RESEARCH ARTICLE

Population genetic structure and landscape connectivity of the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) in the contiguous tallgrass prairie of northeastern Kansas, USA

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Abstract The tallgrass prairie of North America has undergone widespread habitat loss and fragmentation (<4% remains). The Flint Hills region of Kansas and Oklahoma is the largest tallgrass prairie remaining and therefore provides an opportunity to study the population genetic structure of grassland species in a relatively contiguous landscape and set a baseline for evaluating changes when the habitat is fragmented. We adopted a landscape genetics approach to identify how landscape structure affected dispersal, population genetic structure, and landscape connectivity of the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) across a 13,500-km^2 landscape in northeastern Kansas, USA. The racer population had high allelic diversity, high heterozygosity, and was maintaining migration-drift equilibrium. Autocorrelation between genetic and geographic distance revealed that racers exhibited restricted dispersal within 3 km, and isolation-by-distance. Significant isolation-by-distance occurred at broad regional scales (>100 km), but because of sufficient gene flow between locations, we were unable to define discrete subpopulations using Bayesian clustering analyses. Resistance distance, which considers the permeability of habitats, did not explain significant variation in genetic distance beyond Euclidean distance alone, suggesting that racers are not currently influenced by landscape composition. In northeastern Kansas, racers appear to be an abundant and continuously distributed snake that perceives the landscape as well connected with no cover type currently impeding snake dispersal or gene flow.

Keywords Autocorrelation · Conservation · Dispersal · Fragmentation · Gene flow · Grassland · Isolation-by-distance · Landscape genetics · Microsatellites · Snake

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Introduction

Dispersal is the movement of an individual from its natal site to the site where it produces its own offspring or the movement from one breeding site to another (Greenwood and Harvey 1982). Although dispersal ability is an important aspect of an organism's biology, it is often underestimated due to logistical difficulties such as the need to mark numerous individuals, limited size of study areas, and bias toward animals capable of carrying heavy satellite-transmitters (Koenig et al. 1996). An alternative to observational studies of movement is the use of genetic data, which permits the detection of

dispersal and population genetic structure across broad landscape extents. In addition, genetic metrics are able to measure low dispersal rates and only include dispersal events that result in permanent emigration or breeding (Cushman et al. 2006). Both landscape structure and movement behavior greatly influences dispersal success (With and King 1999). Landscape modifications, such as habitat fragmentation, have the potential to influence population genetic structure especially when loss of suitable habitat has created a hostile matrix in which the organism cannot disperse (King and With 2002). Therefore, dispersal is an important life-history trait for understanding the historical, present, and future distribution of genetic variation and how population structure may be impacted by landscape change (Zellmer and Knowles 2009).

Genetic studies often use allele frequencies (e.g., F-statistics; Wright 1943) to quantify genetic differentiation and identify gene flow among isolated and internally panmictic subpopulations. A drawback to this approach is the need to group organisms as discrete subpopulations, which is not realistic for many organisms that have continuously distributed populations or low densities between population clusters. Recent advances in measurements of genetic distance between individuals (Rousset 2000), along with spatial analyses borrowed from landscape ecology, allow us to detect the scale of genetic discontinuities and the correlation between those discontinuities and landscape features (Manel et al. 2003). For example, autocorrelation can be used to measure the spatial scale at which individuals shift from being related to unrelated, thus revealing dispersal range (Sokal and Oden 1978; Epperson and Li 1996). Techniques in landscape genetics also permit an assessment of landscape connectivity for an organism by determining the degree to which the landscape facilitates or impedes dispersal (i.e., gene flow) to shape genetic patterns (Holderegger and Wagner 2006).

Dispersal in snakes is particularly difficult to observe due to the lack of easily identifiable nesting sites in which to mark dispersing juveniles. In addition, mark-recapture methods are often ineffective for estimating densities or delineating population structure because snakes are inconspicuous and have extended periods of inactivity (Parker and Plummer 2001). The few studies that have been conducted on the population genetic structure of snakes suggest that snakes possess limited dispersal abilities, resulting in genetic subpopulation structure occurring at <6 km (Gibbs et al. 1997; Lougheed et al. 1999; Clark et al. 2008). The majority of snake studies involving population genetics evaluate discrete subpopulations determined by the distribution of hibernacula (Clark et al. 2008). The genetic structure of continuously distributed snake populations, or ones that are not clearly delimited by fidelity to hibernacula or other patchily distributed resources (e.g., water bodies, *Thamnophis* spp.; rock outcrops, *Crotalus horridus*), have rarely been studied (Keogh et al. 2007). Therefore, expanding the scope of research to include a habitat generalist occupying a relatively contiguous landscape will give us an understanding of the variety of constraints influencing population genetic structure in snakes.

The population genetic structure of snakes in the tallgrass prairie and how they perceive the connectivity of habitat are not known, but are important questions given that many regions within the historical range of the tallgrass prairie have experienced declines in snake populations, presumably as a consequence of the loss and fragmentation of grasslands by agriculture and urbanization (Cagle 2008). The Flint Hills region of Kansas and Oklahoma represents the largest remaining tract of tallgrass prairie (Knapp and Seastedt 1998), and appears to be a contiguous landscape, in that more than half (52%) of the region is grassland. Understanding the population genetic structure of animals in a relatively contiguous habitat, sets a baseline for evaluating genetic structure when the habitat is fragmented (Banks et al. 2005). However, landscape connectivity is species-specific and may not correspond to our perception; therefore each habitat in the prairie landscape (i.e., grasslands, forests, urban areas, water bodies, and wetlands) may represent different degrees of permeability for a given species (With et al. 1997).

We used spatial modeling and population genetics to elucidate the landscape genetics of a relatively common snake in the tallgrass prairie of Kansas. The goal of this study was to use spatial and genetic information to understand the population genetic structure of the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) and how habitat heterogeneity influenced gene flow. The three main objectives were to (1) identify population subdivisions (or lack thereof) using Bayesian clustering procedures to assign snakes to discrete subpopulations; (2) analyze

fine-scale genetic structuring within snake populations and identify the scale at which individuals within a population are genetically similar, admixed, and differentiated; and (3) define landscape connectivity of racers in the tallgrass prairie by analyzing if the resistance distance, which considers the permeability of habitats, better explains genetic distance between individuals than straight-line distance. By evaluating landscape connectivity, we will be able to determine if landscape structure is limiting dispersal (i.e., gene flow) and impacting genetic structure.

