to 10, 15, 20 and 25, respectively. Based on a bar plot of the Q -matrix, there were five groups of two or more of our original 17 sampling locales: GR1 – Ojibway/Lasalle and Holiday Beach, GR2 – Ruscom, Big Creek, Lambton, and Chatham, GR3 – Rondeau and Sheldon Marsh, GR4 – Maumee Bay, Bass Islands, Kelly's Island and Peele Island and GR5 – Point Peele and Hillman Marsh. These groups were diagnosed in all analyses (Fig. 2) and, thus,

we consider these groups as genetic clusters and defined migrants as individuals not assigned to these. In the BAPS nonadmixture analysis, most individuals had a high probability of belonging to their own genetic cluster with only 14 individuals showing evidence of being a migrant and seven classified as unknown ancestry (<0.80 probability to any cluster). Admixture analysis produced very similar results to the mixture analysis with only nine individuals showing significant evidence ($\alpha = 0.05$) of mixed ancestry (Fig. 2a).

For our TESS analysis, the DIC values and the Q-matrix stabilized with the number of clusters also equal to eight. After running 60 replicates with $k = 8$, we averaged the top 30% (17 clusters) in CLUMPP (Jakobsson & Rosenberg 2007). We chose the top 30% because all of the DIC values were similar up until that point and then increased. The genetic clusters were also similar to the BAPS analysis with the same groupings of populations. There was more uncertainty in the bar plot with 45 individuals classed as having unknown ancestry and 15 identified migrants (seven of

those were the same as in the BAPS analysis). When using the admixture analysis, the DIC values for eight clusters dropped considerably (nonadmixture lowest run = 29 431, admixture lowest run = 28 723) suggesting that the admixture model had a better fit. From the 60 replicates, we only imported the top 10 models into CLUMPP because of a large increase in DIC values after the first 10 clusters. The level of admixture suggested by TESS was greater than suggested by BAPS (Fig. 2b). Using the nonspatial admixture analysis in Structure produced similar results, but only identified seven genetic clusters (see Appendix S2, Supporting information).

Differentiation between genetic clusters was highly significant ($P < 0.001$) for all pairwise comparisons and ranged from 0.04 to 0.28 (Table 2). GR2 (0.09) and GR4 (0.09) had the lowest mean pairwise F_{ST} values, and Cedar (0.17) and Norfolk (0.20) populations had the greatest mean F_{ST} values. Genetic diversity within genetic clusters was similar (Table 3) with the exception of the Norfolk county cluster, which had lower

