

Historical processes and landscape context influence genetic structure in peripheral populations of the collared lizard (*Crotaphytus collaris*)

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Abstract Populations at the periphery of a species' range often show reduced genetic variability within populations and increased genetic divergence among populations compared to those at the core, but the mechanisms that give rise to this core-periphery pattern in genetic structure can be multifaceted. Peripheral population characteristics may be a product of historical processes, such as founder effects or population expansion, or due to the contemporary influence of landscape context on gene flow. We sampled collared lizards (*Crotaphytus collaris*) at four locations within the northern Flint Hills of Kansas, which is at the northern periphery of their range, to determine the genetic variability and extent of genetic divergence among populations for ten microsatellite loci ($n = 229$). We found low genetic variability (average allelic richness = 3.37 ± 0.23 SE; average heterozygosity = 0.54 ± 0.05 SE) and moderate population divergence (average $F_{ST} = 0.08 \pm 0.01$ SE)

among our sample sites relative to estimates reported in the literature at the core of the species' range in Texas. We also identified differences in dispersal rates among sampling locations. Gene flow within the Flint Hills was thus greater than for other peripheral populations of collared lizards, such as the Missouri glade system where most of the mesic grasslands have been converted to forest since the last glacial retreat, which appears to have greatly impeded gene flow among populations. Our findings signify the importance of considering landscape context when evaluating core-peripheral trends in genetic diversity and population structure.

Keywords Microsatellites · Flint Hills · Tallgrass prairie · Collared lizard

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Introduction

Species are often less abundant and more patchily distributed at the periphery of their range than at the core, presumably due to a decline in the quantity and quality of suitable habitat relative to the core of the species' distribution (Whittaker 1956; Hengeveld and Haeck 1982; Brown 1984). Because peripheral populations experience more extreme conditions than populations at the core, population dynamics can also differ between the core and periphery of the range

(Brown 1984; Brown et al. 1995; but see Sagarin and Gaines 2002; Murphy et al. 2006). Small, isolated populations at the periphery are also expected to maintain lower levels of gene flow, to have reduced genetic diversity, and to have higher genetic divergence than populations at the core of a species' range as a result of drift, selection, and founder effects (Bush 1975; Nei et al. 1975; Lesica and Allendorf 1995; Ibrahim et al. 1996; Eckert et al. 2008; Ramakrishnan et al. 2010).

A population's location at the core vs. the periphery of the range is unlikely to fully account for population genetic structure, however. Landscape structure can have a major effect on genetic diversity and gene flow (Storfer et al. 2007). Factors such as recent or historical habitat fragmentation or the presence of barriers to dispersal can limit gene flow among local populations and increase population differentiation (Templeton et al. 1990; Zamudio et al. 1997; Cegelski et al. 2003; Keyghobadi et al. 2005; Spear et al. 2005). Species relying on patchy habitat may be especially influenced by properties of the surrounding landscape in which the habitat patch is located, and this may be reflected in local population genetic structure (Berry et al. 2005; Johansson et al. 2005). Thus, differences in genetic structure among populations may be dependent on both broad-scale factors, such as the location of a population within the species' range, and on local factors, such as the landscape or patch context. Identifying the consequences of both broad-scale and local-scale factors may increase understanding of their interactive effect on the genetic structure of populations (Storfer et al. 2010).

The Collared Lizard (*Crotaphytus collaris*) is well suited for studies of broad- and local-scale factors on genetic structure. The broad distribution of this species across its range permits comparisons of population genetic structure between core and peripheral populations. The species' distribution extends throughout North America, from northern Mexico to northern Kansas and from Arizona east to Missouri (McGuire 1996). Populations within the range are subject to considerable variation in climate, rock habitat, and landscape context (the surrounding habitat or land use), all of which may affect the abundance and distribution of collared lizards at a local scale (Fitch 1956; Templeton et al. 2001; McGuire et al. 2007; Blevins and With 2011).

Collared lizard populations have been examined intensively at the northeastern edge of their range in the Missouri Ozarks, where recent (1950s to the mid-1990s) habitat fragmentation has likely contributed to declining populations, a reduction in body size, decreased seasonal activity, and a reduced breeding season relative to central populations (Sexton et al. 1992). In addition, Hutchison (2003) compared central populations of collared lizards in Texas to peripheral Missouri populations and concluded that Missouri populations appear to represent "evolutionary dead ends," given their dramatically reduced genetic variability as well as their high risk of extinction. Although Hutchison and Templeton (1999) examined genetic diversity in collared lizard populations across their range (including Kansas), they did not examine how characteristics of the landscape might influence genetic structure, particularly at a more local scale; a lack of sampling sites <40 km apart in Kansas precluded their ability to quantify local population genetic structure at a landscape, rather than regional, scale.

