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Integrating individual behaviour and landscape genetics: the population structure of timber rattlesnake hibernacula

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Abstract

Individuals of many species show high levels of fidelity to natal populations, often due to reliance on patchily distributed habitat features. In many of these species, the negative impacts of inbreeding are mitigated through specialized behaviours such as seasonal mating dispersal. Quantifying population structure for species with these characteristics can potentially elucidate social and environmental factors that interact to affect mating behaviour and population connectivity. In the northern part of their range, timber rattlesnakes are communal hibernators with high natal philopatry. Individuals generally recruit to the same hibernaculum as their mother and remain faithful to that hibernaculum throughout their lives. We examined the genetic structure of *Crotalus horridus* hibernacula in the northeastern USA using microsatellite loci. Sampled hibernacula exhibited only modest levels of differentiation, indicating a significant level of gene flow among them. We found no significant correlation between genetic differentiation and geographical distance, but did find significant positive correlation between genetic differentiation and a cost-based distance metric adjusted to include the amount of potential basking habitat between hibernacula. Therefore, thermoregulation sites may increase gene flow by increasing the potential for contact among individuals from different populations. Parentage analyses confirmed high levels of philopatry of both sexes to their maternal hibernaculum; however, approximately one-third of paternity assignments involved individuals between hibernacula, confirming that gene flow among hibernacula occurs largely through seasonal male mating dispersal. Our results underscore the importance of integrating individual-level behaviours and landscape features with studies of fine-scale population genetics in species with high fidelity to patchily distributed habitats.

Keywords: connectivity, *Crotalus horridus*, gene flow, mating dispersal, microsatellites, natal philopatry

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Introduction

Gene flow among subdivided populations is critical for long-term viability: it allows for the rescue and recolonization of declining or extinct populations, and prevents inbreeding effects in small populations (Allendorf & Luikart 2006). Dispersal is typically thought to be the primary behavioural mechanism underlying gene flow. However, some species exhibit very limited dispersal of either sex because of high levels of natal philopatry. Extreme philopatry is often associated with key features of the environment that are patchily distributed and difficult to locate, such as specialized breeding locations or food resources. Examples of such resource-limited dispersal can be found in a diverse array of taxa including birds (Emlen 1982; Van Bekkum *et al*. 2006), bats (Burland *et al*. 2001; Rivers *et al*. 2006), rodents (Nutt 2005), lizards (Gardner *et al*. 2001), and amphibians (Waldman *et al*. 1992).

For many philopatric species, gene flow and effective dispersal is accomplished not through direct migration of individuals to new populations, but through mating events among individuals from different populations.

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Individuals disperse temporarily, encounter each other, mate, and then return to their respective populations of origin. Understanding population genetic structure in taxa with this indirect gene flow is critical because population connectivity may be influenced not only by the environment or landscape, but also by social and behavioural factors that do not limit movements in most species. For example, cooperatively breeding birds may exhibit genetic structure at a fine scale because of the complex ecological and social constraints leading to delayed dispersal and helping at the nest (McDonald *et al*. 1999; Painter *et al*. 2000; Temple *et al*. 2006); likewise, bats with strong natal philopatry rely on autumn aggregative 'swarming' events which mediate gene flow among colonies (Parsons *et al*. 2003).

Regardless of the cause for dispersal, gene flow among populations may be shaped in part by various local geographical features. The relationship between gene flow and geography can be examined by incorporating landscape features into population genetics analyses (Manel *et al*. 2003; Coulon *et al*. 2004; Antolin *et al*. 2006). Incorporating knowledge of the behaviours that influence gene flow into landscape genetics analyses can clarify how these factors interact to create and maintain population structure, and can measure the degree to which populations with high natal philopatry may be susceptible to anthropogenic impacts (e.g. habitat fragmentation).

