Genetic Structure of Western Massasauga Rattlesnakes (Sistrurus catenatus tergeminus)

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ABSTRACT.—Understanding how habitat heterogeneity influences the genetic structure of populations is an important goal of conservation genetics. Species with different evolutionary histories may respond differently to contemporary habitat loss and fragmentation. Recent genetic analyses have shown high levels of genetic structure in two subspecies of Massasauga Rattlesnakes of conservation concern (Eastern Massasaugas, Sistrurus catenatus catenatus and Desert Massasaugas, Sistrurus catenatus edwardsii) living in highly fragmented habitats. Here, we complement those results with an analysis of the genetic structure of the third subspecies (Western Massasaugas, Sistrurus catenatus tergeminus), which has a largely continuous distribution in Kansas but with some isolated populations in Missouri. We found no evidence of genetic structure among the Kansas populations of Western Massasaugas, though our STRUCTURE analysis did identify the two Missouri populations as distinct clusters from each other and from the Kansas populations. Population differentiation estimates were much lower across all Western Massasauga populations compared to those observed in Eastern and Desert Massasaugas. Quantitative analyses of habitat availability and fragmentation confirm that the Kansas landscape is less fragmented than the range occupied by Eastern Massasaugas; this supports a possible influence of habitat fragmentation on genetic structure of these snakes. The more-continuous distribution and relative genetic uniformity of Western Massasaugas found in Kansas contrast with the isolated nature of Desert and Eastern Massasaugas, making the Western subspecies unique within the Massasauga complex.

Understanding of the role that habitat heterogeneity plays in shaping the genetic structure of populations is a long-standing question in evolutionary biology and conservation genetics (Keyghobadi, 2007). The quality of the environment between habitat patches and the distance between patches can have important evolutionary consequences for populations by influencing the frequency of migration (Collingham and Huntley, 2000; Keyghobadi et al., 2005). Species that occupy irregularly distributed habitats can exhibit highly structured populations because often they are isolated and intervening distances between patches prevent gene flow from occurring (Hughes et al., 1999; Barbara et al., 2008; Jones and Gibson, 2011). This contrasts with populations of species that experience regular exchanges of individuals due to more-generalized habitat requirements or reduced distances between habitat patches. Examining similar species occupying different levels of habitat heterogeneity can provide insights into the impact of habitat availability on population genetic structure (Brouat et al., 2003).

Among snakes, three subspecies of Massasauga rattlesnakes (Sistrurus catenatus): Eastern (Sistrurus catenatus catenatus), Western (Sistrurus catenatus tergeminus), and Desert Massasaugas (Sistrurus catenatus edwardsii) (see Kubatko et al., 2011 for a recent phylogenetic analysis of this group) represent an opportunity for evaluating how differences in habitat heterogeneity can influence genetic structure in closely related species. Eastern Massasaugas range from New York, United States and parts of Ontario, Canada westward across the Great Lakes region to eastern Iowa (Szymanski, 1998), whereas Western Massasaugas reach their northern limit in southeastern Nebraska, southwestern Iowa, and parts of Missouri and are found through large sections of Kansas and Oklahoma into Texas (Tennant, 1984). Taxonomists debate the taxonomic status of the Missouri populations, with contrasting claims that both Western Massasaugas and Eastern/Western hybrids occur within the state or only pure Eastern Massasaugas (Evans andloyd, 1948; Szymanski, 1998; Gibbs et al., 2010). Recent genetic data, however, demonstrated that snakes found in Missouri are genetically most similar to Western Massasaugas from Kansas and should be considered as S. c. tergeminus (Gibbs et al., 2010; Gerard et al., 2011). Finally, Desert Massasaugas are found in scattered populations in Colorado, southeastern Arizona, and parts of Mexico in addition to more cohesive population centers in parts of New Mexico and Texas (Tennant, 1984; Anderson et al., 2009).