Our aim in this study was to examine the genetic diversity and genetic population structure of collared lizards from multiple sampling locations (<40 km apart) in the northern Flint Hills of Kansas, a region that contains the largest contiguous tallgrass prairie landscape remaining in North America. We analyzed the genetic structure of four collared lizard populations and evaluated our results within the context of core vs. peripheral populations. We hypothesized that collared lizard population genetics in the northern Flint Hills region should exhibit decreased genetic diversity within populations relative to populations at the core of the species' range, reflecting the peripheral nature of such populations. Additionally, we hypothesized that peripheral populations in the Flint Hills would exhibit low divergence among populations relative to populations at the core and even those along the eastern periphery of their range, assuming that the predominantly tallgrass matrix of this landscape (i.e., the landscape context) is more conducive to lizard dispersal. We consider the potential for regional differences in the genetic structure and diversity of peripheral populations within different parts of the range, particularly given the regional differences in landscape context.

Methods

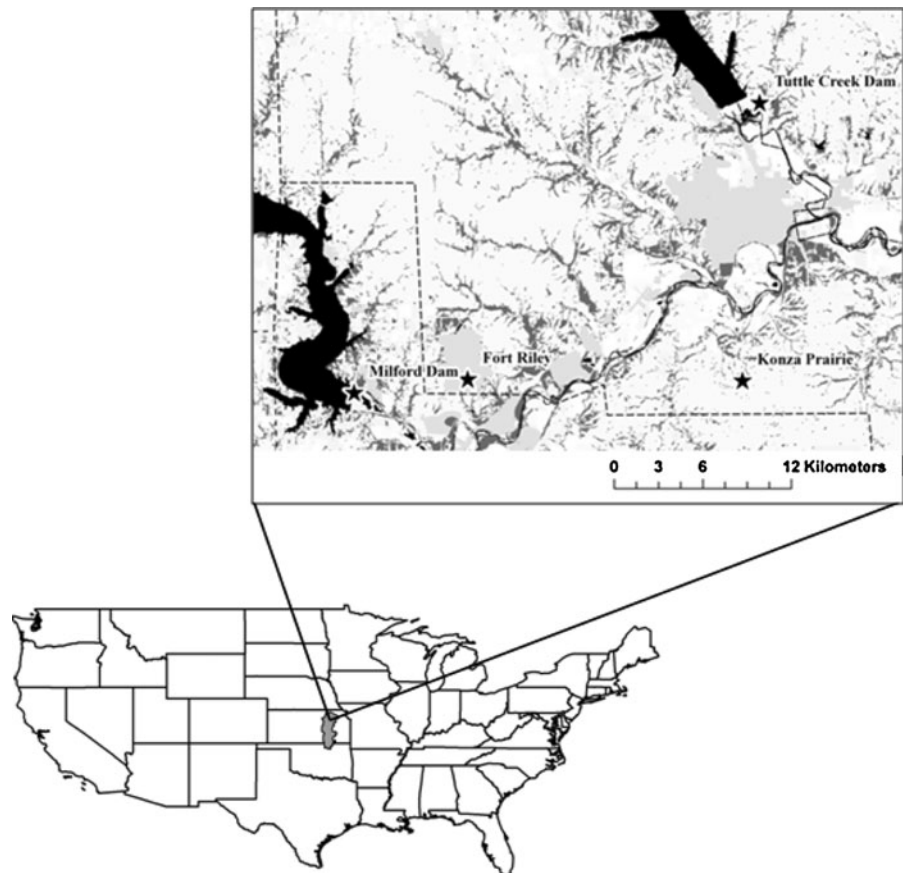
Study sites

Our study populations of collared lizards were located in the northern Flint Hills of Kansas. Collared lizards have experienced multiple range expansions and contractions over the last hundred thousand years and have expanded into their current distribution in northern Kansas only within the last 7,000 years (Hutchison and Templeton 1999; Hutchison et al. 1999; McGuire et al. 2007); we therefore assumed that any observed genetic diversity or local population genetic structure is a result of processes occurring since invasion. We collected DNA from lizards ($n = 229$) at four locations (pair-wise distances between sites ranged from 10 to 35 km) to examine patterns of local genetic structure. The four locations were: the Konza Prairie Biological Station (Konza Prairie) in Riley Co.; the Fort Riley Military Reservation (Fort Riley) in Riley Co.; Milford Dam in

Geary Co.; and Tuttle Creek Dam in Riley/Pottawatomie Cos. (Fig. 1). These sites were chosen because of their close proximity to one another and because each was known to provide suitable habitat that supported populations of collared lizards. Because our samples were collected at the periphery of the collared lizard range, where lizards are only patchily distributed within and among sites, our sampling was necessarily constrained to those locations where collared lizards occurred.