Methods

We collected genetic material from 81 racers in northeastern Kansas encompassing a 75×180 km extent (13,500 km^2). We collected samples of 58 individuals from Riley, Geary, Pottawatomie, and Marshall Counties in the Flint Hills ecoregion, which included the Fort Riley Military Reservation (FRMR; $n = 12$), Konza Prairie Biological Station (KPBS; $n = 30$), and road-killed individuals throughout the region ($n = 16$). The Flint Hills ecoregion is predominately tallgrass prairie and contains limestone and shale hills with steep narrow valleys. We also obtained samples from 23 individuals from Douglas, Jefferson, and Leavenworth Counties in the Central Irregular Plains ecoregion, which included the University of Kansas Field Station and Ecological Reserves (KSR; $n = 21$) and road-killed individuals throughout the region ($n = 2$). The Central Irregular Plains ecoregion also contains native tallgrass prairie, but is a transition zone representing a grassland/forest mosaic. Tissue samples were obtained May–October in 2007 and 2008. We collected tissue from road-killed specimens encountered opportunistically and from live specimens during snake surveys, which included setting out coverboards (60×180 cm plywood; KPBS, KSR) and overturning rocks (KPBS, KSR, FRMR).

Eastern racers have a broad geographic distribution that encompasses a variety of habitat types (i.e., forests, native grasslands, and old fields) and are found from Montana to western North Dakota and from eastern Iowa to southern Texas (Conant and Collins 1998). Although the subspecies known as Eastern Yellowbelly Racer is considered a habitat generalist, in the Great Plains they are mainly found in grasslands (Fitch 1999). Dispersal in racers occurs

in two ways: (1) during the juvenile stage, when young establish summer home ranges and hibernacula, and (2) when adults change hibernacula or summer home ranges, which can occur throughout their lifetime (Fitch 1963). The average distance from summer home ranges to hibernacula is 400 m for snakes in northeastern Kansas, but can range from 0 to 1,225 m (Fitch 1963). Racers breed from May to June after dispersing from the hibernaculum (Fitch 1999). The female lays eggs near a hibernaculum different from her own in late June (Fitch 1963). From long-term data on KSR, racers occur at a density of 4–6 snakes/ha with a sex ratio of 49% males (Fitch 1999).

We used microsatellite markers to elucidate genetic structure of the racer. Microsatellite markers are selectively neutral, have high mutation rates, and exhibit high allelic diversity, which is necessary for genetic studies interested in contemporary, intraspecific population genetic structure as opposed to the geological timescales of phylogeography (Schlötterer 2000). We employed 12 microsatellite markers developed for Eastern Yellowbelly Racers (Molecular Ecology Resources Primer Development Consortium 2009). A full description of the development of these loci is available on the Molecular Ecology Resources database (accessions 37758–37769 http://tomato.biol.trinity.edu/). DNA was extracted from scales or liver using Dneasy™ Tissue Kit (Qiagen). Products were amplified using polymerase chain reactions (PCR), visualized using an ABI PRISM™ 3730 DNA Sequencer with ROX labeled size standard, and analyzed using GENEMARKER® (Softgenetics). We statistically tested for genotyping errors and the presence of null alleles using the Brookfield-1 equation as the null estimator within MICROCHECKER v. 2.2.0 (Van Oosterhout et al. 2004). All laboratory work was conducted in the KSU Conservation Genetic and Molecular Ecology Lab directed by SMW.

Population genetic characteristics

We calculated genetic diversity and tested for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium with GENEPOP (http://wbiomed.curtin.edu.au/genepop; Raymond and Rousset 1995). Linkage disequilibrium was tested with a Fisher's exact test of contingency tables of allele frequencies for all pairs of loci (Markov chain:

1000 dememorizations, 100 batches, and 100 iterations per batch). We tested for heterozygote deficiency using Hardy–Weinberg exact tests for each locus (Markov chain: 1000 dememorizations, 600 batches, 1000 iterations per batch) within the whole sample. We also analyzed Riley/Geary counties and Douglas/Jefferson counties separately because sampling occurred in two ecoregions. If these were two subpopulations in HWE, treating all samples as one population could produce a Wahlund effect (Hardy–Weinberg disequilibrium due to heterozygote deficiency). We qualitatively checked for a Wahlund effect by comparing the entire sample to the two discrete sampling sites.

To analyze if the number of loci with heterozygote excess was significantly higher than expected by chance (50%) at mutation-drift equilibrium, we used the Wilcoxon sign-rank test within BOTTLENECK 1.2.02 (Piry et al. 1999). As suggested for microsatellites, we employed the two-phase mutation model (TPM) with a variance of 12 and 95% of the stepwise mutation model in the TPM (Piry et al. 1999). Recent reductions in effective population size causes a reduction in the number of rare alleles, which creates heterozygote excess calculated via HWE compared to heterozygosity calculated from the allele number (Luikart et al. 1998). We did not expect racers to have experienced a reduction in population size because racers appear to have been common in the Flint Hills for at least the past 80 years (Busby and Parmelee 1996). We tested for a bottleneck given that we do not fully understand the status of the racer population and are unsure of the impact of land-management changes that have occurred within the past few decades.

Assignment tests

Discrete population boundaries could not be distinguished a priori for racers because the snakes did not occur in distinctly patchy habitats where populations could be defined (Fig. 1). Although we hypothesized that the distribution of racers is likely continuous because grassland habitat is relatively intact and racers are habitat generalists, tissue samples from racers were ultimately obtained from two distinct ecoregions (Flint Hills and Central Irregular Plains), which may give rise to subpopulation structure. We used Bayesian clustering within STRUCTURE version 2.3.1 (Pritchard et al. 2000) to deduce if

subpopulations existed or if gene flow was continuous throughout these two regions. In STRUCTURE, the probability of a set of allelic frequencies given K populations ($\ln\Pr(X/K)$) is determined by calculating posterior probability for each value of K and producing estimates of log-likelihood, which are used to discern the optimal number of population clusters (K). We ran 500,000 steps after a burn-in of 100,000 under the admixture model with correlated gene frequencies. We ran five separate iterations for each value of K and used the average value of $\ln\Pr(X|K)$ and the variation to select K in addition to ΔK (Evanno et al. 2005). If the results had indicated genetic population structure, we would have proceeded with traditional estimates of allele frequencies (F_{ST} statistics) between populations to describe the genetic structure of racers. Since the results indicated that the racers were likely one panmictic population (see "Results"), we used the individual-based approach to understand the scale of population genetic structure as explained in the next section.

Spatial autocorrelation

Spatial autocorrelation was used to analyze fine-scale patterns of genetic structure, which is valuable in investigating within-population processes when population boundaries cannot be defined (Peakall et al. 2003; Double et al. 2005). Pair-wise genetic distance was calculated for each locus and summed across all loci for total genetic distance (Smouse and Peakall 1999). Geographic distance was the Euclidean distance between latitude and longitude of each snake on a UTM-coordinate system. A correlation coefficient (r) for each distance class was calculated with a pairwise matrix of genetic and geographic distance in GENALEX 6 (Peakall and Smouse 2006).