Eastern and Desert Massasaugas persist mainly in isolated populations in fragmented habitats while Western Massasauga populations are found in both fragmented and unfragmented habitats (Tennant, 1984; Greene, 1997; Szymanski, 1998). Previous research has assessed population genetic structure in all three subspecies, but this work has been most complete for S. c. catenatus and S. c. edwardsii. Specifically, genetic analyses using microsatellites have revealed a high degree of long-term genetic structure among Eastern Massasauga populations (overall \(F_{ST}\) for regional comparisons = 0.2), even over short distances (<5 km) (Gibbs et al., 1997; Chiuuchi and Gibbs, 2010). Chiuuchi and Gibbs (2010) found evidence that the structure present in Eastern Massasauga populations predates any influence of European colonization. This indicates Eastern Massasaugas have evolved in isolated habitats. Anderson et al. (2009) analyzed genetic structure among three Desert Massasauga populations and also found substantial genetic structure between populations (overall \(F_{ST}=0.126\)). Hart et al. (2008) found some evidence for genetic structure in five populations of Western Massasaugas from Missouri (referred to as Eastern Massasaugas in their paper) and Nebraska. Because of the small number of loci (\(n=3\)) used, however, and because most of their sampled populations were located in a landscape more fragmented than most of the range of this subspecies, this limits the interpretation of their results.

Here we investigate the genetic variation and structuring of Western Massasauga populations in Kansas and Missouri using a larger set of microsatellite loci and more-complete analyses than previously used. Specifically, we tested whether Western Massasauga populations in Kansas, which appear to occupy less-fragmented habitats, showed reduced levels of genetic structure as compared to other species. We used a geographic...
analyzed the PCR product with an ABI 3100 genetic analyzer and used the program GENEMAPPER v.3.7 (Applied Biosystems, Thermo-Fisher Scientific, Carlsbad, CA) to score all individual genotypes. We also included microsatellite data from 56 individuals reported by Gibbs et al. (2010) for two Missouri populations (Pershing [n = 21] and Squaw Creek [n = 35]) that had been generated using the same protocols. Finally, for comparison, we also analyzed a set of microsatellite data generated by Chiucchi and Gibbs (2010) from the same 17 loci for 210 Eastern Massasaugas, representing 11 populations, a subset from their range-wide study. These 11 populations span from southwestern Ohio to western New York and cover a distance similar to the geographic extent of the Western Massasauga samples (584 km and 566 km, respectively). We did not make a direct comparison with Desert Massasaugas because samples from the Anderson et al. (2009) study were not genotyped for our set of 17 microsatellite loci.

Population Genetic Analyses.—We calculated gene diversity measures such as allele frequency and observed and expected heterozygosity using ARLEQUIN v.3.11 (Excoffier et al., 2005). We tested for departures from Hardy-Weinberg equilibrium globally and within the three STRUCTURE-defined clusters using GENEPOP (Raymond and Rousset, 1995) and used FSTAT v.2.9.3 (Goudet, 1995) to calculate allelic richness adjusted for sample size. We assessed genetic differentiation among populations using pairwise $F_{ST}$ calculated with ARLEQUIN v.3.11 (Excoffier et al., 2005). To correct for multiple comparisons associated with Hardy-Weinberg and $F_{ST}$ we applied the Benjamini and Yekutieli (B-Y) method (Benjamini and Yekutieli, 2001; Narum, 2006) to adjust the significance level for numbers of multiple comparisons. Finally, the program FREEWA was used to estimate frequencies of null alleles and assess their impact on $F_{ST}$ (Chapuis and Estoup, 2007).

We employed a Bayesian approach for assigning population clusters using the program STRUCTURE v.2.3.2 (Pritchard et al., 2000). Our STRUCTURE runs incorporated sampling location information (LocPrior; Hubisz et al., 2009) for comparison using the admixture model and correlated alleles. Ten independent runs for each of $K = 1$ through $K = 9$ were conducted using an initial burn-in period of 10,000 iterations followed by a run of 100,000 iterations. We selected $K = 9$ as the maximum because with six sampling areas we felt this was an appropriate upper limit for the number of clusters. We used the $\Delta K$ method developed by Evanno et al. (2005) to identify the number of populations ($K$) as performed in STRUCTURE HARVESTER version 0.56.3 (Earl and vonHoldt, 2011). Finally we also used GENEPOP (Raymond and Rousset, 1995) to examine evidence for an effect of isolation by distance at the population level using $F_{ST}$ differentiation with a Mantel test (1,000 permutations).

Landscape Analysis.—We estimated amounts of suitable habitat available to Eastern and Western Massasaugas in Ohio and Kansas, respectively, at two different spatial scales to compare landscape metrics related to fragmentation (e.g., habitat area, patch size, and connectivity). We selected a scale relevant to habitat use by individual snakes (Durbian et al., 2008) and one more representative of the overall landscape. These landscape metrics influence genetic structure in a variety of animal taxa (Gaines et al., 1997; Coulon et al., 2004; Cresswell and Osborne, 2004) and hence might explain differences in genetic structure observed between the two Massasauga subspecies.