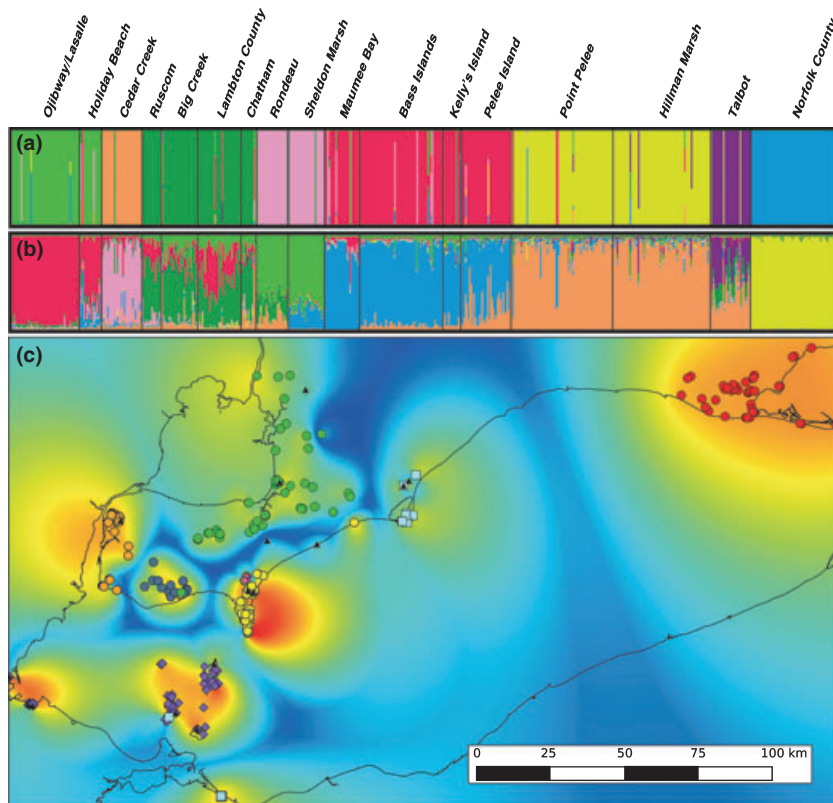


Fig. 2 Bar plots representing admixture coefficients for eastern foxsnakes from a spatial assignment test performed in (a) BAPS 5.1 and (b) TESS 2.3. (c) The geographical representation of admixture coefficients through spatial kriging with low (cool colours) to high (hot colours) representing mean (TESS and BAPS) admixture proportions. Individuals are classed from the non-admixture analysis in BAPS and TESS with different colour and/or shapes representing different clusters. Black triangles represent individuals where there was a discrepancy between the two programs or neither program assigned the individual to a cluster with >80% probability. See Fig. 1 for spatial reference.

Table 2 Pairwise F_{ST} values (bottom) and Jost's D differentiation values (top) between genetic clusters (see Fig. 1 for population distribution and Fig. 2 for cluster results) of eastern foxsnakes in southwestern Ontario and northwestern Ohio

	GR1	Cedar	GR2	GR3	GR4	GR5	Talbot	Norfolk
GR1*		0.18	0.03	0.11	0.07	0.08	0.17	0.11
Cedar	0.18		0.17	0.15	0.17	0.18	0.16	0.20
GR2†	0.04	0.15		0.06	0.04	0.09	0.11	0.14
GR3‡	0.10	0.21	0.06		0.05	0.09	0.16	0.09
GR4§	0.07	0.15	0.05	0.09		0.04	0.12	0.14
GR5¶	0.10	0.18	0.10	0.14	0.05		0.08	0.09
Talbot	0.13	0.16	0.09	0.16	0.09	0.08		0.18
Norfolk	0.19	0.36	0.20	0.22	0.20	0.17	0.28	
Mean F_{ST}	0.10	0.17	0.09	0.12	0.09	0.10	0.12	0.20

All pairwise values were highly significant ($P < 0.001$).

*Ojibway and Holiday Beach populations.

†Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations.

‡Rondeau and Sheldon Marsh populations.

§Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations.

¶Point Pelee and Hillman Marsh populations.

Table 3 Sample size, expected heterozygosity (H_e), mean number of alleles (MNA), allelic richness (AR) and F_{IS} for genetic clusters of eastern foxsnakes (Fig. 2) in southwestern Ontario and northwestern Ohio. Standard deviation is given in brackets

	N	H_e	MNA	AR	F_{IS}
GR1*	62	0.60 (0.13)	4.33 (1.61)	4.06 (1.34)	0.02 (0.13)
Cedar	28	0.53 (0.14)	4.00 (0.95)	3.99 (0.95)	0.07 (0.16)
GR2†	78	0.63 (0.12)	5.42 (1.62)	4.56 (1.11)	0.12 (0.06)
GR3‡	47	0.50 (0.17)	4.08 (1.51)	3.73 (1.29)	0.03 (0.17)
GR4§	126	0.61 (0.13)	5.33 (1.83)	4.55 (1.40)	0.05 (0.04)
GR5¶	141	0.53 (0.21)	4.92 (1.73)	4.19 (1.49)	0.02 (0.05)
Talbot	28	0.58 (0.16)	3.83 (1.40)	3.82 (1.40)	-0.01 (0.15)
Norfolk	79	0.31 (0.19)	3.25 (1.29)	2.88 (0.99)	0.11 (0.10)

*Ojibway and Holiday Beach populations.

†Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations.

‡Rondeau and Sheldon Marsh populations.

§Maumee Bay, Bass Islands, Kelly's Island and Pelee Island populations.

¶Point Pelee and Hillman Marsh populations.

allelic richness and expected heterozygosity than the other populations.

Spatial kriging

Because there were differences in the level of admixture in the BAPS and TESS analyses, we derived separate kriging surface maps for each and then combined the maps by calculating the mean pixel values. This combined surface map and nonadmixture genetic assignments identified a number of boundary regions on the landscape (Fig. 2c). All the island populations were grouped together with the mainland population in far northwestern Ohio and southeastern Michigan. The rest of the mainland populations were grouped separately

from island populations in Ontario and Ohio, but the differences were not as sharp as with some of the mainland populations (Fig. 2c). Seven of the eight genetic populations were distributed across mainland Ontario with steep differences in admixture proportions between most of the clusters.

Admixture proportions varied significantly among the habitat suitability classes ($F_{4,18439} = 3059$, $P < 0.001$), and Tukey HSD tests revealed that the three highest suitability (marginal, suitable, optimal) classes were significantly higher than the two lowest classes (barrier and unsuitable; Fig. 3a). Overlaying the barrier habitat class over the admixture proportions map demonstrated that most regions with low admixture proportions consisted of this barrier habitat (Fig. 3b).

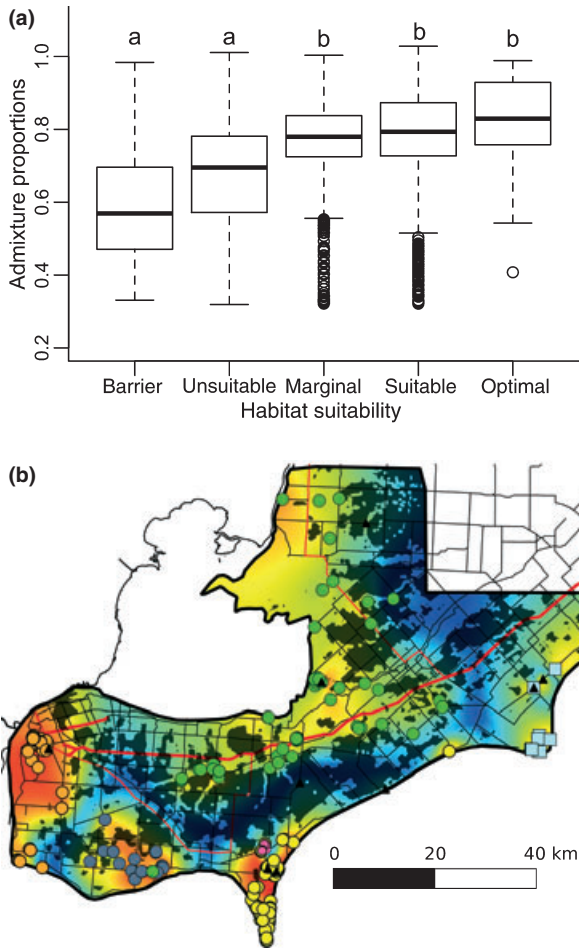


Fig. 3 (a) Box plots of differences in admixture proportions (derived from BAPS and TESS assignment tests and extrapolated onto the landscape using surface interpolation) within habitat suitability classes. Boxes with different symbols are significantly different. (b) Barrier habitat suitability class overlaid on the geographical representation of admixture proportions (see Fig. 2 and text for details).