We created the autocorrelogram by plotting r as a function of geographic distance to elucidate the scale at which genetic and geographic distance are no longer correlated. This transition from positive to no correlation indicates the approximate scale of the genetic neighborhood (Sokal and Wartenberg 1983). The ability to correctly estimate the extent of genetic structure relies on the extent of the actual structure, distance class size, and sample size within each distance class. We chose the size of distance classes so that each class had approximately equal sample sizes and varied the number of distance classes,

Fig. 1 Site map showing land-cover types from the Kansas GAP project that could potentially impact the connectivity of the landscape for Eastern Yellowbelly Racers (*Coluber constrictor flaviventris*). The *outlines* represent counties: Riley/Geary (*n* = 54), Pottawatomie (*n* = 3), Marshall (*n* = 1), Leavenworth (*n* = 1), Douglas/Jefferson (*n* = 22). *Boxes* represent sampling areas. For each of the six habitat types illustrated, no habitat = gray and habitat = black
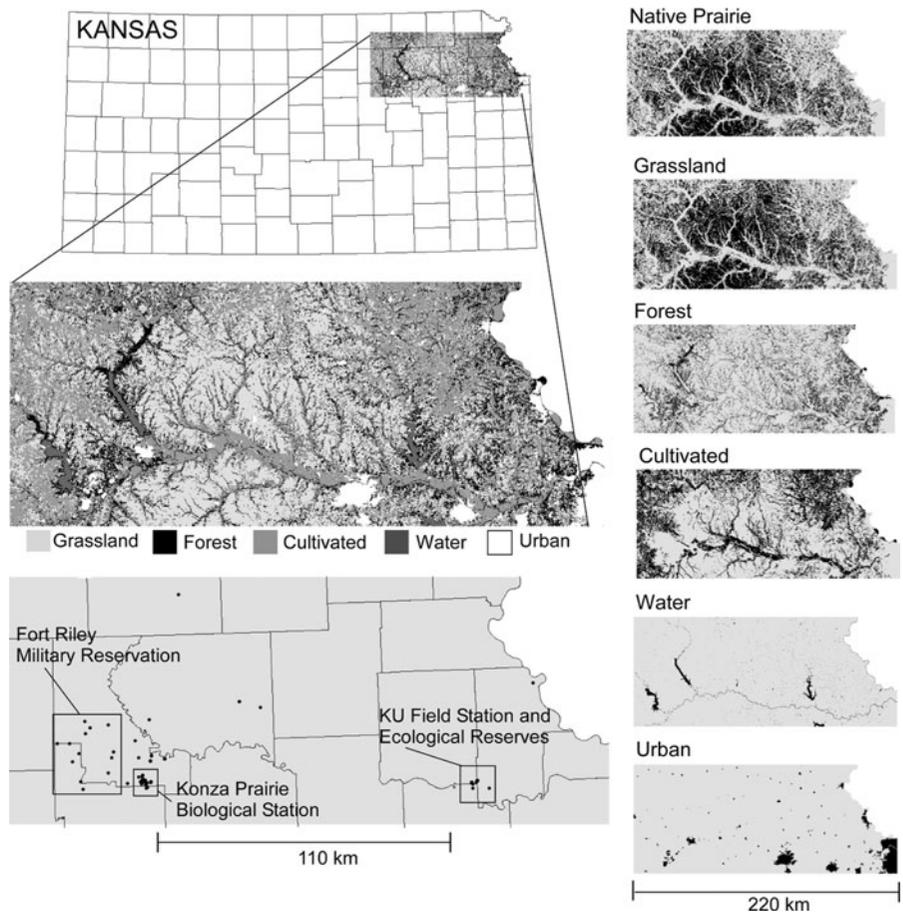
which effectively changed the size of distance classes. In reporting results from multiple correlograms, we are assured that distance class intervals were not greater than the scale of genetic structure or smaller than necessary as to compromise statistical power (Peakall et al. 2003).

We tested significance at each distance class using 1000 random permutations in which individual genotypes were shuffled between the geographic locations to achieve a recalculated estimate of *r*. The upper and lower bounds of the 95% confidence intervals (CI) were defined by the 5th and 95th values of the estimated *r*, sorted in ascending order. The random permutations generated an estimate of *r* around the null hypothesis (no genetic structure). Where *r* was greater than the 95% CI, significant positive autocorrelation occurred, which is expected if dispersal is restricted (Peakall et al. 2003). We also estimated *r* using 1000 bootstraps and used ranked *r*-values to define the 95% CI. We confirmed

significance if *r* exceeded the 95% CI around the null hypothesis (permutation test) and if the 95% CI around *r* did not overlap zero (bootstrapping).

Landscape connectivity

Population genetic structure was correlated to landscape heterogeneity using isolation-by-distance (i.e., genetic vs. geographic distance between individuals) to define the functional landscape connectivity and assess the influence of land cover type on dispersal in racers. We calculated genetic distances between individuals (a_r) as described by Rousset (2000) in which high values indicate dissimilarity between individuals. Previously, we used linear genetic distance (Smouse and Peakall 1999) for the spatial autocorrelation analyses. Therefore we employed a Mantel test to compare the genetic distance measures; a significant correlation ($r = 0.999$, $P < 0.001$) indicated that both measures would give similar results.

We modeled landscape connectivity using CIR-CUITSCAPE, which calculates resistance distances between racers on a raster landscape (McRae and Shah 2009). The resistance distance is the distance between two snakes and takes into account all possible pathways (i.e., uses circuit theory to create a graph-theoretic distance metric). For example, multiple routes and wider habitat swaths allow greater dispersal between snakes (i.e., analogous to multiple and wider conductors connecting electrical nodes allowing for greater electrical current; McRae and Beier 2007). Resistance distances in CIRCUITSCAPE may better evaluate genetic isolation-by-distance than Euclidean distance because the metric is based on assumptions regarding the permeability of land-scape features to dispersal, which ultimately impacts gene flow.