Konza Prairie is a 3,487-ha tallgrass prairie site managed by Kansas State University's Division of Biology. Rock habitat at this site occurs as limestone ledges outcropping along hillsides managed under different bison or cattle grazing and burning regimes (Blevins and With 2011). Konza Prairie is separated from our other sampling sites by the Kansas River, Fort Riley, and the cities of Manhattan and Junction City. Fort Riley is an active military site with over 28,000 ha of tallgrass prairie and gallery forest. Lizards were sampled at several locations across the

Fig. 1 The Collared lizard's (*Crotaphytus collaris*) range extends broadly through the mid- and southwestern United States. Sampling locations for this study (*inset*) occur along the northern periphery of the range in the Flint Hills, Kansas (*dark gray*, state map). Sample site locations are indicated by *stars*. Inset colors indicate land cover (*white* prairie or agriculture, *light gray* urban, *dark gray* forest, *black* water)



site. Milford and Tuttle Creek Dams are managed by the U. S. Army Corps of Engineers. Construction began at Milford Dam in 1962 and at Tuttle Creek Dam in 1952. Although some natural rock ledges occur at both sites, lizards were sampled at riprap along the eastern side of Milford dam (~2 km in length) and within the spillway and at riprap along the eastern lake edge at Tuttle Creek Dam. Upland habitat mainly separates Milford Dam from Fort Riley, while Tuttle Creek Dam is separated from Fort Riley and Milford Dam by Tuttle Creek Lake and the city of Manhattan.

At Konza Prairie, we collected the lizards from which we obtained our DNA samples ($n = 229$ individuals) during repeated surveys of limestone outcrops within 16 watersheds that contained collared lizards during May–August in 2008 and 2009 (Blevins and With 2011). We captured lizards by hand (noosing), and recorded the individual lizard's location (UTM coordinates; accuracy < 5 m) using a handheld GPS unit (Garmin). We also recorded sex (adults are sexually dimorphic, with males being more brightly colored and 10–15 mm larger than females at maturity; Fitch 1956; Yedlin and Ferguson 1973), and age based on size classes (juveniles < 80 mm; Sexton et al. 1992) when possible. Samples from Fort Riley, Milford Dam, and Tuttle Creek Dam were obtained opportunistically and were collected during surveys of lizards at rock habitat. When possible, capture locations were georeferenced, and both sex and age were determined. Tail tips (up to 1 cm) for DNA extraction were clipped from each lizard and stored in tubes containing DryRite for dehydration (IACUC protocol #2297). Four samples from Konza Prairie came from frozen tissue of lizards believed to have died of natural causes. The majority of our samples (77%) came from collared lizards located at Konza Prairie (adults, $n = 113$; juveniles, $n = 64$), and the remainder from Fort Riley (adults, $n = 14$; juveniles, $n = 0$), Milford Dam (adults, $n = 14$; juveniles, $n = 3$), and Tuttle Creek Dam (adults, $n = 20$; juveniles, $n = 1$).

Molecular methods

We used polymorphic microsatellite markers to examine genetic diversity and population differentiation since the most recent range expansion because microsatellites provide relatively contemporary

estimates of genetic diversity and differentiation (Selkoe and Toonen 2006). We used 10 previously published microsatellite primers specific to collared lizards: Orig6, Orig7, Orig11, Orig21, Orig24, Orig25, Orig26, Enr3, Enr48, and N5 (Hutchison et al. 2004). Loci were motifs of 2–4 nucleotide repeats with a minimum of 6 consecutive repeats. We isolated DNA from tail tips (<0.5 cm) by first immersing tail tissue in liquid nitrogen and then crushing it using a mortar and pestle. We used standard proteinase K–phenol–chloroform extraction methods (Sambrook et al. 1989) and amplified DNA using polymerase chain reaction (PCR) using modified specifications from Hutchison et al. (2004). Product was visualized using a 3730 DNA Analyzer (Applied Biosystems) and genotypes were manually scored using GeneMarker version 1.8 (Softgenetics). Samples that failed to amplify or were ambiguous were repeated, either by PCR or DNA extraction.

Statistical methods

Because genotyping errors have the potential to bias final conclusions (Taberlet et al. 1996; Bonin et al. 2004), we ran samples more than once and subsequently calculated the average per locus allelic dropout (ADO) rate (Broquet and Petit 2004). To ensure the quality of our results, we checked our data set for errors in genotyping caused by null alleles, stuttering, and large allele dropout using the software MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004). For each sampling location, we then conducted standard testing for departure from Hardy–Weinberg equilibrium, calculating F_{IS} according to Weir and Cockerham (1984), and for genotypic linkage disequilibrium for each locus pair using Fisher's method (Markov chain with parameters set to default values) within GENEPOP on the web (<http://genepop.curtin.edu.au/>; Raymond and Rousset 1995).