IBR and least-cost analysis

When using both Loiselle’s kinship coefficient and Rousset’s ‘a’ in the IBR analysis, the trends were similar. All Mantel’s tests, including an analysis with an equal landscape (i.e. simple IBD), revealed matrix correlations that were highly significant ($P > 0.001$). Models that used habitat suitability scores (with and without barriers), however, had significantly higher correlations (nonoverlapping 95% confidence intervals) than the model only considering an equal landscape (Fig. 4a). Using habitat groups (without the barrier class) did not significantly increase the correlation over the correlations simply using classes 1–100 (Cond1 vs. Cond2 and Cond4 vs. Cond5; Fig. 4a). When including the barrier class set as an absolute barrier (zero conductivity for

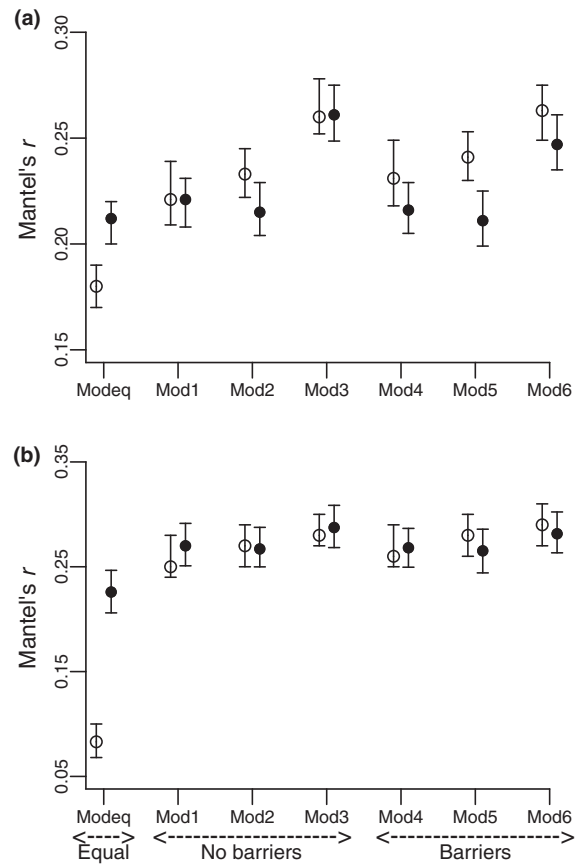


Fig. 4 Absolute values of Mantel’s correlation coefficients (with 95% bootstrap confidence intervals) comparing matrices of pairwise genetic distance for (a) Loiselle’s kinship coefficient and (b) Rousset’s ‘a’ genetic distance. Resistance values were derived from isolation by resistance (IBR) (open circles) and least-cost (closed circles) models. See Table 1 and text for details on models. Modeq and Mod1 through Mod6 are the Cond (IBR data) and Cost (‘least cost’ path data) models in Table 1. See Table 1 and text for additional details.

habitat suitability scores ≥ 2) (Cond3 and Cond6; Fig. 4a), the Mantel’s r was higher than all other models (significantly higher for Loiselle’s kinship, but not for Rousset’s distance). We found similar correlations between models with urban areas and a four-lane highway set as absolute barriers and models without these barriers (Fig. 4a).

Similar patterns were found with LCP analysis with a few minor differences. When using Loiselle’s kinship coefficient, Cost1, Cost2, Cost4 and Cost5 had overlapping confidence intervals with the equal landscape model. As with the IBR models, models with Cost3 and Cost6 had the highest Mantel’s r values (Fig. 4b). The 95% bootstrap confidence intervals of these models did not overlap with the equal landscape model and were similar to the IBR values. When using Rousset’s ‘a’ distance, all cost models were greater than the equal

landscape and were very similar to the values with the IBR analysis (Fig. 4b).

Partial Mantel's tests using the IBR values of the best habitat models (Cond3 and Cond6) and straight-line distance (Condeq model) confirmed the importance of landscape in explaining differentiation patterns between individuals. The correlations between pairwise Rousset's 'a' genetic distance matrices and landscape-derived resistance matrices (Cond3 and Cond6), while controlling for straight-line distance (Condeq), were all significantly positively correlated, as expected (Table 4). None of the partial Mantel's tests, however, showed a significant positive correlation when comparing Rousset's 'a' to straight-line distance (Condeq), when controlling for landscape-derived resistance matrices (Cond3 and Cond6) (Table 4). All partial Mantel's tests were significant (negative correlation expected) when using Loiselle's kinship coefficient, but were significantly higher when comparing genetic distance to Cond3 and Cond6 and controlling for straight-line distance (Condeq) (Table 4).