We made habitat comparisons between our Western Massasauga sampling locations in Kansas and existing Eastern Massasauga populations in Ohio as described in Chiucchi and

**Materials and Methods**

Sample Collection and Genotyping.—We sampled Western Massasaugas from four locations in Kansas in 2004 and 2005 (Fig. 1). Individual snakes were opportunistically sampled during nighttime road and visual encounter surveys over relatively large areas (18–100 km) and then grouped into “populations” defined by a specific geographic location to allow us to evaluate pairwise differentiation using F-statistics. Specifically, we defined populations as samples from individuals from Barber County (Co.) ($n = 8$), Cheyenne Bottoms Wildlife Area ($n = 20$), Ellsworth Co. ($n = 11$), and Russell Co. ($n = 15$). We recognize this pooling of data to estimate a series of landscape metrics related to patch size, connectivity, and fragmentation. We tested whether reduced levels of genetic structure covaried with decreased habitat fragmentation; this would suggest a possible role for habitat heterogeneity in generating structure. In contrast, similar levels of structure in fragmented and unfragmented habitats would imply there is some inherent biological feature of these snakes that results in limited movement regardless of the configuration of the habitat.

![FIG. 1. Sampling locations for Western Massasaugas in Kansas and Missouri. 1) Russell County; 2) Cheyenne Bottoms; 3) Ellsworth County; 4) Barber County; 5) Squaw Creek; and 6) Pershing.](image-url)
Table 1. Allelic diversity, observed heterozygosity, expected heterozygosity information, and null allele estimates across 17 loci for six populations of Western Massasaugas. Values in parentheses represent data from 11 populations of Eastern Massasaugas for the same loci as reported by Chiucchi and Gibbs (2010).

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. of Alleles</th>
<th>$H_{obs}$</th>
<th>$H_{exp}$</th>
<th>Null allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C05</td>
<td>34 (26)</td>
<td>0.52 (0.75)</td>
<td>0.92 (0.77)</td>
<td>0.195</td>
</tr>
<tr>
<td>1E09</td>
<td>18 (12)</td>
<td>0.85 (0.63)</td>
<td>0.87 (0.67)</td>
<td>0.014</td>
</tr>
<tr>
<td>1G05</td>
<td>23 (11)</td>
<td>0.30 (0.55)</td>
<td>0.82 (0.63)</td>
<td>0.278</td>
</tr>
<tr>
<td>1H03</td>
<td>16 (7)</td>
<td>0.53 (0.40)</td>
<td>0.59 (0.47)</td>
<td>0.046</td>
</tr>
<tr>
<td>2A09</td>
<td>11 (9)</td>
<td>0.89 (0.53)</td>
<td>0.86 (0.54)</td>
<td>0.005</td>
</tr>
<tr>
<td>1F04</td>
<td>6 (5)</td>
<td>0.53 (0.24)</td>
<td>0.62 (0.26)</td>
<td>0.059</td>
</tr>
<tr>
<td>1F11</td>
<td>13 (11)</td>
<td>0.45 (0.66)</td>
<td>0.84 (0.70)</td>
<td>0.024</td>
</tr>
<tr>
<td>2A06</td>
<td>16 (11)</td>
<td>0.77 (0.41)</td>
<td>0.85 (0.47)</td>
<td>0.038</td>
</tr>
<tr>
<td>1A06</td>
<td>15 (8)</td>
<td>0.32 (0.43)</td>
<td>0.75 (0.43)</td>
<td>0.240</td>
</tr>
<tr>
<td>2F01</td>
<td>11 (9)</td>
<td>0.75 (0.58)</td>
<td>0.74 (0.58)</td>
<td>0.013</td>
</tr>
<tr>
<td>1D01</td>
<td>19 (18)</td>
<td>0.75 (0.55)</td>
<td>0.80 (0.58)</td>
<td>0.012</td>
</tr>
<tr>
<td>2G06</td>
<td>15 (11)</td>
<td>0.46 (0.75)</td>
<td>0.51 (0.71)</td>
<td>0.014</td>
</tr>
<tr>
<td>1C01</td>
<td>21 (13)</td>
<td>0.53 (0.74)</td>
<td>0.91 (0.71)</td>
<td>0.011</td>
</tr>
<tr>
<td>1F10</td>
<td>34 (17)</td>
<td>0.51 (0.61)</td>
<td>0.87 (0.67)</td>
<td>0.185</td>
</tr>
<tr>
<td>1G08</td>
<td>35 (27)</td>
<td>0.94 (0.64)</td>
<td>0.94 (0.76)</td>
<td>0.003</td>
</tr>
<tr>
<td>2C06</td>
<td>24 (10)</td>
<td>0.66 (0.51)</td>
<td>0.86 (0.60)</td>
<td>0.106</td>
</tr>
<tr>
<td>2H09</td>
<td>17 (9)</td>
<td>0.54 (0.71)</td>
<td>0.78 (0.72)</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Gibbs (2010). Ohio was chosen for comparison because the majority of the populations used in the reanalysis of the genetic data were located there, and the patchy distribution of Eastern Massasauga populations within the state is characteristic of their distribution in the United States. The cropland data layer (CDL) produced by the United States Department of Agriculture (USDA) National Agriculture Statistics Service (NASS) served as the basis for our habitat assessment. This data layer combines data from the 2001 National Land Cover Database (Homer et al., 2004) with current information on the distribution of agriculture, an important driver of habitat fragmentation in both Kansas and Ohio (Doherty and Grubb, 2000; Porej et al., 2004; Klug et al., 2011). By extracting information on the amount of habitat and agriculture, we made direct comparisons between Kansas and Ohio. This national database layer is most appropriate for comparing habitats in Kansas and Ohio because they were produced with a consistent methodology, whereas state-specific land cover maps (e.g., GAP analyses) could introduce user bias. We performed all the following GIS analyses with ArcGIS v.10.1 (ESRI; Redlands, CA).