We used the Kansas GAP Analysis (Egbert et al. 2001), which included 43 land-cover classes at a spatial accuracy of 30 m, to determine landscape composition. We condensed land-cover categories into seven categories: native prairie (never culti-vated), restored grasslands (Conservation Reserve Program [CRP] fields), forests, wetlands/marshes, cultivated fields, water (rivers, lakes, and reservoirs), and urban areas. To assess how racers might respond to landscape heterogeneity, we constructed resistance values for each habitat type by creating multiple hypotheses of the influence of land cover on snake dispersal. Resistance grids were constructed by coding each pixel based on the habitat's resistance to dispersal (1 = most permeable, 100 = least per-meable, barrier = not permeable). We exported eight, hypothetical landscape grids (cell size = 150 m^2) from ArcMap 9.0 that varied in the resistance values for each habitat type (Table 1). Although we hypothesize that movement in racers would be tied to grasslands, it is not known what type of habitat racers prefer to move through during dispersal. Therefore, six of the grids had one habitat as more permeable than all others because we did not want to limit dispersal to one habitat a priori (Table 1). The other two grids comprised a gradient of resistance values depending on the presumed degree of human alteration. The 'Gradient' hypoth-esis coded all natural land cover as least resistant, cultivated fields as intermediate, and urban and water as high resistance. The 'Barrier' hypothesis was similar to 'Gradient' but had urban and water as

Table 1 Resistance values for each land-cover type for cal-culating resistance distances between individual racers. Resistance distances derived from these eight maps were used to correlate geographic distance (Euclidean or resistance dis-tance) and genetic distance (a_r)

Resistance maps	
Native prairie	*Grassland*
Native prairie-10	Native prairie-10
CRP/restored-100	CRP/restored-10
Forests-100	Forests-100
Wetlands/marshes-100	Wetlands/marshes-10
Cultivated-100	Cultivated-100
Water-100	Water-100
Urban-100	Urban-100
Forest	*Cultivated*
Native prairie-100	Native prairie-100
CRP/restored-100	CRP/restored-100
Forests-10	Forests-100
Wetlands/marshes-100	Wetlands/marshes-100
Cultivated-100	Cultivated-10
Water-100	Water-100
Urban-100	Urban-100
Water	*Gradient*
Native prairie-100	Native prairie-10
CRP/restored-100	CRP/restored-10
Forests-100	Forests-10
Wetlands/marshes-100	Wetlands/marshes-10
Cultivated-100	Cultivated-50
Water-10	Water-100
Urban-100	Urban-100
Urban	*Barrier*
Native prairie-100	Native prairie-10
CRP/restored-100	CRP/restored-10
Forests-100	Forests-10
Wetlands/marshes-100	Wetlands/marshes-10
Cultivated-100	Cultivated-50
Water-100	Water-0
Urban-10	Urban-0

complete barriers to racer movement (Table 1). We ran each grid separately in CIRCUITSCAPE to calculate resistance distances between individual racers for a total of eight resistance distances.

We used Mantel tests (Mantel 1967) to compare the correlation between the pair-wise genetic and pair-wise geographical matrices to test for the impact

of landscape heterogeneity on gene flow (Smouse et al. 1986). We analyzed isolation-by-distance in two analyses including the entire study region and the Flint Hills alone. We did not perform a separate analysis for the Central Irregular Plains owing to small sample size ($n = 23$). The Mantel tests were performed in library VEGAN version 1.6-7 (Dixon 2003; Oksanen 2005) of R (R Development Core Team 2005). The nine models tested included the Euclidean distance and each of the eight resistance maps. After assessing significance of the Mantel test for all nine models, we then ran a partial Mantel test, which compared the genetic and resistance matrices while controlling for Euclidean distance. We also ran a partial Mantel test comparing genetic and geographic distance while controlling for ecoregion (1 = individuals in same region or 2 = individuals in different regions). We acknowledge the debate over whether the partial Mantel test produces biased estimates of P values (Raufaste and Rousset 2001; Castellano and Balletto 2002; Rousset 2002). Cushman and Landguth (2010) have shown through simulation modeling that partial Mantel tests have high power and low error when testing drivers of genetic differentiation. Therefore, we considered significant partial Mantel tests an indication of whether resistance maps explained variation in genetic distance beyond Euclidean distance alone. If the resistance distance was a better estimator than

Euclidean distance, we can conclude that landscape structure may have been affecting gene flow, such that habitats were differentially permeable and the landscape was functionally fragmented for racers.

Results

Population genetic characteristics

We sampled 81 racers, with intensive sampling within the Flint Hills ecoregion (1,000 km^2; Fig. 1). All 81 samples were genotyped at 12 microsatellites with no redundant genotypes (i.e., no pairs had completely matching genotypes). Allelic variation ranged from 8 to 51 alleles per locus (mean = 20.8, SD = 11.2). Expected and observed heterozygosities ranged from 0.29 to 0.97 and 0.31 to 0.88, respectively, for the entire sample (Table 2). No pairs of loci exhibited linkage disequilibrium. We found consistent evidence of deviation from HWE for one microsatellite (i.e., GQ371178; Table 2). We found evidence for null alleles at two microsatellites (i.e., GQ371178 and GQ371183; nf > 0.10). Racers in northeastern Kansas apparently have not experienced population numbers low enough to have caused a founder's effect because there were not more heterozygotes than expected based on mutation-drift equilibrium using either the sign test ($P = 0.32$) or the

Table 2 Microsatellite loci in Eastern Yellowbelly Racers (*Coluber constrictor flaviventris*) with allele number and expected and observed heterozygosity for all samples (Total), the Flint Hills ecoregion (Riley/Geary Counties), and the Central Irregular Plains ecoregion (Douglas/Jefferson Counties) in northeastern Kansas

GenBank accession #	Allele no.	Total H$_E$/H$_O$ ($n = 81$)	Flint Hills ecoregion H$_E$/H$_O$ ($n = 54$)	Central Irregular Plains ecoregion H$_E$/H$_O$ ($n = 22$)
GQ371177	13	0.75/0.75	0.77/0.78	0.72/0.73
GQ371178	21	0.89/0.54[a]	0.86/0.59[a]	0.93/0.45[a]
GQ371179	20	0.87/0.81	0.88/0.83	0.84/0.73
GQ371180	21	0.92/0.86	0.92/0.85	0.91/0.95
GQ371181	14	0.90/0.86	0.87/0.81	0.89/0.95
GQ371182	15	0.90/0.72[a]	0.92/0.80	0.77/0.50
GQ371183	29	0.92/0.67[a]	0.93/0.57[a]	0.91/0.86
GQ371184	8	0.29/0.31	0.29/0.30	0.21/0.23
GQ371185	14	0.86/0.88	0.88/0.89	0.76/0.82
GQ371186	51	0.97/0.85	0.97/0.87	0.96/0.82
GQ371187	18	0.80/0.74	0.83/0.76	0.78/0.73
GQ371188	27	0.93/0.81	0.93/0.81[a]	0.91/0.77

[a] Locus deviated significantly from Hardy–Weinberg equilibrium

Wilcoxon test ($P = 0.30$; two-tailed) within BOTTLE-NECK. The expected number of loci displaying heterozygote excess (7.27) was not substantially different from the observed number of loci displaying heterozygote excess (6) or heterozygote deficiency (6).

Assignment tests

We found evidence for population subdivision at $K = 1–2$ (Fig. 2). When STRUCTURE finds clustering solutions with similar probabilities at multiple K, the K with the highest $\ln\text{Pr}(X/K)$ and lowest variation is chosen, which would be $K = 1$ (mean $= -4781.4$, SE $= 0.36$) or $K = 2$ (mean $= -4763.5$, SE $= 1.26$; Pritchard et al. 2000, 2009). When evaluating the percent membership of each racer in the two clusters with the plot of ancestry estimates, we observed a separation between snakes found in the Flint Hills and those in the Central Irregular Plains (Fig. 2c). Due to uncertain results using clustering methods, we continued with individual-based methods to evaluate the scale at which racers exhibit genetic differentiation.

