Herpetological Conservation and Biology 12(2):509–521.
Submitted: 5 August 2016; Accepted: 13 July 2017; Published: 31 August 2017.

Snakes in the City: Population Structure of Sympatric Gartersnakes (*Thamnophis* spp.) in an Urban Landscape

Eric J. Gangloff[1,5], Dawn M. Reding[1,2], David Bertolatus[3,4], Christopher J. Reigel[4], Jennifer L. Gagliardi-Seeley[4], and Anne M. Bronikowski[1]

[1]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011, USA*
[2]*Department of Biology, Luther College, Decorah, Iowa 52101, USA*
[3]*Department of Integrative Biology, University of Colorado Denver, Denver, Colorado 80217, USA*
[4]*Department of Biology, Metropolitan State University of Denver, Denver, Colorado 80217, USA*
[5]*Corresponding author, e-mail: eric.gangloff@sete.cnrs.fr*

***Abstract.*—Despite the intense challenges to wildlife presented by urbanization, many species continue to survive and even to thrive in these highly impacted landscapes. In the urban matrix of Denver, Colorado, USA, two snake species persist in restricted urban habitats near the limit of their geographic ranges: the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*Thamnophis radix*). We used nuclear microsatellite markers to assess the population genetic structure of three populations of both species living syntopically in this highly developed urban environment and one population of *T. elegans* in natural habitat. Our findings indicate strong population structure, limited migration between populations of each species, and evidence of inbreeding and a loss of genetic diversity, with these patterns more pronounced in *T. radix*. Additionally, we do not find evidence of increased hybridization between these species in such confined habitat patches. These results suggest that populations of long-lived vertebrate species may require intervention to escape long-term detrimental effects of habitat fragmentation and subsequent genetic isolation.**

Key Words.—habitat fragmentation; hybridization; inbreeding; *Thamnophis elegans*; *Thamnophis radix*

Introduction

Despite a litany of negative impacts on herpetofauna imposed by urbanization (Bonnet et al. 1999; Shine et al. 2004a; Evans et al. 2011), some reptile species continue to survive in these anthropogenically altered habitats. However, data on population genetic structure in urban centers are lacking for reptiles generally and for snakes specifically (Mitchell and Jung Brown 2008; Seigel and Mullin 2009). While habitat fragmentation due to agriculture (Row et al. 2010; Wood et al. 2015) and other development (Guicking et al. 2004; Jansen et al. 2007; Clark et al. 2010) has been implicated in restricted gene flow and increased genetic differentiation in snake populations, few studies have examined population genetic structure in a strictly urban environment (but see Prior et al. 1997; Xuereb et al. 2015). Importantly, fragmentation does not affect all snake species uniformly (Gibbs and Weatherhead 2001; DiLeo et al. 2010) and population structure is not necessarily influenced by fragmentation if populations can maintain connectivity or have historically limited dispersal (DiLeo et al. 2010; Dubey et al. 2011; Weyer et al. 2014). Thus, quantifying these processes in urban habitats remains an important step in characterizing the potential impacts of development on population persistence (e.g., Lourenço et al. 2017).

Gartersnakes (*Thamnophis* spp.) are widely distributed, can be high in density in some locations, and are seemingly adaptable to urban environments (Rossman et al. 1986; Kjoss and Litvaitis 2001; Mifsund and Mifsund 2008; Patrick and Gibbs 2009). Although some urban populations might appear healthy in the short-term, two key processes can lead to erosion of genetic diversity and population declines: reduced genetic connectivity and hybridization. First, restriction of gene flow combined with genetic drift can lead to loss of heterozygosity, which in turn can lead to inbreeding depression and population declines, as has been documented in a number of snake species (Madsen et al. 1996; Gautschi et al. 2002; Keller and Waller 2002; Ujvari et al. 2002). Second, habitat loss and fragmentation may promote hybridization between species that do not normally interbreed. Hybridization can erode genetic distinctiveness and present challenges for management, but can also be an important source of genetic diversity (Lande 1998; Placyk et al. 2012).

In this study, we examined the population genetic structure of two sympatric gartersnake species, the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*), sharing habitat in a major urban center of Denver, Colorado, USA, as well as an outgroup population of *T. elegans* in unfragmented habitat outside the city. Using nuclear