To test our hypothesis that genetic diversity is lower in peripheral populations relative to populations at the core of the range, we calculated several diversity measures reported in the literature (Hutchison 2003) for comparison. We first calculated allelic richness using a rarefaction method accounting for differences in sample size implemented in F-STAT (Goudet 1995). We also determined the effective number of alleles (n_e) according to the method of Kimura and Crow (1964), where $n_e = 1/[\sum(x_i)^{-2}]$.

Observed and expected heterozygosity were calculated using GENEPOP, and relatedness (r) of individuals within each sampling location was determined using maximum likelihood estimation implemented in the software ML-RELATE (Kalinowski et al. 2006).

Reduced genetic diversity might be the result of a recent (within the last few dozen generations; Luikart et al. 1998) population bottleneck (Nei et al. 1975), independent of the location of a population within a species' range (core vs. periphery). To verify that any reduction in genetic diversity measures (other than observed heterozygosity) we detected relative to core populations was not the result of a recent population bottleneck, we determined whether samples exhibited heterozygote excess relative to allelic richness (Cornuet and Luikart 1996). To calculate heterozygote excess, we used the two-phase mutation (TPM) model available in the software BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996) and assessed significance using a one-tailed Wilcoxon test. Additionally, we explicitly tested for evidence of population expansion by analyzing the distribution of allele lengths using both a within-locus and an interlocus test (Reich and Goldstein 1998; Reich et al. 1999) conducted using an Excel macro (Kgtests; Bilgin 2007).

To test our hypothesis that collared lizard populations should exhibit relatively low interpopulation divergence in the Flint Hills region, we conducted several analyses to identify the amount of divergence among our sample populations for comparison with values reported in the literature for populations in other regions. We first measured F_{ST} (Wright 1951; significance testing implemented in F-STAT) and calculated isolation by distance (IBD; Wright 1943), comparing pairwise genetic distance ($F_{ST}/1 - F_{ST}$) with log-geographic (Euclidean) distance matrices. We tested for significance of IBD by conducting 1,000 permutations using Mantel's test (Mantel 1967) in GENEPOP. We also conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to identify whether the majority of genetic variation was partitioned within or among populations.

Additionally, because we hypothesized that the tallgrass matrix of the Flint Hills landscape may be more conducive to gene flow than the landscape context of some other peripheral populations (e.g., Missouri glade populations), we used individual-

based tests such as principal coordinates analysis (GENALEX version 6.2; Peakall and Smouse 2006) and Bayesian clustering (Pritchard et al. 2000) to identify whether sampled individuals could be definitively assigned to populations based on their multilocus genotypes. Bayesian clustering (implemented in program STRUCTURE version 2.1.3; Pritchard et al. 2000) was conducted using the admixture model, with K (the number of clusters) ranging from one to four (for each of our sample sites). We performed 30 independent runs with an initial burn-in of 100,000 followed by 1,000,000 iterations. The number of populations considered as the best fit to our data set was determined by the K value with the highest average log probability [$\Pr(XK)$] over the thirty runs and by examination of bar plots for predicted ancestry of samples.

Since we expect that populations in the Flint Hills will have higher levels of gene flow relative to peripheral populations in other landscape contexts, we also attempted to quantify gene flow. We calculated pairwise past migration rates (m_i) and $\Theta = 4N\mu$ using the coalescent approach available in program MIGRATE-N (Beerli and Felsenstein 2001). We first used F_{ST} values to calculate m_i and Θ using the Brownian motion method. Model-estimated values were used as starting values for three additional runs to obtain reliable parameter estimates. Parameter estimates were determined using the full migration model and default values for search parameters. We used maximum likelihood estimation, and results for the final three models were equivalent.

Because samples from Konza Prairie were individually georeferenced, we were also able to calculate dispersal occurring at the finest scale, along outcrops within a sampling location, and consider how differences in landscape context might differentially affect dispersal at different locations within the collared lizard range. We performed global spatial autocorrelation analyses on samples and calculated pairwise geographic and genetic distance matrices among individuals. We then generated the autocorrelation coefficient (r) using GENALEX. Statistical significance was assessed by generating 999 random permutations and by performing 1,000 bootstrap trials to determine the 95% confidence interval for each permutation. The selection of distance classes (30, 60, 100, 300, and 1,000 m) was based on observed movements of 44 uniquely marked lizards

during this study (total number of re-sightings = 50; Fig. 2) and on dispersal distances reported in the literature (Hranitz and Baird 2000).