Spatial autocorrelation

We present multiple correlograms to assure the chosen distance classes do not influence the scale of genetic structuring but reflect the true genetic structure (Fig. 3). For example, correlogram A gives the first three distance classes as 0–2, 2–3, and 3–7 km; correlogram B gives 0–1, 1–3, and 3–7 km; and correlogram C gives 0–1, 1–2, and 2–3 km. Comparisons among these correlograms enabled us to pinpoint the scale at which positive genetic structure occurred below 7 km. For correlogram A, the r-values are significantly positive at 3 km (Fig. 3a). For correlogram B, the r-values are significantly positive at 1 km (Fig. 3b). For correlogram C, the r-values are significantly positive at 1 and 3 km (Fig. 3c). Through the use of multiple correlograms, we deduced that snakes within 3 km of each other showed significant genetic similarity. The correlograms maintain positive genetic structure with few 95% CI and few r-values dropping below $r = 0$ until a distance class of 117 km (i.e., distance between Central Irregular Plains and Flint Hills samples), where r-values become consistently negative. Although pair-wise comparisons between 40 and 100 km are underrepresented due to a discrete
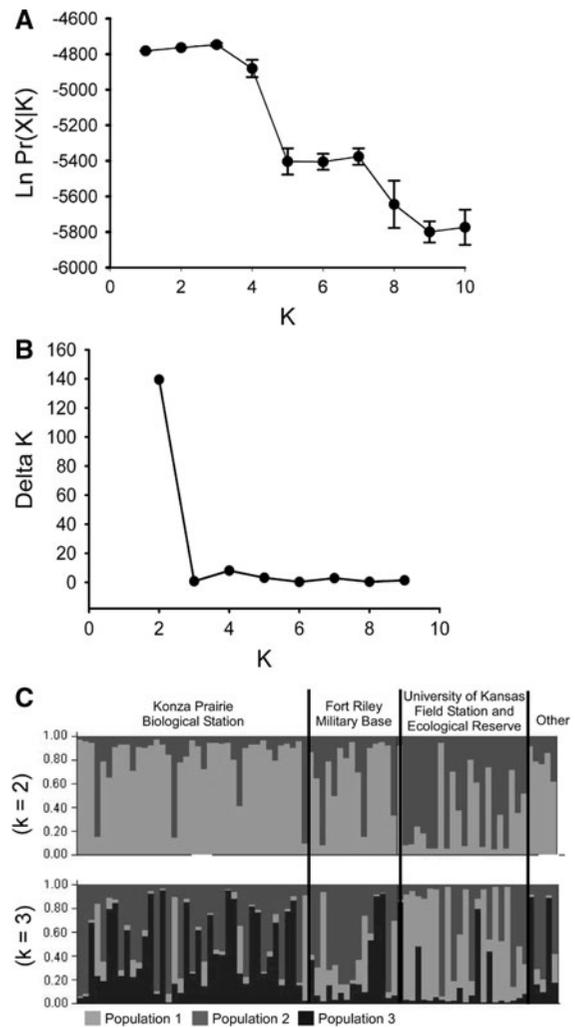

Fig. 2 In STRUCTURE we used the average of 5 iterations for $K = 1–10$. (**a**) Plot of the log-likelihood value ($\ln\text{Pr}(X|K)$) versus the number of potential subpopulations (K). (**b**) The Evanno et al. (2005) ΔK method to evaluate the most supported K based on the rate of change in the likelihood as a function of K. (**c**) The plots of ancestry estimates, which represent the estimated membership for each racer in each of the K-inferred clusters

sampling distribution, the correlograms indicate restricted dispersal within 3 km and a cline of genetic differentiation where genetic distances increase with geographic distance.

Landscape connectivity

Mantel tests indicated that isolation-by-distance partially explained the genetic structure of racers when considering all samples ($n = 81$, $r = 0.075$,

Fig. 3 Correlograms of the correlation coefficient (*r*) for genetic distance as a function of geographic distance for racers in northeastern Kansas. The permuted 95% confidence intervals (upper = dark gray; lower = light gray) and bootstrapped 95% error bars (black) are illustrated. The number of pairwise comparisons within each distance class is listed in parentheses
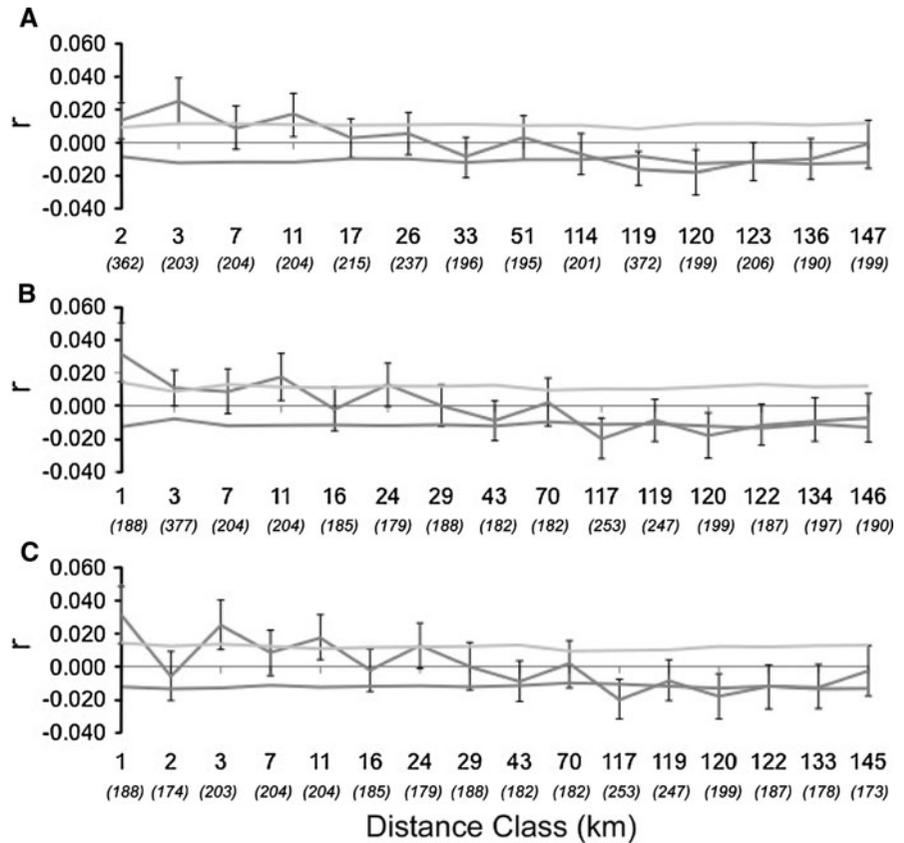
$P = 0.006$; Table 3a) at the broadest landscape scale (75 × 180 km), but not when considering only those samples in the Flint Hills (25 × 40 km; $n = 57$, $r = 0.042$, $P = 0.286$; Table 3b). The genetic distance was also partially explained by distance in space for the eight resistance distances when considering the entire data set (Table 3a) but not the restricted data set (Table 3b). None of the resistance maps explained further variation in genetic distance after controlling for Euclidean distance (Table 3). In addition, ecoregion did not explain genetic distance indicating one population experiencing isolation-by-distance (Table 4). Although low *r*-values from the Mantel tests do not appear to explain a large amount of the variation, this is a common finding in individual-based models (Rousset 2000). Therefore, racers in this study are exhibiting a gradient of genetic differentiation as a function of distance even when potential habitat resistance is considered. These finding are likely due to the resistance distances being highly correlated with Euclidean distance ($r > 0.6$),