The timber rattlesnake (*Crotalus horridus*) is an ideal candidate species for assessing spatial genetic structure and evaluating the possible landscape and social dynamics of gene flow. Northern populations of timber rattlesnakes overwinter in communal hibernacula, generally located around patchily distributed rocky escarpments that serve as the geographical centres of local populations. During the 5-month summer active season, individuals migrate seasonally to surrounding areas for foraging and mating, with adult females typically moving 1–3 km and adult males 3–6 km from their hibernacula (Brown 1993). Although they move widely, individuals can be found throughout the active season thermoregulating at communal basking areas. Such basking habitats are often associated with steep, south-facing exposures that provide natural shelter rocks and breaks in the forest canopy. The majority of individuals return to their hibernaculum in the fall. Field observations also indicate that newborns generally recruit to their maternal hibernaculum (Cobb *et al*. 2005; Brown *et al*. 2007). Genetic sampling in this system is facilitated by these predictable seasonal behaviours; samples can be collected with relative ease from individuals during spring egress and fall ingress, and from shed skins left at basking habitats.

Most snake species are under-studied because they are perceived as intractable research subjects for ecological research (Bonnet *et al*. 2002). However, timber rattlesnakes have been the focus of several detailed field and laboratory behavioural and ecological studies (reviewed in Reinert & Zappalorti 1988b; Brown 1993; Zaidan & Beaupre 2003; Clark 2006), allowing us to propose hypotheses about habitat use and the factors limiting gene flow in natural populations. Comprehensive understanding of population genetic structure is also important for informing management and conservation plans for threatened populations. Although formerly abundant, *C. horridus* has suffered drastic population declines throughout its range (Stechert 1982; Brown 1993), and hibernacula are increasingly isolated from each other by various forms of anthropogenic habitat fragmentation.

Here, we assess the overall genetic structure among five groups of hibernacula in the northeastern portion of the species' range. We use molecular markers to examine the relative contributions of interpopulation dispersal and mating behaviour to gene flow, and implement a (geographical information system) GIS-based cost-distance model to quantify how landscape attributes may affect genetic connectivity among hibernacula in different regions. We test the hypotheses that (i) hibernacula are individual genetic demes due to high rates of natal philopatry, (ii) the type of intervening habitat between hibernacula affects their degree of genetic connectivity, and (iii) effective migration occurs through mating behaviour rather than direct migration.

Materials and methods

Population sampling

This study includes 373 timber rattlesnakes sampled from 14 hibernacula in New York. Our sampling sites are primarily located in undisturbed habitat, but in some cases were subdivided by paved roadways. Our objective here is to assess genetic structure in the absence of anthropogenic barriers for a better understanding of patterns expected in undisturbed populations. Because of evidence that roads exert a strong negative influence on timber rattlesnake movement and survival (Andrews & Gibbons 2005), in this analysis we compare only populations in contiguous natural habitat (not separated by roads). Tests of the genetic consequences of roads is a topic of an independent study of these and other populations.

Our 14 sampled hibernacula are located in three different areas in the state: five in the Adirondacks (AD), three in Sterling Forest west (SFW), two in Sterling Forest east (SFE), two in Bear Mountain north (BMN), and two in Bear Mountain south (BMS) (Fig. 1). We used the same alphanumeric numbering system for hibernacula reported in Brown in press). Sample sizes ranged from 15 to 57 individuals per hibernaculum (mean ± SD = 27 ± 13).

Samples were collected from live snakes and shed skins. We drew blood from the caudal vein or clipped ventral