Results

Although we documented a high rate of ADO (8%), we conducted replicate genotyping when possible (samples were re-run on average 1.8 times \pm 0.04 SE) to minimize the influence of ADO on the results. We identified two loci, Orig11 and N5, as monomorphic and excluded them from all further analyses except tests for recent population expansion, which require inclusion of such loci (Reich et al. 1999). We found evidence for the presence of a null allele, which is distinguishable from biological causes for Hardy–Weinberg disequilibrium such as the Wahlund effect (Van Oosterhout et al. 2004), at a single locus (Orig7; frequency in sampling locations = 0.23–0.44, average = 0.36 \pm 0.05 SE). After removal of this locus, all sampling locations were found to be in Hardy–Weinberg equilibrium. We therefore calculated and applied a correction to that locus using the Van Oosterhout method available in the software MICRO-CHECKER for our population-level analyses. The software ML-RELATE provides a correction for the presence of null alleles, but for all other analyses requiring genotypic data, we either compared results with or without the inclusion of this locus or simply excluded it, depending upon the underlying assumptions of the analyses. The test for linkage disequilibrium indicated that two loci, Orig24 and Orig25, were

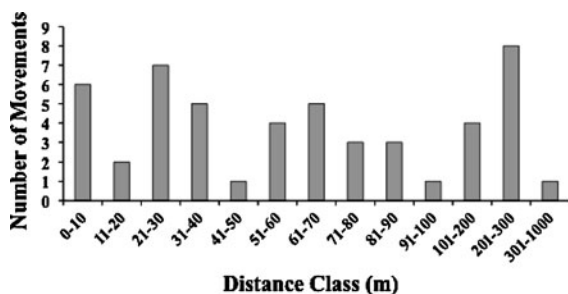


Fig. 2 Frequency distribution of distances moved by collared lizards at the Konza Prairie Biological Station in the northern Flint Hills, based on resightings of marked individuals ($n = 50$)

significantly linked in one of our four populations (Konza Prairie). Hutchison (2003) also found evidence for linkage disequilibrium at Orig24 and Orig25 (they are indeed located on the same chromosome; Hutchison et al. 2004), but in only one population of 42 sampled, and concluded these loci were evolving independently. Thus, we also retained these loci in our analyses.

Measures of genetic diversity were similar for Konza Prairie, Fort Riley, and Milford Dam, while Tuttle Creek Dam demonstrated lower estimates of genetic diversity and higher inbreeding and relatedness estimates (Table 1). Because contemporary disturbances at Tuttle Creek Dam, including a rock-scouring flood event that occurred in 1993, may have decreased the genetic diversity of this site relative to our other three sites, we grouped the Konza Prairie, Fort Riley, and Milford Dam sampling locations together and compared them to results for Tuttle Creek Dam. We used a two-sample *t*-test (data normally distributed: H_O , H_E , and F_{IS}) and a nonparametric two-sample Kolmogorov–Smirnov test (data non-normally distributed: allelic richness, effective number of alleles). Relatedness was compared using a *z*-test for two proportions. Only observed heterozygosity was significantly different between the two groups ($P = 0.035$), and we found evidence for a recent population bottleneck at Tuttle Creek Dam ($P = 0.04$; all other sampling locations, $P > 0.05$). With the exclusion of this site, we found evidence for recent population expansion [within locus (*k*) test, $P = 0.04$]. The interlocus (*g*) test for population expansion was not significant (significance assessed based on Table 1, Reich et al. 1999); however, other studies have found similar results, suggesting decreased power of the test when mutation rates among loci are variable (i.e., data sets that, like ours, contain dinucleotide, trinucleotide, and tetranucleotide microsatellite loci; Donnelly et al. 2001).

Modest genetic differentiation was evident among sites, with F_{ST} values ranging from 0.05 to 0.13 (average including Tuttle Creek Dam = 0.08 \pm 0.01 SE; average excluding Tuttle Creek Dam = 0.05 \pm 0.00 SE). All pairwise estimates except between Fort Riley and Milford Dam were significantly >0 after Bonferroni adjustment (Table 2). However, we found no evidence to support a hypothesis of isolation by distance ($P = 0.08$), and the AMOVA revealed that the majority (86%) of the molecular variance

Table 1 Average (\pm SE) allelic richness adjusted by sample size (AR), effective number of alleles (N_e), average observed and expected heterozygosity, inbreeding coefficient (F_{IS}), and relatedness (r) of four sampling locations for adult collared