which indicates a permeable landscape across a large, functionally contiguous geographical extent (Table 3).

Discussion

We adopted a landscape genetics approach to explore how habitat heterogeneity at a regional scale influenced gene flow, population genetic structure, and landscape connectivity for racers in the largest tallgrass prairie in North America. A large number of individuals in a near-continuous distribution must exist for a population with dispersal-limited individuals to show isolation-by-distance as observed in this population of racers in northeast Kansas (Table 3, Fig. 3).

A large number of individuals are likely contributing to the gene pool given the population exhibited a high number of alleles per locus (range = 8–51 mean = 20.9, SE = 3.2). This is higher than values

Table 3 Correlation coefficient and corresponding significance for Mantel test comparing Euclidean distance and resistance distance as well as Mantel tests and partial Mantel tests comparing genetic and geographic distance for racers assuming either the Euclidean model or one of the eight resistance models (cf. Table 1). The partial Mantel test compares genetic and resistance distance while controlling for Euclidean distance. (A) 81 samples (B) Flint Hills region

	Correlation with Euclidean dist.		Mantel test		Partial Mantel	
	r	P-value	r	P-value	r	P-value
(A) All samples						
Euclidean	–	–	0.075	0.006	–	–
Barrier	0.811	0.001	0.084	0.013	0.041	0.139
Gradient	0.865	0.001	0.084	0.009	0.040	0.127
Prairie	0.790	0.001	0.076	0.014	0.029	0.212
Forest	0.736	0.001	0.073	0.002	0.026	0.149
Urban	0.735	0.001	0.070	0.002	0.022	0.161
Grassland	0.789	0.001	0.071	0.033	0.021	0.305
Water	0.737	0.001	0.069	0.001	0.021	0.183
Cultivated	0.666	0.001	0.059	0.010	0.013	0.302
(B) Riley/Geary						
Euclidean	–	–	0.042	0.286	–	–
Gradient	0.759	0.001	0.051	0.198	0.030	0.326
Prairie	0.741	0.001	0.046	0.246	0.022	0.369
Barrier	0.632	0.001	0.038	0.272	0.016	0.422
Grassland	0.750	0.001	0.039	0.301	0.011	0.412
Forest	0.559	0.001	−0.002	0.530	−0.031	0.763
Water	0.656	0.001	−0.003	0.517	−0.040	0.828
Urban	0.646	0.001	−0.006	0.555	−0.042	0.840
Cultivated	0.563	0.001	−0.016	0.615	−0.047	0.875

Table 4 Correlation coefficient and corresponding significance for partial Mantel test comparing genetic and geographic distance for racers assuming either the Euclidean model or one of the eight resistance distance models and controlling for ecoregion

Geographic distance	Partial Mantel test	
	r	P-value
Euclidean	0.027	0.224
Barrier	0.047	0.211
Gradient	0.060	0.083
Prairie	0.063	0.106
Forest	0.040	0.086
Urban	0.046	0.070
Grassland	0.059	0.128
Water	0.043	0.088
Cultivated	0.021	0.270

observed in snakes of restricted distribution such as garter snakes (*Thamnophis* spp.), massasauga rattlesnakes (*Sistrurus catenatus*), and water snakes ([*Nerodia sipedon*]; Gibbs et al. 1997; Prosser et al. 1999; Manier and Arnold 2005), but comparable to

black rat snakes ([*Elaphe obsoleta*]; Lougheed et al. 1999) which are abundant and widely distributed. In addition, the population showed no signs of a bottleneck and was maintaining migration-drift equilibrium, indicating no subpopulation structure due to limited gene flow. Genetic evidence, in conjunction with survey data (i.e., racers were 47% of total snake captures, Klug et al. 2010), indicates the racer is relatively abundant and has a continuous distribution across this predominantly grassland region. At present, the tallgrass prairie of Kansas seems capable of maintaining high rates of genetic exchange for racers across a broad extent and could serve as a baseline for evaluating genetic structure where grassland habitat is extremely fragmented.

As STRUCTURE uses the frequency of alleles, limited gene flow is necessary between subpopulations for genetic drift to occur and allele frequency to change (and be detected in STRUCTURE). We found that gene flow occurred throughout the study area, as we were unable to confidently define discrete subpopulations in STRUCTURE (i.e., K could have been 1–2). The inability to assign discrete subpopulations

using Bayesian clustering methods for a species that shows limited dispersal is surprising, especially given the size of the study area (13,500 km^2; Fig. 2). In previous studies on snakes, genetic differentiation was found between populations of massasauga rattlesnakes within 2 km (Gibbs et al. 1997), timber rattlesnakes (*Crotalus horridus*) within 8 km (Clark et al. 2008), and black rat snakes within 6 km (Lougheed et al. 1999), all of which were attributed to limited dispersal ability. A shared characteristic of these snakes is that they are habitat specialists or exhibit high philopatry to communal hibernacula. For example, population divisions were evident in water snakes and garter snakes, which are strongly associated with patchily distributed water sources (Prosser et al. 1999, Manier and Arnold 2005). On the contrary, a lack of genetic differentiation between subpopulations was found in the small-eyed snake (*Rhinoplocephalus nigrescens*), a habitat generalist (Keogh et al. 2007). Given that the racer is a habitat generalist and does not exhibit philopatry to summer home ranges or hibernacula (Fitch 1963), perhaps it is not surprising that subpopulations were not evident, despite limited dispersal. Likewise, Gauffre et al. (2008) have shown that recent barriers to dispersal are difficult to detect in clustering methods employing F_{ST} when effective population size is large, as might be the case for racers. Schwartz and McKelvey (2009), have also shown that STRUCTURE may report $K = 2$ as equally likely as $K = 1$ when patterns of isolation-by-distance are present, such as found in our study. Due to these uncertainties, we also used individual-based methods to evaluate population genetic structure of racers.