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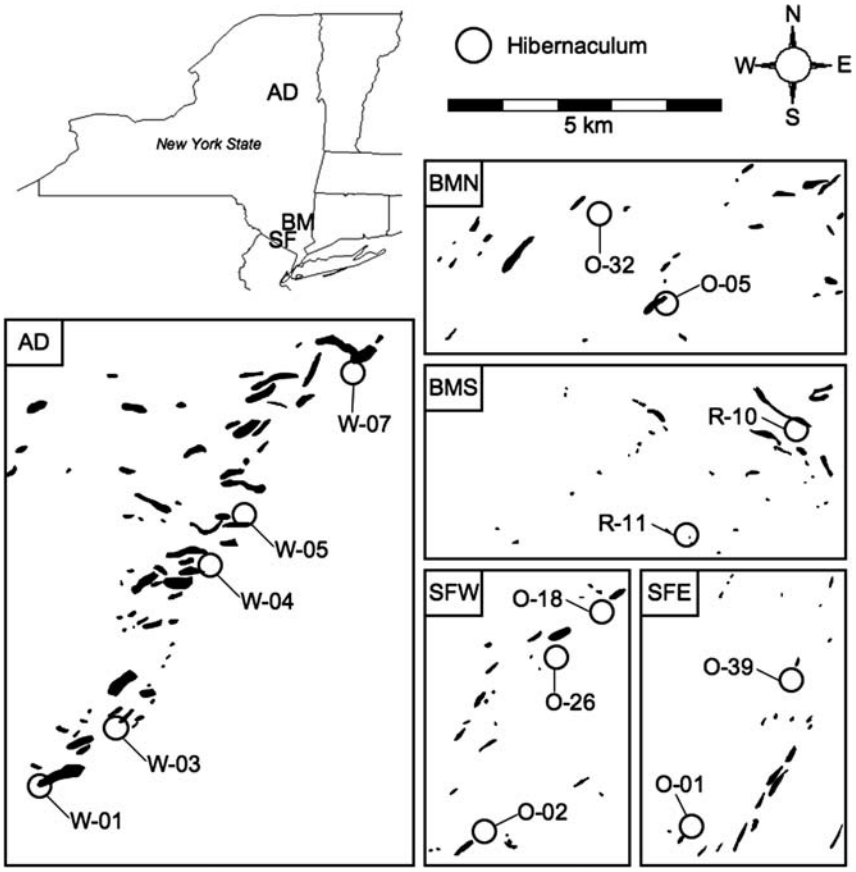

Fig. 1 Location of the three areas (AD, Adirondacks; BM, Bear Mountain; SF, Sterling Forest) in New York containing five regions where *Crotalus horridus* hibernacula were sampled in this study. Black areas indicate habitat defined as potential basking areas in GIS model (details provided in text), shown in relation to the location of hibernacula in each region.

scales from snakes captured in the field and preserved tissue in 95% ethanol for later genetic analysis. Sampled snakes were sexed, weighed, measured, and permanently marked by a scale-clipping method (Brown & Parker 1976). The number of rattle segments and overall taper of the rattle string was recorded for age estimation following Brown (1991). The approximate age class and sex of those individuals that produced shed skins were noted when possible by measuring the skin and counting subcaudal tail scales. Some samples were collected from aggregated litters of newborn snakes with or without an attending female, presumably the mother of that litter (Greene *et al*. 2002). In such cases, to avoid introducing a bias due to a high proportion of siblings, we used only one tissue sample per litter.

All timber rattlesnake hibernacula in our study area were identified by long-term surveys conducted by R. Stechert (BM and SF areas) and W. S. Brown (AD area) in cooperation with the New York Department of Environmental Conservation. Collected samples were assigned to the nearest hibernaculum as their hibernaculum of origin. A hand-held global positioning system (GPS) or USGS 1:24 000 topographic maps were used to identify exact coordinates of individual capture locations. All hibernating crevices used by snakes that were within 500 m of each other were classified as a single hibernaculum. In most cases, this assignment was unambiguous as most hibernacula are associated with cliffs and ledges with south-facing exposures, and snakes were collected within 100–200 m of these areas. In contrast, the average distance between adjacent hibernacula in this study was 3.2 km (ranging from 1.2 to 9.9 km). Therefore, we are confident that our hibernaculum assignment accurately portrays individual population affiliations.