Estimating Suitable Habitat.—To differentiate between suitable and nonsuitable habitat, we used 450 point locations accumulated for Western Massasaugas from Kansas by the Kansas State Herpetological Atlas project (Taggart et al., 2011) and 259 points collected during survey efforts for Eastern Massasaugas from Ohio (Wynn and Lipps, unpubl. data). We overlaid the point locations with the CDL maps for both states and then extracted the specific land cover class information associated with each point location. Forested and active agricultural areas were not considered suitable habitats in either Kansas or Ohio because the vast majority of points located on either of these land cover classes were within 100–200 m of characteristic Massasauga grassland habitat. This could result from a combination of classification errors along the border of grassland habitat and forested or agricultural areas (Smith et al., 2003) and the temporary utilization by snakes of forested and agricultural areas adjacent to core habitat. We identified two habitat classes in Kansas and three in Ohio that contained the majority of points to represent suitable habitat.

After selecting our suitable habitat classes, we reclassified the Kansas and Ohio CDL maps into three classes representing suitable habitat, unsuitable habitat, and agriculture. We then created 1-km and 10-km buffers around six Ohio populations and the four Kansas sampling areas. Because sampling for two of the designated Kansas populations occurred over large areas, we split each of these into three buffered areas, based on snake locations, which allowed for nonoverlapping 10-km buffers, thus improving the spatial extent of our analysis. This subdivision of sampling areas resulted in a total of eight buffers for Kansas.

We used each buffer as a mask to create 1 km and 10 km grid files which were imported individually into FRAGSTATS v.3.3 (McGarigal and Marks, 1994). We then used FRAGSTATS to calculate landscape metrics for each grid file to compare amounts of habitat and agriculture, patch size, connectivity, and fragmentation of Massasauga habitat in Kansas and Ohio. We estimated abundance of suitable habitat in each state by calculating the percentage of suitable habitat (PLAND) within each grid file. We then made patch size comparisons by calculating mean patch size for both states. For connectivity we estimated the patch cohesion index (COHESION), a measure of the physical connectedness of a patch type, and connectance (CONNECT), a proportion of how many patches are within 200 m of each other. For our final landscape metric we estimated an index of fragmentation called the percentage of like adjacencies (PLADJ), which measures the level of aggregation for a patch type of interest. More-detailed descriptions of these metrics and how they are calculated are found in McGarigal et al. (2002). We analyzed differences in each landscape metric between Kansas and Ohio with a Mann-Whitney U-test using MINITAB v.16 software.