Spatial autocorrelation analysis

Using Euclidean distances, the scale of spatial autocorrelation steadily declines and the kinship coefficient drops below zero between 16.2 and 20.9 km; thereafter, the kinship coefficient declines and remains below zero (Fig 5a). We therefore considered the scale of spatial genetic structure to be 18.5 km (mid-point between last category with relatedness >0 and first point <0). When

Table 4 Results of partial Mantel's test comparing matrices of pairwise genetic distance [Rousset's 'a' (Rou 'a'); Loiselle's kinship (Kin)] and resistance values derived from isolation by resistance

Correlation	Controlled	Mantel's <i>r</i>	<i>P</i> -value >0*
Rou 'a' × Condeq	Cond3	-0.06	0.98
Rou 'a' × Condeq	Cond6	-0.06	0.98
Rou 'a' × Cond3	Condeq	0.28	0.0001
Rou 'a' × Cond6	Condeq	0.29	0.0001
Correlation	Controlled	Mantel's <i>r</i>	<i>P</i> -value <0†
Kin × Condeq	Cond3	-0.07	0.0001
Kin × Condeq	Cond6	-0.07	0.0001
Kin × Cost 3	Condeq	-0.21	0.0001
Kin × Cost 6	Condeq	-0.20	0.0001

Condeq model included an equal landscape (all values = 1) and therefore analogous to straight-line distance. See Table 1 and text for details on additional models.

*Rousset's *a* should increase with increasing distance.

†Loiselles kinship coefficient should decrease with distance.

using pairwise resistance from the values derived from the Cond3 model (provided best results in the Mantel's analysis), there was a sharp decline with the kinship coefficient dropping below zero between resistance values of 2.04 and 3.78 (Fig 5b) and we thus considered the scale of genetic structure to be 2.91 (mid-point between 2.04 and 3.78). We subsequently compared plots with lines connecting individuals greater than 18.5 km apart (Fig. 5c) and with lines connecting individuals that had greater than 2.91 Ω between them (Fig. 5d) to the assignment results. All genetic clusters were connected when using straight lines distances, but when using resistance values, only individuals from the Cedar and Holiday Beach populations and Talbot and Hillman are connected despite being classed in different genetic clusters.

Discussion

Role of landscape features on dispersal and population structure

As expected from the preliminary work presented by DiLeo *et al.* (2010), we found striking genetic population structure across fine geographical scales (tens of kilometres) for eastern foxsnakes. Using additional samples (589 samples vs. 114 samples) and populations (Lake Erie island populations, Norfolk population), we found an additional three genetic clusters not identified in DiLeo *et al.* (2010). Our main goal here was to use the results of habitat suitability modelling to determine whether habitat distribution and quality has impacted dispersal patterns, leading to this structure. A pilot study based on 40 eastern foxsnakes from across this region showed that all possessed identical haplotypes for 700 bp of the mtDNA cytochrome *b* region (J. R. Row & S. C. Loughheed, unpublished data). Therefore, deep historical factors (e.g. separate glacial refugia and subsequent contact zones, which are common in this area (Austin *et al.* 2002) were unlikely to confound our analyses aimed at evaluating the effects of habitat distribution on dispersal.

The majority of landscape genetic studies to date have used a series of models based on broad notions of habitat use to determine which habitat types (e.g. forest cover, marsh distribution) or landscape features impact genetic differentiation between individuals or populations (e.g. Lee-Yaw *et al.* 2009; Schwartz *et al.* 2009; Quéméré *et al.* 2010). By combining our genetic results with explicit habitat suitability modelling, we objectively established the effects of habitat distribution and quality on population structure and dispersal.