DNA extraction and microsatellite amplification

DNA was extracted from all tissue types using QIAGEN DNeasy Tissue Kits (QIAGEN) and the eluted product was used directly as template in polymerase chain reactions (PCR). Nine microsatellite loci were amplified: *Cw*A29f, *Cw*B6, *Cw*B23, *Cw*C24, and *Cw*D15 (Holycross *et al*. 2002), and 5a, 5–183, 7–144, and 7–87 (Villarreal *et al*. 1996). PCRs were performed in 10 μL reaction volumes containing 1× PCR buffer (Invitrogen), 1.0–2.0 mM $MgCl_2$, 0.02 mM each forward and reverse primer, 0.1 mM dNTP mix, 0.25 U Invitrogen Platinum *Taq* DNA polymerase, and 1 μL template DNA (~10 ng/μL). The PCR temperature profile was 94 °C for 5 min; 35 cycles of 90 °C for 1 min, either 60 °C (*Cw*A29f and 7–144), 56 (*Cw*D15 and 7–87) or 50 °C (all other loci) for 1 min, and 72 °C for 1 min; followed by

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a final 10-min extension at 72 °C. Fragment sizes were calculated by comparison with an internal size standard (LIZ500, Applied Biosystems) after electrophoreses on an ABI 3100 automated capillary sequencer. Allele sizes were scored using GENEMAPPER 3.0 (Applied Biosystems).

Genetic diversity and differentiation

We first used GIMLET (Valiere 2002) to identify samples from shed skins that had duplicate genotypes to those collected from live animals, and removed them from subsequent analysis. Genotypes at all loci and all populations were then analysed with the program MICROCHECKER 2.0 (Van Oosterhout *et al*. 2004) to estimate null allele frequency and other potential genotyping problems. We calculated observed and expected heterozygosities, and tested for deviations from Hardy–Weinberg (HW) expectations at each locus and hibernaculum using GENEPOP 3.1 (Raymond & Rousset 1995). We used a Monte Carlo chain method (1000 dememorizations, 100 batches, 1000 iterations) following the algorithm of Guo & Thompson (1992), and applied a Bonferroni correction for multiple tests for a table-wide significance level of 0.05 (adjusted P value = 0.0004). We used FSTAT 2.1 (Goudet 1995) to test for linkage disequilibrium among all loci, as well as to calculate allelic richness for each hibernaculum and pairwise F_{ST} values and their significance. Although F_{ST} is still widely used for measuring pairwise population differentiation, it incorporates many unrealistic assumptions, and assignment-based methods may be a more powerful alternative (Pearse & Crandall 2004). Thus, we used the DOH calculator (Brzustowski 2002) to calculate D_{LR}, the genotype likelihood ratio distance of Paetkau *et al*. (1995). D_{LR} is the likelihood that a given genotype originated from the population where it was sampled relative to other populations, and performs well at fine scales where populations may show little differentiation (Paetkau *et al*. 1997). The overall significance of these assignment tests was determined by using DOH to create random genotypes from pooled populations in each region and recalculating population assignments for 1000 randomized data sets, allowing us to determine whether the actual level of self-assignment in hibernacula is higher than in randomly constructed populations.

Migration and gene flow

We examined patterns of gene flow and migration using two approaches. Contemporary levels of gene flow were estimated with individual-based assignment techniques implemented in GENECLASS 2.0 (Piry *et al*. 2004) and STRUCTURE 2.1 (Pritchard *et al*. 2000). Long-term estimates of gene flow among populations were estimated based on coalescent analyses implemented in MIGRATE 2.1.3 (Beerli & Felsenstein 2001).

GENECLASS and STRUCTURE both use Bayesian assignment to identify migrant individuals, or individuals with mixed ancestry. Because they include different assumptions, these two programs can be used in concert to characterize current migration. STRUCTURE assumes that all populations in an area have been sampled, whereas GENECLASS can potentially identify individuals that do not strongly assign to any population. Both programs perform well only when populations are sufficiently differentiated such that high levels of gene flow will not confound the identification of migrant genotypes (Berry *et al*. 2004). Therefore, we first used the Bayesian algorithm in STRUCTURE to identify the most likely number of genetic clusters (K) represented by the genotypes in the absence of any geographical information. We used the model allowing admixture and correlated gene frequencies with a migration rate of 0.1, and ran 500 000 steps after a burn-in of 100 000. We determined these values were sufficient for accurate estimation of K by establishing that values of key summary statistics converged (Pritchard *et al*. 2000). We conducted 10 independent runs for each value of K and retained the run with the highest likelihood. We also applied the Evanno *et al*. (2005) method using a second-order rate of change to determine the most likely value of K. Both methods consistently resulted in the same number of estimated genetic demes.