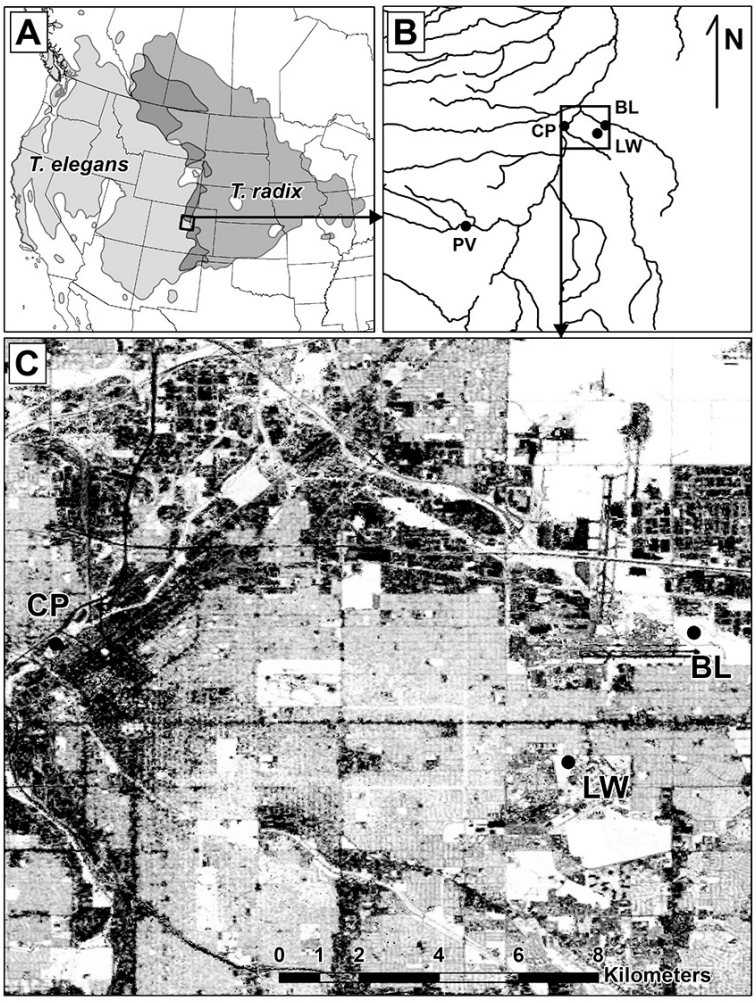

Figure 1. (A) Range map of the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*) in western North America. (B) Regional map showing study populations in north-central Colorado, USA, including waterways (dark lines). (C) City map of Denver showing three urban populations. Darker shading indicates greater urban development. Abbreviations of sites are BL = Bluff Lake Nature Center, CP = Commons Park, LW = Lowry Wetlands, PV = Pine Valley Ranch Park.

microsatellite markers, we tested four hypotheses of population genetic structure: (1) urban populations are genetically differentiated; (2) urban populations show indications of inbreeding and reduced genetic diversity; (3) migration between populations is limited; and (4) population isolation in small habitat fragments will lead to increased hybridization. Exploring these questions in multiple taxa simultaneously allows for a greater understanding of population genetic structures in urban landscapes and provides much-needed insight into how population dynamics are shaped by urbanization (e.g., Delaney et al. 2010; Reid et al. 2017).

Materials and Methods

Study system and field methods.—*Thamnophis elegans* and *T. radix* are closely related congeners (Alfaro and Arnold 2001; de Queiroz et al. 2002) and, within the overlap of their geographic ranges, share habitat in a rapidly developing urban area along the Front Range of Colorado. Both species eat a diversity of prey items, occupy a wide variety of habitats across their broad geographic ranges, and are generally found close to bodies of water, although *T. elegans* more readily uses aquatic habitat and food sources (Rossman et al. 1986; Hammerson 1999; Ernst and Ernst 2003). The three urban sites we surveyed represent a sampling of urban habitats (4.2–12.5 km apart) of Denver: Commons Park (CP), a 10-ha downtown city park; Lowry Wetlands (LW), a 20-ha unimproved watershed on a former U.S. Air Force base; and Bluff Lake Nature Center (BL), a 50-ha urban nature preserve formerly part of Stapleton International Airport. Additionally, we include *T. elegans* samples from Pine Valley Ranch Park (PV), a 332 ha site 70 km southwest of the city center, representing a population in natural, largely intact habitat (Fig. 1; see also detailed descriptions in Gangloff et al. 2014). Each of these sites is transected

Table 1. Habitat description and summary of genetic variability measures for the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*) from populations in and around Denver, Colorado, USA. The abbreviations n = number of samples and F_{IS} = system of mating inbreeding coefficient. Private alleles and allelic richness calculated with PopGenReport (Adamack et al. 2014); average observed and expected heterozygosities calculated with Arlequin (version 3.5, Excoffier and Lischer 2010) after removing one loci (see text for details). Inbreeding coefficients estimated with program BayesAss (version 3.0, Wilson and Rannala 2003), shown with 95% credible set (Bayesian confidence intervals) in parentheses, with asterisk if significantly different from zero.

Population	Years Sampled	Lat/Long (datum = WGS84)		n	Private Alleles	Allelic Richness	Avg H_O	Avg H_E	F_{IS}
Bluff Lake Nature Center (BL)	2010–2012	39°45'31.95"N 104°51'27.40"W	*T. elegans*	30	4	3.97	0.50	0.50	0.06 (-0.02–0.14)
			T. radix	10	6	6.16	0.60	0.78	0.30* (0.09–0.52)
Commons Park (CP)	2010–2012	39°45'29.12"N 105°0'17.44"W	*T. elegans*	14	4	3.98	0.62	0.59	0.18 (-0.10–0.46)
			T. radix	29	12	5.78	0.72	0.78	0.10* (0.02–0.17)
Lowry Wetlands (LW)	2009–2012	39°43'38.35"N 104°53'23.07"W	*T. elegans*	21	3	4.31	0.40	0.41	0.17* (0.03–0.32)
			T. radix	15	5	5.35	0.62	0.76	0.25* (0.02–0.38)
Pine Valley Ranch Park (PV)	2011–2012	39°24'32.31"N 105°20'59.25"W	*T. elegans*	40	5	2.87	0.55	0.52	0.05 (-0.03–0.13)
Average/Total	2009–2012		*T. elegans*	105	16	3.78	0.52	0.51	0.12
			T. radix	54	23	5.76	0.64	0.77	0.22

by or contiguous with a stream or river, which presents the opportunity for snakes to travel via these waterways. These study sites were identified based on our own experience surveying urban parks and undeveloped lots across Denver. We note that there are likely other sites in the Denver Metro area containing gartersnakes, especially between the Pine Valley Ranch site and the city of Denver. As such, the populations included in this study should be treated as a representative sample of urban populations, not the complete set of gartersnake populations in this city.

We located snakes on open ground or under cover objects during the active season (March-November) from 2009–2012, often with the assistance of high school students (Gangloff 2011). We note that our unbalanced sample sizes are not due to differences in sampling effort among populations. In fact, the population with the largest sample size (Pine Valley Ranch for *T. elegans*) is the location at which we spent the fewest person-hours surveying. Thus, our sample sizes are likely commensurate with small population sizes in these urban habitats. After collecting morphometric and behavioral data, we marked snakes by ventral scale clipping (Brown and Parker 1976) and released all animals at the point of capture. Tissue samples were stored in 95% ethanol until lab analysis. In addition to samples from live snakes, we collected tissue samples from deceased animals when it was possible to ensure that they were not previously marked individuals (see Table 1 for site and sampling details).