Results

Genetic Variation.—The Western Massasauga samples displayed high levels of genetic diversity but also some heterozygosity deficits relative to Hardy-Weinberg expectations. We observed an average of 19.3 alleles per locus with overall mean observed heterozygosity ($H_{obs}$) 0.63 lower than expected heterozygosity ($H_{exp}$) 0.80 (Table 1). At a population level, allele frequencies among the six Western Massasauga sampling locations ranged from 7.3–11.2 with heterozygosity deficits present in each population (Table 2). Overall, 8 of 17 loci were out of Hardy-Weinberg equilibrium after correcting the critical P-value for the number of multiple comparisons using the B-Y method. Deviations from Hardy-Weinberg expectations were observed in 34 of 102 locus/population tests following correction.
For the STRUCTURE-defined clusters we observed deviations from Hardy-Weinberg in 6 of 17 loci for Squaw Creek, 5 of 17 loci for Pershing, and 8 of 17 loci for the Kansas group. Overall estimates of null allele frequencies across the 17 loci ranged from 0.003 to 0.278 with a mean of 0.09 (Table 1). Within population null allele frequencies estimated using FreeNA were uniform and small (0.07–0.10).

Western Massasauga populations showed elevated levels of genetic diversity relative to Eastern Massasauga populations (Table 1). Though our Eastern Massasauga sample size is nearly twice that of the Western Massasauga sample size, they exhibited a marked reduction in allelic diversity across the 17 loci (12.6 vs. 19.3 alleles/locus) (Table 1). Allelic richness estimates adjusted for sample size for the six Western Massasauga populations (5.74–7.25; Table 2) exceed all those reported for Eastern Massasaugas (2.86–5.23) by Chiucchi and Gibbs (2010). Finally, the level of mean observed heterozygosity (0.57) in the Eastern Massasauga samples was similar to that of the Western Massasauga samples (0.63).

Genetic Differentiation.—Western Massasauga populations in Kansas and Missouri show low levels of genetic structure relative to the degree of differentiation shown in other Sistrurus. Pair-wise $F_{ST}$ estimates for Western Massasaugas were low to moderate (0.009–0.083) with a global $F_{ST}$ of 0.047 (Table 3). The FREEENA adjusted global $F_{ST}$, which accounts for the null alleles, was only slightly smaller than the original estimate ($F_{ST} = 0.043$). Because of the apparent minor influence of the null alleles on $F_{ST}$ we elected to include all 17 loci in our analysis. All but two pair-wise comparisons among Western Massasauga populations were significant (comparisons of Barber Co. to Russell Co. and Cheyenne Bottoms to Russell Co. were exceptions). Pershing exhibited the highest levels of differentiation among population comparisons for both indices ($F_{ST} = 0.088$). The Mantel test for isolation by distance showed a positive correlation between geographic distance and genetic distance ($r = 0.75, P < 0.01$).

In contrast, the pairwise $F_{ST}$ estimates for Eastern Massasaugas (0.01–0.39, global = 0.22) were almost four times greater than those for Western Massasaugas. All pairwise comparisons were significant after correction except for one (KL-1 to KL-2). The STRUCTURE analysis identified three genetic clusters in our data set consisting of the Kansas groups (Barber Co., Cheyenne Bottoms, Ellsworth Co., Russell Co.) as a single cluster and the two Missouri populations, Squaw Creek and Pershing, each as a separate cluster (Fig. 2).

Landscape Analysis.—Based on our land cover analysis at both fine (1 km) and broad (10 km) spatial scales, Massasauga habitat appears more abundant and less fragmented in Kansas compared to Ohio (Table 4). We observed an increase in mean patch size for Kansas (11.6 ha to 27.0 ha) when moving from fine to broad scale, while mean patch size in Ohio remained fairly constant (1.8 ha to 1.7 ha) over the same extent. Our statistical comparisons of landscape metrics between the two states revealed more habitat in Kansas that was less fragmented and available to Western Massasaugas at both spatial scales (though with considerable variability for some metrics; Table 4). Mann-Whitney U-test results showed the proportion of area utilized for agriculture did not differ significantly for the selected regions in Kansas and Ohio included in our spatial analysis at either fine ($p = 0.8$) or broad scales ($p = 0.3$).