After grouping hibernacula into genetic demes, we identified likely migrants among them. In STRUCTURE, we used the USEPOPINFO option to assign each of our samples to its cluster of origin so that the program would calculate the posterior probability that each individual arose from its sampled cluster vs. a neighbouring cluster. In GENECLASS, we computed the probability that individuals were migrants by using a likelihood ratio of source population to the highest likelihood value for all populations within each region. We used a frequency-based method to calculate likelihood ratios and Monte Carlo resampling to calculate 0.01 significance levels (Paetkau *et al*. 2004). We also performed an exclusion test in GENECLASS to identify clusters that could be statistically ruled out as the origin of each individual. We calculated the probability of exclusion again using a simulated null (Paetkau *et al*. 2004).

We used the program MIGRATE (Beerli & Felsenstein 2001) to estimate the effective population size and rate of migration among hibernacula. We used Bayesian analysis because this method may be more accurate under a wide range of conditions (Beerli 2006). We used F_{ST} estimates for starting values of M (ratio of migration rate, m, to mutation rate, μ) and θ ($\theta = 4N_e\mu$, where N_e is effective population size). We monitored the logfile of initial runs to make sure the data-likelihood of the start tree for each chain reached convergence. After this initial analysis, parameter estimates were used as starting parameters for the next run until results equilibrated at approximately the same values. For each analysis, we ran four long chains of 100 000 genealogies

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with one of every 20 genealogies sampled. To increase the genealogy space explored, we used the adaptive heating scheme with four chains at temperatures of 1.0, 1.2, 1.5, and 3.0.

Landscape genetics analysis

Locations for our sampled hibernacula were plotted in a GIS using ARCGIS 9.0. These locations were overlaid with 10-m digital elevation models (DEM) from the US Geological Survey (USGS). Previous studies indicate that hibernacula with shared basking habitat might be more closely related (Bushar *et al*. 1998). Therefore, we estimated two pairwise measures of distance between hibernacula: a straight-line Euclidean distance, and a least-cost habitat-path distance taking into account the amount of likely basking habitat between hibernacula. The habitat-path distance was based on the fact that timber rattlesnakes require steep, south-facing, exposed rocky outcroppings for thermoregulation where they aggregate and potentially encounter individuals from other hibernacula. Using reclassified DEMs, we defined potential basking habitat as 10 × 10 m grid cells with a slope of 30° or greater, and a SE-to-SW aspect (aspect between 112.5° and 247.5°). A cost surface was then created that assigned no cost to landscape cells that contained potential basking habitat. The total cost of the lowest value path between hibernacula was then taken as the least-cost path between those two sites. Because potential basking habitat was assigned no cost, the value of the least-cost path is thus equivalent to the distance between hibernacula minus the amount of potential basking habitat between hibernacula. We tested a null hypothesis of a nonsignificant Pearson correlation coefficient between geographical distance (either Euclidean or least-cost distance) and genetic distance (F_{ST}) with a nonparametric permutation test. Because regions containing more than two populations resulted in matrices of nonindependent data, pairwise values were permuted within those regions. Values for regions with only one pairwise measure were permuted independently. An overall null distribution of correlation coefficients was generated by 1000 permutations using RESAMPLING STATS (Simon 1997).

Parentage analysis

Due to their longevity and philopatry, timber rattlesnake parents and their offspring are likely to be present in the same or neighbouring populations, even after offspring have reached maturity (Brown 1993). We used parentage analyses to estimate parent–offspring relationships and examine gene flow among hibernacula in each region. The program NEWPAT (Wilmer *et al*. 1999) was used to find the genotypes of individuals that are potential mothers or fathers of sampled individuals, and to estimate the significance of the pairings using a randomization test. Parentage analyses can be misleading when populations include clusters of highly related individuals; however, in our study, the age of all individuals was estimated from a combination of rattle characteristics (relative width of basal vs. terminal segment of rattle) and body size (length and weight) (Brown 1991). In addition, demographic studies show that minimum age at maturity in most populations is approximately 5 years (Brown 1991; Martin 1993; Aldridge & Brown 1995). Therefore, parent–offspring pairs uncovered in NEWPAT were only considered valid if they were significant at the 0.05 level and if the estimated ages of the pair were at least 5 years apart. As a test of the power of our markers for parentage assignment, we included 11 neonatal snakes that were captured in the field soon after birth with their mother in attendance.