Laboratory methods.—We extracted DNA from tissue samples following a salting-out protocol modified from Sunnucks and Hales (1996). We incubated diced tissue samples with 20 µL proteinase K and 480 µL TNES buffer overnight at 37° C with continuous shaking. We added 140 µL 5 M NaCl and centrifuged at 14,000 rpm for 20 min. We removed the supernatant, added it to 700 µL cold 100% ethanol, and centrifuged it at 14,000 rpm for 5 min. We then removed the ethanol, added 500 µL of 70% ethanol to the pellet, centrifuged at 14,000 rpm for 5 min, and dried the inverted tube on a hot block (about 55° C) for 10 min. We measured DNA concentration after resuspension in 25 µL of TE-lite buffer with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and diluted samples to 25 ng/µL with ultrapure water.

We used eight previously published microsatellite loci designed for gartersnakes and closely related species, with slight modification (Table 2). We amplified loci in

Table 2. Properties of the microsatellite loci used in this study. The letter F refers to forward primer; R refers to reverse primer.

Locus	Multiplex	Repeat Motif	Primer concentration used (nM)	Reference
Nsµ10	3	$(TG)_{22}$	F = 0.03, R = 0.45	Prosser et al. (1999)
Nsµ2	2	$(AC)_{18}$	F = 0.03, R = 0.45	Prosser et al. (1999)
Nsµ3	3	$(ATCT)_{14}AT(CA)_{20}$	F = 0.03, R = 0.35	Prosser et al. (1999)
TS10	1	$(ATGG)_3(ATGA)_6$	F = 0.03, R = 0.35	Manier and Arnold (2005)
TS2	2	$(AAT)_{10}(AAT)_3$	F = 0.025, R = 0.1	McCracken et al. (1999)
Te1Ca18	1	$(CA)_{22}(GA)_2(C)_6$	F = 0.03, R = 0.28	Garner et al. (2004)
Te1Ca2	1	$(CA)_{20}$	F = 0.03, R = 0.50	Garner et al. (2004)
Te1Ca3	2	$(CA)_{13}$	F = 0.04, R = 0.65	Garner et al. (2004)

three multiplexes using a Type-it Microsatellite PCR Kit (Qiagen, Venlo, Netherlands) in 7 µL reactions using 25 ng DNA, 0.03–0.65 nM of forward and reverse primer for each locus, 0.5–0.6 nM of M13-labeled primer, and 3 mM $MgCl_2$ (from Type-it Master Mix; Table 2). We performed amplifications on a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, California, USA) with an annealing temperature of 57° C. Diluted PCR products (1:20) were then electrophoresed using the ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) at the Iowa State University DNA Facility. We used GeneMapper (version 4.0, Applied Biosystems) software to define allelic bins and score alleles, which we verified by visual inspection. We included only samples for which ≥ 6 loci amplified for analysis, which minimizes the effect of missing data and keeps our study on par with the number of microsatellite markers that have typically been used in population genetic studies (Koskinen et al. 2004). Finally, we amplified a randomly selected 10% of our samples and we scored them a second time to validate scoring protocols (DeWoody et al. 2006).

Data analysis.—We consolidated and manipulated data format using GenAlEx (version 6.41, Peakall and Smouse 2006), Formatomatic (version 8.8.1, Manoukis 2007), and the R programming language (version 3.1.2, R Core Team 2014). We checked the data for possible scoring error sources, including null alleles, 'stutter' bands, and large-allele dropout using Microchecker (version 2.2.3, Van Oosterhout et al. 2004) and tested for linkage disequilibrium using GenePop (version 4.3, Rousset 2008). Analyzing each species independently, we used the R package PopGenReport (version 1.6.6, Adamack et al. 2014) to determine the number of alleles and to estimate null allele frequencies (Brookfield 1996), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness, and number of private alleles for each population. We estimated departures from Hardy-Weinberg equilibrium (Exact test with Markov chain lengths of 10^4 and 10^3 dememorization steps), conducted an AMOVA, and computed genetic distance between populations (pairwise F_{ST} values with bootstrap confidence intervals calculated using 1,000 permutations) using Arlequin (version 3.5, Excoffier and Lischer 2010). In addition to F_{ST}, we report the statistic F'_{ST}, which gives a standardized measure of genetic structure independent of within-population diversity (Meirmans and Hedrick 2011). We calculated an unbiased estimator of F'_{ST} using estimates of heterozygosity from the program smogd (Crawford 2010) following the equations of Meirmans and Hedrick (2011).

To test for hybridization between these species and population structure within species, we used the program Structure (version 2.3.4, Pritchard et al. 2000), employing an admixture model, which accounts for correlated allele frequencies and detects admixture at any point in time (Falush et al. 2003; Putman and Carbone 2014). We followed standard methods, performing five replicates for values of K (the number of potential genetic units) from 1 to 7, with 10^5 burn-ins and 10^5 replicates for each run. For the analysis of hybridization including all individuals, we did not include a priori species assignments. We calculated *Q*-values for all individuals, which represent the estimated likelihood that an individual belongs to a given genetic unit, using a value of 80% to identify putative hybrids (Vähä and Primmer 2006; Kapfer et al. 2013). For the analysis of population structure within each species, we used the usepopinfo option so that we could calculate the posterior probability that a given individual arose from its assigned population. We interpreted the output results with the program Structure Harvester (web version 0.6.94, Earl and vonHoldt 2011) to visualize the estimated probability of K [mean ln Pr(K)] and implement the Delta K [ΔK] method, which uses the rate of change of the log probability of the number of genetic clusters to provide more accurate estimates of K (Evanno et al. 2005).