Our Bayesian assignment tests revealed that seven of eight genetic clusters were distributed across southwest-

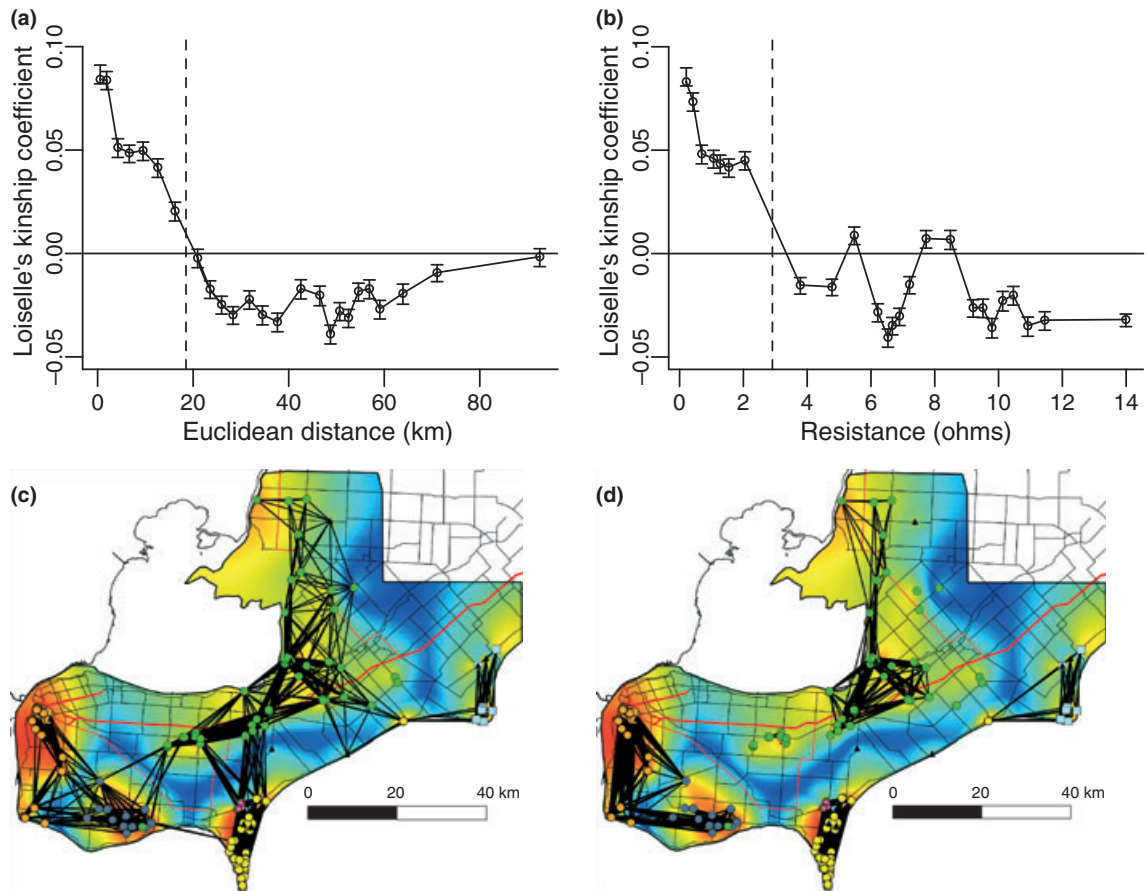


Fig. 5 Spatial autocorrelation correlograms of Loisé's kinship coefficient with (a) straight-line geographic distance and (b) resistance values from *CIRCUITSCAPE* using the *cond3* model (Table 1). Spatial scale of positive autocorrelation was determined as the mid-point distance or resistance between the kinship coefficients above and below zero and is marked with a dotted line. Connecting individuals (black lines) that are less than the scale of positive autocorrelation, matches better when using (d) resistance values than (c) geographic distances. See Fig. 1 for spatial reference and Fig. 2 for assignment results.

ern Ontario where habitat for foxsnakes has been significantly reduced and fragmented. Boundary regions between these clusters were comprised of low suitability habitat demonstrating that low-quality habitat is probably restricting gene flow between these clusters. Supporting these results, both IBR and LCP analysis found that matrices of individual genetic distance were significantly more correlated with matrices of resistance values, derived from habitat suitability scores (higher suitability scores = lower resistance), than models with an undifferentiated landscape (i.e. straight-line distance). Further, models with very low suitable habitat set as absolute barriers to any movement had the highest correlation coefficients suggesting that individuals are unwilling or unable to travel through, and/or populations are not present in this low-quality habitat.