Table 1 Genetic diversity of 14 *Crotalus horridus* hibernacula included in this study. For each region and hibernaculum sampled (Hib), we list number of individuals genotyped (*N*), expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (A), and loci with significant Hardy–Weinberg disequilibrium after Bonferroni corrections (HW)

Region	Hib	*N*	H_E	H_O	A	HW
AD	W-01	57	0.55	0.50	4.83	*Cw*C24f, 7–144
	W-03	18	0.57	0.58	5.07	
	W-04	15	0.60	0.59	5.55	
	W-05	32	0.56	0.53	5.06	*Cw*A29f
	W-07	18	0.59	0.51	5.12	
SFW	O-18	22	0.63	0.59	5.98	*Cw*A29f
	O-26	27	0.66	0.63	6.21	
	O-02	54	0.61	0.57	5.87	*Cw*A29f
SFE	O-01	31	0.63	0.60	5.58	
	O-39	18	0.55	0.49	4.60	
BMN	O-32	17	0.57	0.57	4.17	
	O-05	24	0.59	0.61	3.82	
BMS	R-10	19	0.61	0.60	5.08	
	R-11	21	0.60	0.59	4.44	*Cw*A29f

AD, Adirondacks; SFW, Sterling Forest west; SFE, Sterling Forest east; BMN, Bear Mountain north; BMS, Bear Mountain south.

Results

Genetic diversity and differentiation

After Bonferroni corrections, significant departures from Hardy–Weinberg (HW) equilibrium were observed for *Cw*a29f at four of the 14 populations, and *Cw*C24f and 7–144 at one population each (Table 1). MICROCHECKER revealed that *Cw*a29f had a significant probability of null alleles at four populations, and 5a, 7–144, and 7–87 showed a significant probability of null alleles at one population each. FSTAT indicated no overall linkage disequilibrium between any of the loci. Because we detected no consistent patterns of

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Table 2 Pairwise population F_{ST} (below diagonal) and D_{LR} (above diagonal) for sampled *C. horridus* hibernacula in five study regions. Significant F_{ST} values are in bold; sampled hibernacula region abbreviations are as defined in Table 1

	AD						SFW		
	W-01	W-03	W-04	W-05	W-07		O-18	O-26	O-02
W-01		0.28	0.36	0.60	0.53	O-18		0.01	1.13
W-03	0.01		0.00	0.50	0.53	O-26	0.00		1.37
W-04	0.00	0.00		0.03	0.32	O-02	**0.04**	**0.02**	
W-05	0.01	0.01	0.00		0.25				
W-07	**0.03**	**0.03**	0.02	0.03					

	SFE			BMN			BMS	
	O-01	O-39		O-05	O-32		R-10	R-11
O-01		1.54	O-05		1.29	R-10		1.48
O-39	**0.05**		O-32	**0.05**		R-11	**0.05**	

HW disequilibrium or null alleles across all populations, we retained all loci for analysis.

Population divergences as measured by F_{ST} revealed generally low but significant levels of differentiation between hibernacula (F_{ST} = 0.00–0.05), with the exception of the sites in the Adirondack region (Table 2). Here, most pairwise F_{ST} values were not significant. Results from D_{LR} measures also indicated low differentiation among hibernacula in AD, and modest differentiation in other regions. In AD, pairwise D_{LR} values were < 1, indicating low average likelihood of observing genotypes in the hibernaculum from where they were collected to that of other hibernacula in the region. In other regions, D_{LR} ranged from 1.0 to 1.5, indicating only moderately higher likelihood that individual genotypes assign to their hibernacula of origin. The randomization procedure showed significant ($P < 0.05$) self-assignment in most hibernacula, suggesting that they are genetically distinct. The four exceptions, W-04, W-05, O-18, and O-26, are in close geographical proximity to other hibernacula (Fig. 1).