We used the program BayesAss (verision 3.0, Wilson and Rannala 2003) to estimate contemporary migration rates and system of mating inbreeding coefficients (F_{IS}). In BayesAss, we first optimized delta values to ensure that 40–60% of the total changes were accepted, then inspected traces and effective sample size values in the program Tracer (version 1.6, http://beast.bio.ed.ac.uk/Tracer). We completed 10 runs for each species with different initial seed values, with runs consisting of 8×10^6 iterations, sampled every 1,000 iterations, and a burn-in of 2×10^6. We then compared the models for each species using a posterior simulation-based analogue of Akaike's information criterion through Markov Chain Monte Carlo (AICM) implemented in Tracer (Baele et al. 2012). Using the seed from the best-fit run for each species, we ran the analyses again with 8×10^7 iterations, sampled every 1,000 iterations, and a burn-in of 2×10^6 (Faubet et al. 2007). Results presented here are from these final runs for each species, using the value *m*, which represents the proportion of genetic migrants per generation.

Results

Tests of disequilibrium and allelic variation.—After correction for multiple comparisons (sequential Bonferroni), we identified eight locus-population combinations out of Hardy-Weinberg equilibrium, including one locus out of equilibrium at five of the seven species-population combinations (Table 3). This

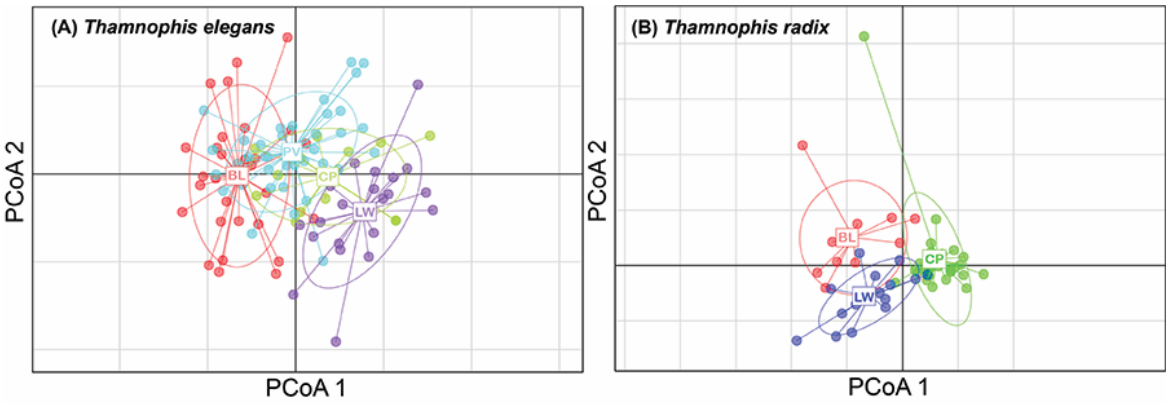

Figure 2. Plots of the first two axes of a principal coordinate analysis to visualize genetic diversity among samples and clustering by population for (A) the Western Terrestrial Gartersnake (*Thamnophis elegans*) and (B) the Plains Gartersnake (*T. radix*) from populations in and around Denver, Colorado, USA. Plots produced with PopGenReport (Adamack et al. 2014). Abbreviations of sites are BL = Bluff Lake Nature Center, CP = Commons Park, LW = Lowry Wetlands, PV = Pine Valley Ranch Park.

locus, TS2, also showed a statistically significant null allele frequency for both species and was therefore removed from further analyses. One additional locus in *T. elegans* and four others for *T. radix* showed a null allele frequency significantly different from zero (Table 3), with an overall null allele frequency of 4.5% for *T. elegans* and 11.0% for *T. radix*. With one exception (estimate of 0.28 at locus Nsμ10 in *T. radix*), the point estimates for null allele frequencies were ≤ 25%. Null alleles at these frequencies should only minimally impact estimates of coarse population structure, so we retained these loci in later analyses (Kelly et al. 2011; Bushar et al. 2015). After correcting for multiple comparisons (sequential Bonferroni), no loci were in linkage disequilibrium. The total number of alleles and the number of private alleles per population were not affected by sample size as determined by visual inspection of scatterplots, indicating that each of the populations contains a unique subset of the overall genetic variation (Table 1; Gibbs et al. 1997). The total number of alleles at each locus varied from two to 11 in *T. elegans* and from six to 14 in *T. radix*. Additionally, we calculated observed and expected heterozygosities for each locus (Table 3) and for each population (after removing the TS2 locus). Observed heterozygosity was lower than expected in *T. radix* but not in *T. elegans* (% difference in observed vs. expected heterozygosity -16.9% and + 2.0%, respectively; Table 1).

Table 3. Summary statistics for eight microsatellite loci in the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*) from populations in and around Denver, Colorado, USA. The abbreviations AS = allele size (bp), TA = total number of alleles, AH_O, = average observed heterozygosity, AH_E = average expected heterozygosity, NAF = null allele frequencies, and PH = the number of populations out of Hardy Weinberg Equilibrium. Null allele frequencies calculated following Brookfield (1996) with 95% confidence intervals in parentheses. All estimates made using the R package PopGenReport (version 1.6.6, Adamack et al. 2014).