Although much of the genetic structure across south-western Ontario could be explained by a lack of suitable habitat, in some cases, other factors appear to have

played a role. For example, the Talbot population was differentiated from the Point Pelee/Hillman population despite being connected by a significant swath of suitable habitat. These populations, however, are separated by a busy two-lane highway implying that the highway is a significant impediment to movement. This is further supported by the fact that the two road kills found on this road were assigned to the different genetic clusters. Other populations were separated by a major 4-lane freeway (Ontario Provincial Highway 401) and were not genetically differentiated; however, underpasses for large creeks and agricultural drains with riparian habitat passing under the highway near these populations probably serve as conduits for movement of foxsnakes.

Island biogeography has been cited in the interpretation of genetic population structure for many species (e.g. Kozakiewicz *et al.* 2009; Sebastian *et al.* 2009), including snakes species in this region (King & Lawson

2001). Despite the fact that lake barriers have probably been in place much longer than current habitat distribution patterns and anthropogenic landscape features, they do not appear to be acting as strong barriers for foxsnakes. We found no differentiation between island populations using assignment tests, but did find some differentiation between island populations and neighbouring mainland populations. King & Lawson (2001) found lower F_{ST} values between populations of garter snakes (*Thamnophis sirtalis*) separated by terrestrial habitats than island populations separated by comparable distances of water. In our study, some of the highest F_{ST} values were populations separated by terrestrial habitats, and the island population cluster was one of the least differentiated from other populations (tied with the same mean pairwise F_{ST} values as the largest (by area) mainland cluster) despite not being a central population. Foxsnakes can swim long distances over water (MacKinnon *et al.* 2006) and so this lack of differentiation over water is not surprising, but suggests that individuals are not as willing and/or able to travel across large patches of unsuitable terrestrial habitat and roads as they are willing to traverse open water.

Over 80% of the terrestrial landscape in our study area has been converted to agriculture and because foxsnakes avoid agricultural fields, low suitability habitat was mainly made up of this landcover type. Habitat fragmentation, conversion and isolation across this region, therefore, seem to have played a major role in restricting gene flow and shaping the mainland population structure for foxsnakes. Habitat fragmentation has been shown to reduce dispersal and affect population structure for a number of terrestrial species (e.g. Cegelski *et al.* 2003), but an increasing number of studies document large effects of fragmentation on population structure and genetic diversity of terrestrial squamates (Berry *et al.* 2005; Jansen *et al.* 2008; Marshall *et al.* 2009; Clark *et al.* 2010; Dubey & Shine 2010). Squamates may be particularly impacted by habitat loss and fragmentation possibly because of their thermoregulatory requirements (Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006). Our results also suggest, however, that habitat corridors may be an effective method for maintaining and improving connectivity for eastern foxsnakes. For example, the strongest barriers appear to be large swaths of very low suitable habitat, and even marginal habitat appears to maintain connections between populations (e.g. Ruscom, Big Creek, Chatham, Lambton populations and Norfolk population; Fig. 2), despite extensive habitat fragmentation. Similar studies are required on additional terrestrial squamates to determine whether this is specific to foxsnakes or a more common attribute. Foxsnakes are regularly found

along riparian habitat and large drainage ditches, which may make them particularly suited to habitat corridors.