Population	AR	N_e	H_o	H_E	F_{IS}	r
Konza Prairie	3.63	2.91	0.59	0.57	-0.03	0.11
Fort Riley	3.64	2.85	0.57	0.57	0.01	0.09
Milford Dam	3.49	2.65	0.58	0.56	-0.04	0.10
Tuttle Creek Dam	2.70	2.08	0.40	0.45	0.11	0.15
Average	3.37	2.62	0.54	0.54	0.01	0.11
SE	0.23	0.19	0.05	0.03	0.03	0.01

lizards (sample sizes: Konza Prairie, $n = 113$; Fort Riley, $n = 14$; Milford Dam, $n = 14$; and Tuttle Creek Dam, $n = 20$) using eight microsatellite DNA loci

Table 2 Pairwise comparisons of genetic distance between four sampling locations for adult collared lizards (for sample sizes, see Table 1) based on eight microsatellite DNA loci

Population	Konza Prairie	Fort Riley	Milford Dam
Konza Prairie	-		
Fort Riley	0.06*	-	
Milford Dam	0.05*	0.05	-
Tuttle Creek Dam	0.09*	0.09*	0.13*

Significant ($P < 0.05$ after Bonferroni correction) differences are indicated by asterisks

occurred within populations rather than among populations. The principal coordinates analysis returned six principal coordinates with eigenvalues >1 , with the first three explaining 59% of the variation in genotypes. A plot of the first two coordinates indicated that there was little structure, as demonstrated by the large amount of overlap of individuals from different sampling locations. However, some separation occurred between Tuttle Creek Dam and Konza Prairie along coordinate one (Fig. 3). Results from Bayesian genetic clustering indicated that there was little structure in our samples, with average log-likelihoods nearly indistinguishable for $K = 1, 2,$ or 3 .

Estimated migration rates differed among sampling locations (Table 3) and indicated gene flow had occurred (average N_m per generation = 1.56 ± 0.47 SE). The highest number of migrants was predicted from Tuttle Creek Dam to all other sites (average N_m per generation = 3.76 ± 0.53 SE) and between Milford Dam and Fort Riley (average N_m per generation = 1.73 ± 0.10 SE). Results from spatial autocorrelation analysis of samples from Konza Prairie indicated that neither positive nor negative

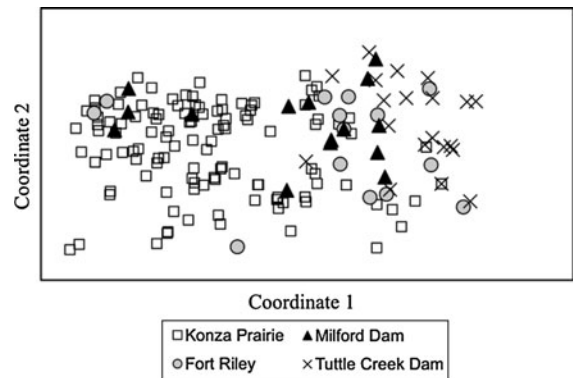


Fig. 3 Principle coordinates analysis conducted on DNA samples from adult collared lizards indicating relative genetic similarity of individuals across sample locations (for sample sizes, see Table 1)

Table 3 Estimates of theta ($4 N\mu$) and number of migrants per generation ($N_m = \Theta M, M = mi/\mu$) in program MIGRATE

Population	Θ	N_m			
		1, x	2, x	3, x	4, x
Konza Prairie	3.08	-	0.52	1.11	5.39
Fort Riley	3.10	0.50	-	1.98	4.12
Milford Dam	0.91	0.00	1.48	-	1.77
Tuttle Creek Dam	0.21	0.71	0.52	0.55	-

Estimates are based on DNA samples from adult collared lizards at four sampling locations in northeastern Kansas using eight microsatellites (for sample sizes, see Table 1)

spatial autocorrelation was evident in the four smallest distance classes (<1 km). However, results for spatial autocorrelation analysis at the 1-km distance class demonstrated positive correlation in distance class 1 (0–1 km) and negative correlation in distance classes 4 and 5 (3–5 km) (Fig. 4).

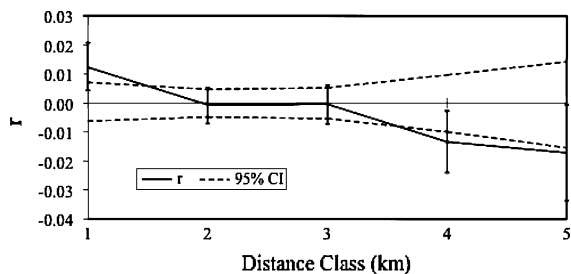


Fig. 4 Spatial autocorrelation analysis (with bootstrapped 95% error bars) conducted on collared lizard DNA samples collected at Konza Prairie ($n = 177$) grouped into distance classes of 1 km (i.e., distance class 1 indicates the correlation between genetic and geographic distance for samples collected between 0 and 1 km)