	Thamnophis elegans						*Thamnophis radix*					
Locus	AS	TA	AH_O	AH_E	NAF	PH	AS	TA	AH_O	AH_E	NAF	PH
Nsμ10	138–154	5	0.410	0.478	0.05 (0.00–0.11)	0	140–158	7	0.408	0.800	0.28 (0.16–0.41)	2
Nsμ2	181–229	6	0.369	0.372	0.002 (0.00–0.05)	0	177–215	8	0.654	0.719	0.039 (0.00–0.10)	0
Nsμ3	195–231	10	0.876	0.839	0.00 (0.00–0.01)	0	179–219	11	0.907	0.834	0.00 (0.00–0.002)	0
TS10	155–179	6	0.573	0.572	0.00 (0.00–0.04)	0	151–199	11	0.627	0.840	0.13 (0.03–0.24)	1
TS2	139–175	11	0.356	0.644	0.21 (0.13–0.30)	3	136–160	6	0.420	0.669	0.18 (0.06–0.30)	2
Te1Ca18	96–98	2	0.098	0.093	0.00 (0.00–0.00)	0	90–133	12	0.630	0.848	0.13 (0.05–0.23)	0
Te1Ca2	236–254	8	0.547	0.660	0.07 (0.02–0.13)	0	220–272	14	0.840	0.835	0.001 (0.00–0.05)	0
Te1Ca3	91–119	10	0.723	0.766	0.03 (0.00–0.07)	0	87–119	9	0.635	0.833	0.12 (0.04–0.21)	0
Avg/Tot	—	58	0.494	0.553	0.045	3	—	78	0.640	0.797	0.11	5

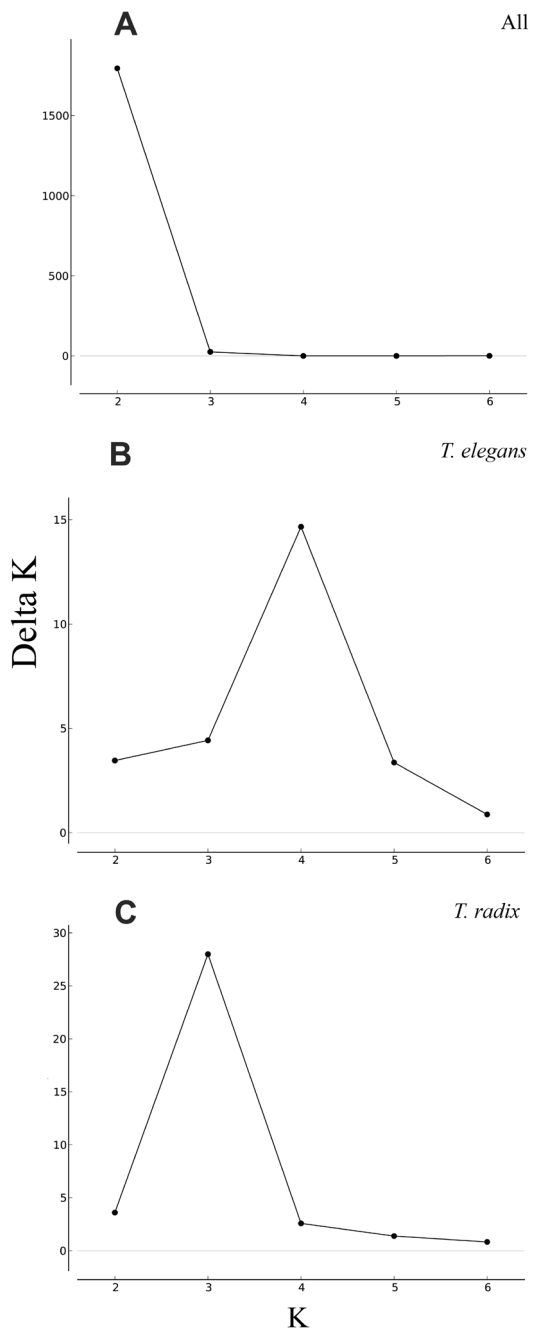

Figure 3. Plots of ΔK (Evanno et al. 2005) for values of K ranging from 1 to 7, where K is the number of genetic units for (A) all snakes, (B) the Western Terrestrial Gartersnake (*Thamnophis elegans*) , and (C) the Plains Gartersnake (*T. radix*) collected near Denver, Colorado, USA. Values calculated using Structure (version 2.3.4, Pritchard et al. 2000) and Structure Harvester (web version 0.6.94, Earl and vonHoldt 2011). Notice the peak in ΔK at 2 in the figure including all snakes (A), indicating strong support for two clusters aligned with the two species; peaks at 4 for *T. elegans* (B) and at 3 for *T. radix* (C) indicate strong support for genetic clusters aligned with the sampled populations of both species.

Table 4. Pairwise population differentiation statistics for the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*) from populations in and around Denver, Colorado, USA. F_{ST} calculated in Arlequin (version 3.5, Excoffier and Lischer 2010; see text for statistical methods). Below diagonals: *T. elegans*; Above diagonals: *T. radix*. *P*-values (uncorrected) shown in parenthesis below estimates; F_{ST} estimates significantly different from zero shown with one ($P < 0.05$) or two ($P < 0.01$) asterisks. Abbreviations of sites are BL = Bluff Lake Nature Center, CP = Commons Park, LW = Lowry Wetlands, PV = Pine Valley Ranch Park.

	BL	CP	LW
BL	—	0.058** ($P < 0.001$)	0.039* ($P = 0.0342$)
CP	0.084** ($P < 0.001$)	—	0.059** ($P < 0.001$)
LW	0.126** ($P < 0.001$)	0.084** ($P < 0.001$)	—
PV	0.056** ($P < 0.001$)	0.039** ($P = 0.002$)	0.085** ($P < 0.001$)

Population structure, inbreeding, migration rates, and hybridization.—Global estimates of genetic differentiation among populations are moderate to high for both species (*T. elegans* $F_{ST} = 0.077$, $F'_{ST} = 0.21$; *T. radix* $F_{ST} = 0.053$, $F'_{ST} = 0.43$). Pairwise F_{ST} estimates between populations ranged from 0.039 to 0.126 for *T. elegans* and 0.039 to 0.059 for *T. radix*, with all pairwise combinations statistically significant after sequential Bonferroni correction for multiple comparisons. The highest and lowest pairwise F_{ST} estimates occurred between the two closest populations (Bluff Lake and Lowry Wetlands), but in different species (F_{ST} of 0.126 for *T. elegans* and 0.039 for *T. radix*; Fig. 2, Table 4).