IBR vs. least-cost analysis

Initial tests comparing IBR and LCP analysis demonstrated that IBR produced significantly better results (higher Mantel correlation coefficient's) than LCPs for simulations (McRae 2006) and coarse scale (5 and 50 km resolution) empirical data sets (McRae & Beier 2007). IBR was originally developed for population analysis, but with an individual-based data set, Schwartz *et al.* (2009) found similar results between the two methods, but needed to decrease the resolution of the IBR analysis because of computational constraints of CIRCUITSCAPE. We also found similar results between the two methods with an individual-based data set, both finding a significant result and selecting the same model with the highest Mantel's correlation coefficient. Because of the extensive fragmentation across this region, it is possible that there are few possible habitat corridors and thus the scenario we present may not be a rigorous test of these methods as dispersal is forced through a small portion of the total region. Certainly, we second the view of Schwartz *et al.* (2009) that more simulation and empirical studies are required to fully compare these two methods. For conservation purposes, IBR has the added benefit of mapping and quantifying all habitat corridors instead of a single least-cost path, which will be useful in conservation planning (McRae *et al.* 2008).

Resistance values in spatial autocorrelation analysis

We expected that the scale of spatial genetic structure would closely match genetic populations identified through assignment tests (i.e. individuals separated by greater than the scale of autocorrelation would be grouped in separate genetic clusters). We found significant evidence for spatial genetic structure using both straight-line geographical distances and resistance values derived from CIRCUITSCAPE (Fig. 5), but only when using resistance values did the results match with assignment tests. Individuals separated by $<2.91 \Omega$ (the extent of spatial genetic structure using resistance values) were generally grouped into the same genetic clusters, and individuals separated by >2.91 were from different clusters (Fig. 5). This was not the case when mapping individuals separated by 18.5 km (the extent of spatial genetic structure using straight-line distances) (Fig. 5) implying that using resistance values may be more biologically realistic. We suggest that more empirical studies compare results of spatial autocorrelation analysis using both straight-line and resistance values.

Further, simulation studies on complex landscapes may better establish the relationship between spatial genetic scale and genetic populations identified through assignment tests and determine the benefits of using resistance values when comparing between sexes or groups.

Conclusions

The importance of landscape variables in shaping dispersal patterns and population genetic structure is becoming increasingly clear for a variety of taxa (Cegelski *et al.* 2003; Berry *et al.* 2005; Lee-Yaw *et al.* 2009). Combining well-derived ecological and spatial techniques (e.g. habitat suitability modelling) with detailed surveys of genetic population structure is a promising method to understand how landscape features and habitat distribution impacts population structure, but has not been well utilized in the literature. Eastern foxsnakes (*M. gloydi*) have persisted to this point across a heavily fragmented region despite being marsh and prairie specialists. Through habitat suitability modelling and genetic analysis, we have demonstrated that habitat degradation and fragmentation limit dispersal for foxsnakes, which has had a strong effect on the genetic population structure across this region. Without active efforts to halt habitat modification, or restore portions of the large swaths of very unsuitable habitat that we identify here as impediments to dispersal, it is probable that isolation among these populations will remain or increase with clear negative consequences for the persistence of foxsnakes across this region.

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This research was part of Jeffrey R. Row's PhD research on the landscape genetics and phylogeography of foxsnakes. His research interests are centred on combining ecological, spatial and genetic analysis to understand how geographic, demographic and ecological factors influence genetic variation within a species. Gabriel Blouin-Demers studies the behavioural and physiological ecology of reptiles He is interested in how behaviour and phenotype, modulated by physiological constraints, affect whole-organism performance and, thus, fitness-related life-history traits. Stephen C. Lougheed is interested in the evolutionary processes that cause phyletic and adaptive diversification of frogs, snakes and birds. His research lies at the nexus of landscape genetics and phylogeography, with a particular interest in species of conservation concern in Canada.

Supporting information

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

Appendix S1 Ecological Niche Factor Analysis methods and results for eastern foxsnakes across southwestern Ontario.

Appendix S2 Nonspatial assignment test results for eastern foxsnakes across southwestern Ontario and northwestern Ohio.

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