Discussion

This study tested the hypothesis that peripheral populations within a species' distribution have reduced genetic variability and increased population differentiation relative to core populations at the center of the range (Eckert et al. 2008; but see Gaston 2003, Table 2). Additionally, because landscape structure can variously influence the dispersal of organisms, gene flow within or among populations, and selection, consideration of landscape context can provide insight into the different mechanisms influencing population genetic structure throughout a species' range (Manel et al. 2003). For instance, Johansson et al. (2005) found differences in common frog (*Rana temporaria*) population genetic diversity and differentiation across regions in response to landscape context (i.e. agricultural intensity). Similarly, Berry et al. (2005) found that grand skink (*Oligosoma grande*) populations in exotic pastureland were more genetically structured and had less genetic variation than populations in native tussock grassland, although this study did not explore differences across regions. Our analysis allows for an examination of historical influence on population genetics, while also providing a basis for comparison of population genetics among populations existing within different landscape contexts in different regions.

Indeed, we observed estimates of allelic diversity and population structure for several collared lizard populations sampled at the northern extent of the distribution in the northern Flint Hills that were intermediate between estimates for central

populations in Texas and peripheral populations in the southwestern Ozarks (Hutchison 2003). We identified two loci (Orig11 and N5) that were monomorphic for all four sampling locations, and thus lacked any genetic variation. Hutchison (2003) found core-peripheral trends in monomorphism among collared lizard populations, with peripheral populations in the northeastern Ozarks having significantly more monomorphic loci than core populations in central Texas, which had none. Because we found evidence of recent population expansion at three of our sites, low genetic diversity in those collared lizard populations does not appear to be the result of a recent population bottleneck. Rather, with the exclusion of Tuttle Creek Dam, our results support the hypothesis of a recent range expansion for collared lizards (Hutchison et al. 1999). Hutchison and Templeton (1999) postulated that collared lizard populations, having only expanded into the Kansas region during the Holocene ($\sim 7,000$ ybp), have not been present long enough to have attained drift-migration equilibrium conditions (as demonstrated by the presence of increased genetic isolation between populations over greater distances; that is, isolation by distance), and indeed, we found no evidence for isolation by distance among our populations over shorter distances.

Our results suggest that a moderate degree of population structure may exist among our sample sites (pairwise estimates of F_{ST} were significant between all but two sites). However, we did not observe the high levels of differentiation characteristic of peripheral Missouri populations [southwestern Ozarks ($n = 28$), average $F_{ST} = 0.14 \pm 0.03$ SE; northeastern Ozarks ($n = 53$), $F_{ST} = 0.34 \pm 0.03$ SE] over the same distance (<40 km; Hutchison and Templeton 1999). Our average F_{ST} (0.08 ± 0.01 SE with Tuttle Creek Dam, 0.05 ± 0.01 SE without) appears intermediate between Missouri peripheral and Texas central ($n = 12$, average $F_{ST} = 0.02 \pm 0.00$ SE) populations (Hutchison and Templeton 1999).

This intermediate degree of population structure appears to be at least partially influenced by contemporary landscape and demographic processes. Multiple lines of evidence indicate that two of our study sites, Fort Riley and Milford Dam, display high rates of gene flow (nonsignificant pairwise F_{ST} and 1–2 migrants exchanged per generation). We also found evidence

that one sample site, Tuttle Creek Dam, has undergone a recent population bottleneck. This bottleneck may have resulted from a large flooding event in 1993, which raised the lake level 19 m and necessitated opening the dam's spillway (producing a flow-rate of 1,700 m³/s at peak) that drowned and eroded rock habitat (and presumably collared lizards within that habitat) where we collected our samples. Such a rapid decrease in population size would account for the signature of low genetic diversity at this site, while the high level of inbreeding and relatedness suggests that little dispersal from other populations has yet occurred. Although migration estimates for Tuttle Creek Dam suggest that this site has indeed experienced high rates of gene flow, low sample size for this site (number of samples with complete genotype = 6), likely impaired our ability to derive robust estimates of migration rates or ancestry.

Interestingly, the evidence for moderate population structure among populations seems, at first appearance, contradictory to our measures of gene flow occurring among the sites. In fact, populations that have been founded by a small, genetically homogeneous group during a range expansion are expected to have a high degree of genetic similarity, which could be mistaken for high rates of gene flow that are not actually occurring (Ibrahim et al. 1996; Pogson et al. 2001; Duvernell et al. 2008). This has been suggested for species that have recently expanded into habitat that was formerly unsuitable due to the presence of glaciers (Larson et al. 1984; Highton 1995).