Further support for the existence of a large and continuous population comes from the Mantel tests for isolation-by-distance, in which we found a significant positive relationship between genetic and geographic distance when using the entire study area, but not when the analysis was limited to the Flint Hills (25 × 40 km). The fact that isolation-by-distance was not significant within the Flint Hills suggests the 1,000-km^2 extent of that study area was not large enough to observe isolation-by-distance. The results from the genetic autocorrelation analyses show a genetic gradient where the *r*-value became negative around 117 km, which indicates the point at which individuals become more genetically different than average. Therefore, genetic

dissimilarity occurring between individuals from different sampling locales (i.e., Flint Hills and Central Irregular Plains) contributed to the significance of isolation-by-distance. Although racers in the Flint Hills and Central Irregular Plains are genetically differentiated (i.e., isolation-by-distance from Mantel tests and genetic neighborhoods from autocorrelation) enough gene flow must occur in the large and continuous population of racers for migration to offset drift and subpopulations not to be detected in Bayesian clustering analyses (i.e., STRUCTURE).

Our results indicated that population genetic structure was not influenced by landscape composition in northeastern Kansas. We found no natural or anthropogenic land cover currently limiting snake dispersal or gene flow. Although the resistance distances obtained from maps describing the landscape as a gradient of permeability (i.e., "Barrier" and "Gradient"; Table 1) had correlation coefficients that were slightly stronger than Euclidean distance, the partial Mantel tests were not significant. Therefore, it can be inferred the landscape is functionally connected, and gene flow is not limited by landscape structure. This does not mean that racers are unresponsive to land cover but that limited gene flow has not been experienced to elicit a distinguishable effect in terms of the composition and scale of habitat measured. It is possible that areas with greater grassland fragmentation or future changes to the Kansas landscape (e.g., increased urban or forest cover) could result in impeded dispersal and therefore gene flow (Fitch 1999; Short Bull et al., in press).

The greatest challenge in landscape genetics is matching the temporal scale of the observed genetic patterns to the temporal scale of landscape modification (Balkenhol et al. 2009). Although, we are unable to identify the temporal scale at which the observed genetic patterns occurred, general land cover within the Flint Hills has changed relatively little during the past 50 years and we feel our results are robust to past changes in land cover at the scale examined. For example, barriers such as urban centers (e.g., Manhattan, Kansas) have been well established for the past 50 years, the conversion to row crop agriculture was mostly completed by the 1940s (Waisanen and Bliss 2002), and water bodies such as the Tuttle Creek Reservoir was established in the early 1960s. Landguth et al. (2010) found that in using individual-based statistics (e.g., Mantel's *r*) a lag time of only

1–15 generations was needed to establish a genetic signal to a landscape barrier. Therefore, a time frame of 50 years is reasonable for genetic patterns to respond to landscape change given we used individual-based analysis and racers experience a generation time of about three years (Fitch 1963). A corresponding lag time ranging from 3 to 45 years could be sufficient to alter the genetic signal of the racer population despite a large effective population size.

Although the general land cover categories have changed little over the past 50 years, land-management practices within grasslands have experienced more recent changes. Currently, 86% of the Flint Hills is grassland and managed for cattle production (With, unpubl. data). Season- or year-long grazing has been in practice since the late 1800s and still occupies 65% of the Flint Hills landscape, but more recent changes in land management have occurred since the 1980s (With et al. 2008). The practice of annual burning coupled with early-intensive stocking (i.e., cattle stocked at twice the rate for half the season) now makes up 25% of managed grasslands (With et al. 2008). This intensive use of fire and grazing has resulted in reduced vegetation structure that has proven detrimental to many native species (With et al. 2008). A reduction in vegetative cover could potentially expose dispersing racers to temperature extremes and higher predation risk (Wilgers and Horne 2007) during emergence from hibernacula after spring burns. In addition, racers have an affinity for increased vegetation structure (Klug et al. 2010), and thus may avoid grasslands managed with annual burns and early-intensive stocking. Although this land-management may negatively impact racer populations, it may not act as a complete barrier to dispersal, given these intensively managed grasslands are left to recover after cattle are removed mid-July. Previous studies in the Flint Hills have shown that racers avoid recently burned areas but are able to recolonize later in the growing season (Cavitt 2000). In addition, female racers lay eggs in early August and hatching occurs in September (Fitch 1963), after the peak of the growing season when sufficient vegetation is present to facilitate juvenile dispersal to a hibernaculum. Had we the resolution to include grassland management as a variable, we may have been better able to deduce the impact of land mangement on gene flow in racers by evaluating the genetic structure under both current land use and historical land use within the past 100 years (e.g., Zellmer and Knowles 2009).

At the other extreme, increased exurban development curtails burning and grazing, which promotes shrub encroachment and the succession of grasslands to forests. In addition to grasslands, racers are able to use other habitats (e.g., forests) to disperse and may actually prefer a mosaic of habitats, which offer a variety of environments for thermoregulation (Wilgers and Horne 2006). Therefore, the increased woody cover in the Flint Hills (Briggs et al. 2005) and the mosaic of forest and grasslands in the Central Irregular Plains may benefit racer populations and facilitate dispersal until a threshold at which grasslands have converted to forests and grassland species (e.g., racers) are excluded (Fitch 1999). Currently, we have not shown forests to restrict gene flow in racers, but if the intensity and scale of forest cover increases beyond the scale of a racer's ability to disperse, forests could become a barrier. The same could be said for other land-cover types that are not preferred habitat (i.e., cultivated fields and urban centers). Future research should evaluate the influence of shifts in grazing regimes, increased woody cover, exurban expansion, and increased fragmentation on gene flow to deduce how land modification may alter the connectivity and population genetic structure of native species like the racer.

References

Balkenhol N, Gugerli F, Cushman SA, Waits LP, Coulon A, Arntzen JW, Holderegger R, Wagner HH, Participants of the Landscape Genetics Research Agenda Workshop 2007 (2009) Identifying future research needs in landscape

genetics: where to from here? Landscape Ecol 24:455–463

Banks SC, Lindenmayer DB, Ward SJ, Taylor AC (2005) The effects of habitat fragmentation via forestry plantation establishment on spatial genotypic structure in the small marsupial carnivore, *Antechinus agilis*. Mol Ecol 14:1667–1680

Briggs JM, Knapp AK, Brock BL (2005) An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. Bioscience 55:243–254

Busby WH, Parmelee JR (1996) Historical changes in a herpetofaunal assemblage in the Flint Hills of Kansas. Am Midl Nat 135:81–91

Cagle NL (2008) Snake species distributions and temperate grasslands: a case study from the American tallgrass prairie. Biol Conserv 141:744–755

Castellano S, Balletto E (2002) Is the partial Mantel test inadequate? Evolution 56:1871–1873