**DISCUSSION**

Concerning genetic diversity within the Massasauga complex, Western Massasaugas appear more similar to Desert Massasaugas than to Eastern Massasaugas. Desert Massasauga populations in Arizona ($H_{obs} = 0.764, H_{exp} = 0.80$) and New Mexico ($H_{obs} = 0.695, H_{exp} = 0.74$) retain higher levels of genetic diversity than what we estimated for 11 populations of Eastern Massasaugas ($H_{obs} = 0.57, H_{exp} = 0.61$) (Anderson et al., 2009). From a broad perspective, our genetic diversity estimates for Western Massasaugas fall within the range observed for other North American snake species such as Timber Rattlesnakes (Crotalus horridus) ($H_{obs} = 0.49–0.63, H_{exp} = 0.55–0.66$) and Copper-bellied Water Snakes (Nerodia erythrogaster neglecta) ($H_{obs} = 0.53–0.70, H_{exp} = 0.57–0.76$) (Clark et al., 2008; Marshall et al., 2009).

The increased allelic diversity in Western Massasaugas relative to Eastern Massasaugas is consistent with the general lack of genetic differentiation among Western Massasauga populations, particularly those from Kansas. Both results suggest that Western Massasauga populations are large relative to Eastern Massasauga populations and, hence, the role of genetic drift in reducing genetic variation is less of a factor in Western Massasauga than in Eastern Massasauga populations (Chiucchi and Gibbs, 2010).

The difference in structure between Western and Eastern Massasauga populations is most striking for the two Missouri populations. These populations have a geographic conformation that is similar to the Eastern Massasauga populations in that each population is isolated with a low probability of any contemporary exchange of individuals. However, the degree of genetic divergence between Pershing and Squaw Creek populations ($F_{ST} = 0.076; 175$ km) is lower than that between Eastern Massasauga populations of a similar distance from each other; for example, that of Willard Marsh and Rome populations in Ohio ($F_{ST} = 0.21; 170$ km; see Chiucchi and Gibbs, 2010). The Pershing and Squaw Creek populations also show limited divergence from the Kansas groups (Table 3), though the
STRUCTURE analysis separates each of the Missouri populations into separate clusters (Fig. 2).

Our GIS-based analysis of habitat continuity is qualitatively consistent with the idea that Massasauga habitat is more continuously distributed in Kansas than in Ohio and, hence, could underlie observed differences in genetic structure. At both 1 km and 10 km spatial scales, Kansas has larger estimates of overall habitat abundance, patch size, connectivity, and lower levels of fragmentation. Fewer barriers to dispersal could increase the likelihood of mixing between neighboring populations of Western Massasaugas, resulting in lower levels of differentiation compared to Eastern Massasauga populations.

Klug et al. (2011) found a similar lack of genetic structure in Eastern Yellow-bellied Racers (Coluber constrictor flaviventris) inhabiting Kansas’ grasslands. Based on microsatellite data, they also found little evidence for discrete subpopulations with STRUCTURE (K = 1–2), which is comparable to our results based only on Kansas samples (results not shown). Their scale of analysis (75 × 180 km) also is comparable to our study (70 × 215 km). Klug et al. (2011) argue that the lack of genetic structure over a large area of contiguous habitat is because racers are habitat generalists without philopatric ties to specific home ranges or hibernacula.

Based on our analyses, we think differences in habitat fragmentation could be an important cause of the differences in genetic structure between Western and Eastern Massasauga populations. These differences in genetic structure, however, did not necessarily originate from the effects of conventional anthropogenic disturbances on contemporary gene flow. Chiucchi and Gibbs (2010) showed that historical levels of gene flow between Eastern Massasauga populations also were low, suggesting that human-related impacts on landscapes cannot be the only factor leading to high levels of differentiation seen in Kansas or in Missouri. These differences in genetic structure, however, did not necessarily originate from the effects of conventional anthropogenic disturbances on contemporary gene flow. Chiucchi and Gibbs (2010) showed that historical levels of gene flow between Eastern Massasauga populations also were low, suggesting that human-related impacts on landscapes cannot be the only factor leading to high levels of differentiation seen in Kansas or in Missouri.

In summary, high levels of genetic differentiation do not appear to be characteristic of all Massasauga subspecies. Eastern Massasaugas seem to have evolved in small, isolated populations in which individuals are strongly philopatric and show limited movement among populations (Szymanski, 1998; Marshall et al., 2006; Moore and Gillingham, 2006). In contrast, the Western Massasauga populations we analyzed show little structure and seem to consist of large populations with high levels of migration between them, possibly as a result of the less-fragmented habitats they occupy.

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