The program BayesAss estimates migration rates based on transient disequilibrium in genotypes of migrants or their recent descendants and thus reflects migration in the most recent few generations, which in these snakes we interpret as the past several decades (Wilson and Rannala 2003; Chiucchi and Gibbs 2010). Point estimates of contemporary migration rates ranged from 0.008–0.216, with only migration from the Pine Valley Ranch to the Commons Park populations of *T. elegans* significantly different from zero (Table 5). Inbreeding coefficient (F_{IS}) estimates from the program BayesAss were significantly different from zero for one *T. elegans* population and all three *T. radix* populations (Table 1). The lowest point estimate of F_{IS} is found at the Pine Valley Ranch population of *T. elegans* in continuous habitat outside the urban matrix.

Results of the analysis of all individuals in Structure show a clear genetic division between these two species, although there is evidence of occasional hybridization. Both the ΔK and ln Pr(K) methods offer strong support for two distinct genetic groups aligned with species identification (Fig. 3A). Of 159

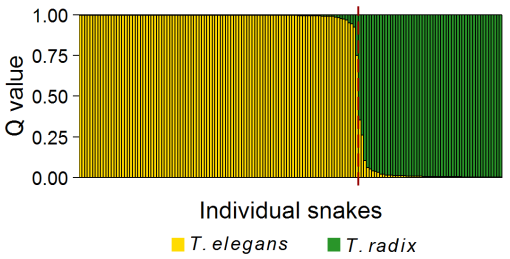

Figure 4. Plots of *Q*-values (percentage of ancestry) from Structure (version 2.3.4, Pritchard et al. 2000) for all individuals. Data are from analysis of gartersnakes (*Thamnophis* spp.) collected near Denver, Colorado, USA. Each individual is represented by a single bar, with yellow bars representing Western Terrestrial Gartersnake (*T. elegans*) ancestry and green bars representing Plains Gartersnake (*T. radix*) ancestry. Red vertical dashed line shows division of species assignments based on morphology.

individuals included in this analysis, three individuals were identified as putative hybrids with a *Q* value of < 80%: individuals field-identified as *T. elegans* and *T. radix* from the Commons Park population and an individual identified as *T. radix* from the Lowry Wetlands population (Fig. 4). Analysis in Structure also supports each sampled population being an isolated genetic unit within each species. Both the ΔK and ln Pr(K) methods offer support for four genetic clusters for *T. elegans* (Fig. 3B) and three genetic clusters for *T. radix* (Fig. 3C), which aligned exactly with population of origin with one exception: each of the five runs of the best-supported models identified one potential migrant in *T. radix*, an adult male caught at Commons Park but assigned to the Bluff Lake cluster with an average probability of 80.8%. Across all individuals within each species, the probability of assigning an individual to its population of origin was 98.0% for *T. elegans* and 97.9% for *T. radix*.

Discussion

Our results show that these urban populations exhibit strong genetic structure and suggest that populations of *T. radix* are genetically isolated, in this case likely due to contemporary urbanization and habitat fragmentation. First, we found support for our hypothesis that populations show a signature of genetic differentiation: estimates of F_{ST} and F'_{ST}, as well as the identification of clear genetic clusters, indicate that there is strong genetic structure among populations in close geographic proximity. Second, we found evidence of reduced genetic diversity and high estimates of inbreeding coefficients, including statistically significant estimates for all three populations of *T. radix* and one in *T. elegans*. In support of our third hypothesis, we found that migration between populations is minimal. Estimates of current migration rates between urban

Table 5. Contemporary migration rates between populations of the Western Terrestrial Gartersnake (*Thamnophis elegans*) and the Plains Gartersnake (*T. radix*) in and around Denver, Colorado, USA. Contemporary migration rates estimated using BayesAss (version 3.0, Wilson and Rannala 2003). The letter *m* = the proportion of each population consisting of genetic migrants per generation and parentheses show 95% credible set (Bayesian confidence intervals). Estimates significantly different from zero shown with asterisk. Abbreviations of sites are BL = Bluff Lake Nature Center, CP = Commons Park, LW = Lowry Wetlands, PV = Pine Valley Ranch Park.

		Thamnophis elegans	*Thamnophis radix*
From	To	*m*	*m*
BL	CP	0.032 (0.00–0.095)	0.018 (0.00–0.048)
BL	LW	0.022 (0.00–0.060)	0.031 (0.00–0.096)
CP	BL	0.022 (0.00–0.050)	0.039 (0.00–0.105)
CP	LW	0.014 (0.00–0.039)	0.044 (0.00–0.119)
LW	BL	0.026 (0.00–0.067)	0.105 (0.00–0.270)
LW	CP	0.065 (0.00–0.175)	0.016 (0.00–0.045)
BL	PV	0.063 (0.00–0.129)	—
CP	PV	0.008 (0.00–0.024)	—
PV	BL	0.022 (0.00–0.059)	—
PV	CP	0.216* (0.087–0.344)	—
PV	LW	0.027 (0.00–0.073)	—
LW	PV	0.047 (0.00–0.127)	—

populations overlapped zero in each pairwise case and the only migration rate significantly different from zero involved individuals moving into the city from an outside population. Population assignment based on genotype identified only one potential migrant snake. Finally, we did not find evidence that hybridization between two species confined to these urban pockets was increased beyond estimates made by other studies of populations in continuous habitat (Placyk et al. 2012; Kapfer et al. 2013). Overall, these results demonstrate that while snakes may be present in urban populations, the population genetic structure suggests that isolation and inbreeding may not be conducive to long-term persistence in some species and that even closely related syntopic species may respond differently to fragmentation caused by urbanization.