Cavitt JF (2000) Fire and a tallgrass prairie reptile community: effects on relative abundance and seasonal activity. J Herpetol 34:12–20

Clark RW, Brown WS, Stechert R, Zamudio KR (2008) Integrating individual behaviour and landscape genetics: the population structure of timber rattlesnake hibernacula. Mol Ecol 17:719–730

Conant R, Collins JT (1998) A field guide to reptiles & amphibians of eastern and central North America. Houghton Mifflin, New York

Cushman SA, Landguth EL (2010) Spurious correlations and inference in landscape genetics. Mol Ecol 19:3592–3602

Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. Am Nat 168:486–499

Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veg Sci 14:927–930

Double MC, Peakall R, Beck NR, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). Evolution 59:625–635

Egbert SL, Peterson DL, Stewart AM, Lauver CL, Blodgett CF, Price KP, Martinko EA (2001) The Kansas GAP land cover map: final report. Kansas Biological Survey Report 98

Epperson BK, Li T (1996) Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. Proc Natl Acad Sci 93:10528–10532

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fitch HS (1963) Natural history of the racer *Coluber constrictor*. Univ Kansas Publ Mus Nat Hist 15:351–468

Fitch HS (1999) Coluber constrictor. In: A Kansas snake community: composition and changes over 50 years. Krieger Publishing Company, Malabar, FL, pp 29–39

Gauffre B, Estoup A, Bretagnolle V, Cosson JF (2008) Spatial genetic structure of a small rodent in a heterogeneous landscape. Mol Ecol 17:4619–4629

Gibbs HL, Prior KA, Weatherhead PJ, Johnson G (1997) Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus c. catenatus*: evidence from microsatellite DNA markers. Mol Ecol 6:1123–1132

Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. Annu Rev Ecol Syst 13:1–21

Holderegger R, Wagner HH (2006) A brief guide to landscape genetics. Landscape Ecol 21:793–796

Keogh JS, Webb JK, Shine R (2007) Spatial genetic analysis and long-term mark-recapture data demonstrate male-biased dispersal in a snake. Biol Lett 3:33–35

King AW, With KA (2002) Dispersal success on spatially structured landscapes: when do spatial pattern and dispersal behavior really matter? Ecol Model 147:23–39

Klug PE, Jackrel SL, With KA (2010) Linking snake habitat use to nest predation risk in grassland birds: the danger of shrub cover. Oecologia 162:803–813

Knapp AK, Seastedt TR (1998) Grasslands, Konza Prairie, and long-term ecological research. In: Knapp AK, Briggs JM, Hartnett DC, Collins SL (eds) Grassland dynamics: long-term ecological research in the tallgrass prairie. Oxford University Press, New York, pp 3–15

Koenig WD, Van Vuren D, Hooge PN (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol Evol 11:514–517

Landguth EL, Cushman SA, Schwartz MK, McKelvey KS, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. Mol Ecol 19:4179–4191

Lougheed SC, Gibbs HL, Prior KA, Weatherhead PJ (1999) Hierarchical patterns of genetic population structure in black rat snakes (*Elaphe obsoleta obsoleta*) as revealed by microsatellite DNA analysis. Evolution 53:1995–2001

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Mol Ecol 7:963–974

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197

Manier MK, Arnold SJ (2005) Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnophis elegans* and *Thamnophis sirtalis*). Mol Ecol 14:3965–3976

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. Proc Natl Acad Sci 104:19885–19890

McRae BH, Shah V (2009) CIRCUITSCAPE user's guide. ONLINE. The University of California, Santa Barbara. Available at: http://www.circuitscape.org

Molecular Ecology Resources Primer Development Consortium (2009) Permanent Genetic Resources added to Molecular Ecology Resources Database 1 May 2009–31 July 2009. Mol Ecol Resour 9:1460–1559

Oksanen J (2005) The VEGAN: Community Ecology Package. Version 1.6-7. Available at http://www.cran.r-project.org/

Parker WS, Plummer MV (2001) Population ecology. In: Seigel RA, Collins JT, Novak SS (eds) Snakes: ecology and evolutionary biology. The Blackburn Press, Caldwell, NJ, pp 253–301

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in EXCEL. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial auto-correlation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57:1182–1195

Piry S, Luikart G, Cornuet JM (1999) Computer note. BOT-TLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered 90:499–503

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Wen X, Falush D (2009) Documentation for STRUCTURE software: version 2.3. Universtiy of Chicago, Chicago, pp 1–37

Prosser MR, Gibbs HL, Weatherhead PJ (1999) Microgeographic population genetic structure in the northern water snake, *Nerodia sipedon sipedon* detected using microsatellite DNA loci. Mol Ecol 8:329–333

R Development Core Team (2005) R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria

Raufaste N, Rousset F (2001) Are partial Mantel tests adequate? Evolution 55:1703–1705

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact test and ecumenicism. J Hered 86:248–249

Rousset F (2000) Genetic differentiation between individuals. J Evolution Biol 13:58–62

Rousset F (2002) Partial Mantel tests: reply to Castellano and Balletto. Evolution 56:1874–1875

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365–371

Schwartz MK, McKelvey KS (2009) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conserv Genet 10:441–452

Short Bull RA, Cushman SA, Mace R, Chilton T, Kendall K, Landguth EL, Schwartz MK, McKelvey K, Allendorf FW, Luikart G (in press) Why replication is important in landscape genetics: American black bear in the Rocky Mountains. Mol Ecol

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–573

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlaiton extensions of the Mantel test of matrix correspondence. Syst Zool 35:627–632

Sokal RR, Oden NL (1978) Spatial autocorrelation in biology I. Methodology. Biol J Linn Soc 10:199–228

Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. Genetics 105:219–237

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Waisanen PJ, Bliss NB (2002) Changes in population and agricultural land in conterminous United States counties, 1790–1997. Global Biogeochem Cycles 16:1137–1156

Wilgers DJ, Horne EA (2006) Effects of different burn regimes on tallgrass prairie herpetofaunal species diversity and community composition in the Flint Hills, Kansas. J Herpetol 40:73–84

Wilgers DJ, Horne EA (2007) Spatial variation in predation attempts on artificial snakes in a fire-disturbed tallgrass prairie. Southwest Nat 52:263–270

With KA, King AW (1999) Dispersal success on fractal landscapes: a consequence of lacunarity thresholds. Landscape Ecol 14:73–82

With KA, Gardner RH, Turner MG (1997) Landscape connectivity and population distributions in heterogeneous landscapes. Oikos 78:151–169

With KA, King AW, Jensen WE (2008) Remaining large grasslands may not be sufficient to prevent grassland bird declines. Biol Conserv 141:3152–3167

Wright S (1943) Isolation by distance. Genetics 28:114–138

Zellmer AJ, Knowles LL (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. Mol Ecol 18:3593–3602