Indeed, several of our results suggest that gene flow has been overestimated in our study. High levels of gene flow were suggested by the AMOVA, which indicated that the majority of molecular variance for samples occurred within rather than among sampling locations, and by MIGRATE-N, where the highest gene flow is suggested between Milford Dam and Fort Riley (excluding results for Tuttle Creek Dam, as discussed above). In addition, the principal coordinates analysis was unable to clearly partition samples into groups based on sampling sites, and we were unable to definitively assign individuals to populations using Bayesian clustering analysis, which suggested comparable likelihoods of one, two or three populations. However, we also note that the inability to assign individuals to unique populations may be the result of using a small number of loci (Pritchard et al. 2000).

Genetic divergence among populations may remain low in the Flint Hills region due to the potential use of rock outcrops and gravel roads as “dispersal avenues” through open grassland, which has been maintained historically—as at present—by fire and grazing, thus preventing the expansion of closed-canopy forest (Freeman 1998; Hartnett and Fay 1998), and future availability of fine-scale spatial data coverages, which would permit the mapping of individual limestone outcrops, would allow for a more rigorous analysis of the effect of landscape context at a local scale, permitting a test of this hypothesis. In comparison, populations of collared lizards in other regions, such as at the eastern periphery of their range in Missouri, occupy rock habitat within an oak-hickory forest matrix, where dispersal and gene flow are highly restricted over short distances (as little as 50 m apart; Hutchison and Templeton 1999; Templeton et al. 2001; Brisson et al. 2003). Collared lizards thus do not appear to disperse far in the context of a forested matrix.

Other rock-dwelling species appear to face similar obstacles to dispersal. For example, Levy et al. (2010) found that ornate dragon lizard (*Ctenophorus ornatus*) populations inhabiting rock outcrops surrounded by cleared agricultural land rather than native vegetation exhibited reduced genetic variation and increased genetic differentiation. Likewise, New Zealand grand skinks (*O. grande*; Whitaker 1996; Berry et al. 2005), Cunningham's skink (*Egernia cunninghami*; Stow et al. 2001) and collared pikas (*Ochotona collaris*; Franken and Hik 2004) are all rock-dwelling species whose dispersal is affected differently by the type of matrix surrounding habitat patches.

Although we did not directly observe lizards using outcrops for dispersal, we were able to examine gene flow over fine spatial scales (0–1 km) at Konza Prairie, a site with ample rock habitat, using spatial autocorrelation analysis. We detected significant negative correlations at greater distances (3–5 km), which may reflect a spatial limit to population admixture at this site. These results are consistent with our observations of movements by marked lizards at the Konza Prairie (100% of recorded movements $\leq 1,000$ m, with 74% ≤ 100 m; Fig. 2). Similar within-population movements have also been reported for central populations in Oklahoma, with lizards dispersing ~ 200 m but not more than 350 m

among rock ledges, with no evidence of population genetic substructure (Hranitz and Baird 2000). We also observed what appears to be a single long-distance dispersal event (1,000 m) by a male having reached sexual maturity, the point at which collared lizards most often disperse (Fitch 1956).

We are able to draw several conclusions based on our results. First, the genetic diversity and population genetic structure of collared lizards in the Kansas Flint Hills offer partial support for the core-peripheral hypothesis, as reported for other taxa (Eckert et al. 2008), and this “edge” effect is most likely a result of founder effects coupled with population expansion into habitat that was historically unsuitable owing to climate change and associated shifts in the biome (i.e., the last glacial retreat started about 12,000 ybp). Second, even though dispersal and gene flow appear to occur readily among rock habitat patches within sites, particularly in comparison to habitat patches within a different (forested) landscape context in other peripheral regions, gene flow does not appear to occur widely enough among sites to mitigate detectable population divergence.

Genetic diversity and population differentiation among our sampling sites may increase over time, especially due to changes in grassland management practices (e.g., forest expansion as a result of fire suppression) and increased exurban development, which can fragment grassland habitat and result in decreased connectivity and gene flow among populations. We conclude that while our results are generally consistent with expected trends in genetic diversity and differentiation for core vs. peripheral populations, not all peripheral populations at the margins of a species' range will necessarily exhibit the same level of genetic diversity or degree of genetic differentiation, an important consideration for the development of future studies of core-peripheral trends in population genetics (Eckert et al. 2008) and for setting conservation targets (Lesica and Allendorf 1995). Among different peripheral regions, local population differences and the degree of genetic differentiation among populations may also be influenced by landscape context (e.g., whether the population is embedded within tallgrass prairie vs. forest matrix, type of land use or land management, degree of fragmentation). More attention, then, should be given to landscape context, including landscape

history, in evaluating current patterns of genetic structure within and among populations.

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