While the use of different markers and estimation methods make direct comparison of F_{ST} with other

studies difficult, qualitative comparisons demonstrate that these urban populations show strong genetic structure. For example, populations of *T. elegans* in continuous habitat in Northern California exhibit a global F_{ST} of 0.024 (Manier and Arnold 2005). Pairwise estimates of F_{ST} in populations of *T. radix* range up to 0.22, but these populations are separated by 150+ km (reviewed in King 2009). In congeneric species, studies using microsatellite markers show that the Common Gartersnake (*Thamnophis sirtalis*) exchanges genes and exhibits little differentiation across the Pacific Northwest of North America (global F_{ST} of 0.036; Ridenhour et al. 2007), among Lake Erie islands and mainland populations of North America (global F_{ST} of 0.037; Bittner and King 2003), and in populations in the mountains of northern California, USA (global F_{ST} of 0.035; Manier and Arnold 2005). However, populations of the Giant Gartersnake (*T. gigas*) inhabiting fragmented agricultural landscapes in the Central Valley of California, USA, show much greater global differentiation (global F_{ST} of 0.108; Wood et al. 2015). The analysis of genetic clustering performed in Structure corroborates this finding of genetic isolation, with the optimum number of genetic clusters matching the number of populations sampled and an average probability of 98% of assigning an individual to its origin population. Furthermore, the principal coordinate analysis visualization demonstrates genetic distinction between populations. Notably, the two populations for which the clusters show the most overlap are between the two populations with a statistically significant migration rate (Pine Valley Ranch and Commons Park in *T. elegans*).

Two lines of evidence support the conclusion that *T. radix* population structure has been more affected by fragmentation than populations of *T. elegans*. First, we found evidence of reduced heterozygosity in *T. radix* but not *T. elegans*. Additionally, we found a reduction in genetic divergence between mating pairs in *T. radix*, with significant estimates of F_{IS} for all three sampled populations of *T. radix* but only one population of *T. elegans* (Nei 1987). We speculate that the reduced population genetic effects in *T. elegans*, including differences in observed heterozygosities and inbreeding, are due to the differential use of waterways for migration. This species is more often found near water, is known to swim adeptly, and sometimes feeds almost exclusively on fish (Bronikowski and Arnold 1999; Ernst and Ernst 2003). In our observations, we never located *T. radix* in the water and only *T. elegans* were observed fleeing toward and into the water. In both species, the F_{IS} estimates are much higher than generally reported for other snake populations, for which the median F_{IS} value is 0.08 (range = -0.11 to 0.30; reviewed in King 2009). The pattern of reduced genetic variation observed here corroborates findings from other snake populations in fragmented landscapes (Jäggi et al. 2000; Ujvari et al. 2002; Wood et al. 2015; Xuereb et al. 2015). Reduced genetic diversity among mating individuals can lead to a reduction in population viability, through mechanisms such as reduced variability at major histocompatibility complex loci (Madsen et al. 2000) or through anatomical anomalies (Merilä et al. 1992; Gautschi et al. 2002). Further studies are warranted on these gartersnake species, as well as other species in impacted landscapes, to assess the effects of continued genetic isolation and inbreeding on both individual development and population viability.

The genetic isolation of these populations is most likely driven by the limited opportunity for migration in an urban matrix. This is supported by our findings of contemporary migration rates not significantly different from zero, with the exception of migration from the Pine Valley Ranch population to the Commons Park population in *T. elegans*. We see two possible non-exclusive reasons for this higher migration rate. First, if *T. elegans* are migrating in waterways, either intentionally or during flooding events, they are likely to be carried downstream from the foothills into urban areas such as Commons Park, which lies on the bank of the largest river in Denver, the South Platte. Additionally, there are likely intermediate, unsampled populations in the large swath of potential habitat found between the city and Pine Valley Ranch. The presence of significant migration does indicate that minimal gene flow is possible from areas outside the urban matrix, at least in *T. elegans*. This migration is most likely to occur along waterways, as snakes avoid roads (Shine et al. 2004a; Andrews and Gibbons 2005; Shepard et al. 2008b) and suffer great mortality when attempting to cross (Rosen and Lowe 1994; Shepard et al. 2008a; Evans et al. 2011; Weyer et al. 2014). Assignment analysis in Structure revealed one putative migrant among the urban populations, an adult male *T. radix* found at Commons Park but assigned to the Bluff Lake genetic cluster. While we cannot rule out the possibility of natural dispersal, there is also a strong chance that this individual may have been relocated directly by humans, as is likely the reason for otherwise seemingly impossible migration events in other reptile species (e.g., Schwartz and Karl 2006). Both areas are highly used urban parks in which children catch wild snakes and potentially relocate them (Eric Gangloff, pers. obs.). No marked snakes in the study were recaptured at another population in our four years of mark-recapture work, in concordance with the genetically based estimates of migration rate.

Despite this restriction to small habitat patches for both species, we found little evidence of hybridization. Based on genetic cluster analysis in Structure, 1.9%

(three out of 159) of individuals were putatively hybrid, though inspection of the field photographs of these individuals does not reveal any evidence of hybridization in color morphology. This number is concordant with rates of hybridization in sympatric gartersnakes reported elsewhere (1.5%; Kapfer et al. 2013), demonstrating that hybridization is potentially possible between these species, though isolating mechanisms maintain species identity in sympatry (e.g., Shine et al. 2004b). For example, we have identified *T. elegans* hibernacula at two of these study populations. Only individuals of this species have been found in and around these burrows in the early spring and late fall. Because gartersnakes generally mate upon emergence from hibernation in the spring (Rossman et al. 1986), the species-specificity of hibernation burrows likely acts as a pre-zygotic barrier to hybridization.

Recent studies demonstrate that urban populations can be essential to conservation (Miller and Hobbs 2002; Sanderson and Huron 2011) and fragmented landscapes may provide important sources of genetic variation (Driscoll and Hardy 2005). As such, it becomes more important to implement strategies to recognize and preserve species in degraded and fragmented habitats. Contrasting patterns of migration and genetic isolation, as demonstrated in these two snake species, can inform management decisions. For example, our results underscore the need to understand how small pockets of habitat are used by different species and how this, in turn, affects gene flow among populations (e.g., Munshi-South and Nagy 2014). This is especially important for long-lived vertebrates, where the effects of isolation and inbreeding can take many years to manifest as population-level changes (Keyghobadi 2007; Kuussaari et al. 2009). Additionally, such small populations are susceptible to demographic stochasticity (Lande 1998) and are at risk of extirpation (Brook et al. 2008; King 2009). For populations in a growing urban center such as these, where there is little possibility of corridor construction to facilitate migration, more direct measures of genetic restoration may be necessary to ensure long-term persistence (e.g., Madsen et al. 1999). This may be especially true for populations of *T. radix*, which display extremely high levels of inbreeding, low levels of migration, and little opportunity for gene flow from outbred populations outside the urban center.

Acknowledgments.—For assistance in the lab and field, we thank Jen Derosby, Joseph Gangloff, HaLea Messersmith, Lisa Roth, Becky Slater, Courtney Tomlin, Tuan Tran, Alex Wendt, Jamica Zion, and P.S. 1 Charter School students. Additionally, we thank Dean Adams for statistical advice, Aaron Bakker for mapping assistance, Shane Nehring for computational support, and John Nason and Jessica Judson for manuscript comments. Bluff Lake Nature Center (especially Chris Story), Denver Parks & Recreation, Lowry Redevelopment Authority, and Jefferson County Open Space (especially Mike Morin) generously allowed access to their land and snakes. EJG was supported by fellowships from the Iowa State University Ecology & Evolutionary Biology Program and from the ISU Office of Biotechnology. Financial support was provided by the ISU Ecology, Evolution, & Organismal Biology Graduate Students Research Grant, the Lois Webster Fund of the Audubon Society of Greater Denver, Prairie Biotic Research, the Marv Aures/Bob Krantz Grant Fund of the Western New York Herpetological Society, and Metropolitan State University of Denver. All research conducted under license from the Colorado Division of Wildlife under permit HP939 (all years) and in accordance with Iowa State University IACUC (1-12-7285-J; 2012 fieldwork).

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Eric J. Gangloff is currently a Post-doctoral Research Associate at Iowa State University, Ames, USA, working in the lab of Anne Bronikowski on the intersection of physiology, behavior, and life-history traits in reptiles. He earned his Ph.D. in Ecology & Evolutionary Biology from Iowa State University in 2016, studying how aspects of physiological stress response and behavior co-vary with life-history traits in the gartersnake *Thamnophis elegans*. (Photo-graphed by Jennifer Steadman).

Dawn M. Reding is an Assistant Professor of Biology at Luther College in Decorah, Iowa, USA. She received her M.S. in Zoology at the University of Hawaii and her Ph.D. in Ecology & Evolutionary Biology at Iowa State University, Ames, USA. She teaches courses in Vertebrate Biology, GIS, and Genomics. Her research interests involve using molecular tools to address basic and applied questions relating to the evolution and conservation of wildlife. Her work has included studies on a diversity of vertebrates, including honeycreepers, bobcats, gar-tersnakes, and bats. (Photograph courtesy of Luther College).

David Bertolatus is a Ph.D. Trainee in Integrative and Systems Biology, working with Dr. Alan Vajda at the University of Colorado Denver, USA. As an ecotoxicologist, his research seeks to better understand the relationships between landscape patterning, chemical occurrence, and biological effects in freshwater ecosystems. Currently, David is using genomic tools to characterize the biological effects of exposure to complex chemical mixtures in freshwater ecosystems. (Photographed by Eric Gangloff).

Christopher J. Reigel is a Microbiologist at Deibel Laboratories in Madison, Wisconsin, USA. He returned to his home state in 2013 after obtaining a degree in biology from the Metropolitan State University of Denver, Colorado, USA. An emphasis in wildlife biology gave Chris the opportunity to assist in gartersnake research under Eric Gangloff and Jennifer Gagliardi-Seeley. While he no longer wrangles snakes in the field, he has significant interest in virology and hopes to integrate his laboratory skills into a career in environmental conservation. (Photographed by Catherine Reigel).

Jennifer L. Gagliardi-Seeley earned her Ph.D. at Lehigh University, Bethlehem, Pennsylvania, USA, in integrative biology with a focus in behavioral ecology. She is currently an Associate Professor at Metropolitan State University of Denver, Colorado, USA. Her main research is on aggression and mate choice behavior in the monogamous, biparental convict cichlid fish. She also works with students on gartersnake behavior, genetics, and ecology in urban environments using genetic techniques and geographic information systems. (Photographed by Christy Carello).

Anne M. Bronikowski is a Professor in the Department of Ecology, Evolution, & Organismal Biology at Iowa State University, Ames, USA. She earned both her M.S. and Ph.D. at the University of Chicago, Chicago, Illinois, USA, in Evolutionary Biology, where her work characterized life-history variation in populations of the gartersnake *Thamnophis elegans*. She has authored over 70 peer-reviewed publications and is currently President of the American Genetic Association. (Photographed by Fred Janzen).