Contemporary estimates of gene flow

The clustering algorithm in STRUCTURE identified a single genetic cluster in three regions, AD, BMN, and BMS (likelihood ~1.0), corroborating that gene flow among hibernacula is sufficient to maintain admixture. Therefore, most hibernacula at the geographical scale sampled in this study form panmictic demes, despite the patchiness of appropriate habitat and high levels of philopatry.

Gene flow was more limited in SFE and SFW. In SFE, STRUCTURE identified two genetic clusters (likelihood ~1.0). Individuals from the two hibernacula generally assigned to separate clusters, but with evidence of admixture between them: individuals from O-01 showed an average cluster membership Q of 0.68 ± 0.24 to cluster 1, and individuals from O-39 showed Q of 0.74 ± 0.23 to cluster 2. Exclusion tests in GENECLASS also indicated that the level of differentiation between the hibernacula is moderate: 40% of the individuals from O-01 could be excluded from O-39, and none of the O-39 individuals could be statistically excluded from O-01. STRUCTURE identified three of 49 (6.1%) individuals from these two hibernacula as migrants. GENECLASS identified two of the same three migrant genotypes with less than 0.01 probability, with the third classified as a migrant genotype with a probability of 0.03.

In the SFW region, STRUCTURE also identified two genetic clusters. Individuals from O-02 showed an average Q of 0.63 ± 0.32 to cluster 1, and individuals from O-18 and O-26 showed respective average Q values of 0.72 ± 0.28 and 0.70 ± 0.31 to cluster 2. Thus, O-26 and O-18 generally assigned to a one genetic cluster, with O-2 forming a second cluster. Exclusion tests in GENECLASS also showed that most individuals (> 85%) could not be excluded from either cluster, confirming the significant level of admixture between them. With O-26 and O-18 collapsed into a single cluster and O-02 as a second cluster, both STRUCTURE and GENECLASS identified the same six of 103 (5.8%) migrant genotypes.

Long-term estimates of gene flow

Estimates of θ values from MIGRATE 2.1 ranged from 0.019 to 0.127. Assuming a typical vertebrate microsatellite mutation rate of 10^{-4}, this translates to effective population sizes (N_e) of hibernacula that range from 47 to 317, with a mean of 156 ± 80 (n = 14). However, these estimates should be considered approximate at best because the actual mutation rate for these loci is unknown, and slight variations significantly alter the estimates of effective population sizes. The number of migrants (Nm) between hibernacula ranged from 0.3 to 2.8, with an average of 1 ± 0.7 (n = 14) (Table 3). Although there are no cases of significant asymmetry in

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Table 3 Estimates of effective population size (N_e) and asymmetric migration (Nm) for *Crotalus horridus* populations in the five study regions inferred from Bayesian analyses implemented in MIGRATE. The hibernacula receiving migrants are in rows, and the hibernacula from which migrants originated from are in columns. Effective population sizes assume a mutation rate of 10^{-4}. Parenthetical values indicate 95% confidence intervals; sampled hibernacula region abbreviations are as defined in Table 1

	AD					
	N_e	W-01	W-03	W-04	W-05	W-07
W-01	317 (244, 369)		0.4 (0.1, 1.0)	0.4 (0.1, 1.0)	1.4 (0.5, 2.3)	0.4 (0.1, 1.0)
W-03	107 (44, 156)	2.8 (0.9, 5.3)		0.5 (0.1, 1.4)	1.2 (0.3, 2.3)	0.6 (0.1, 1.4)
W-04	78 (19, 119)	1.6 (0.2, 2.3)	0.8 (0.1, 1.7)		0.7 (0.1, 1.5)	1.0 (0.1, 2.1)
W-05	173 (94, 232)	2.4 (1.0, 4.0)	0.9 (0.3, 1.8)	0.4 (0, 0.9)		0.4 (0, 0.9)
W-07	94 (31, 144)	1.8 (0.4, 3.5)	0.3 (0, 0.9)	0.8 (0.2, 1.5)	0.8 (0.2, 1.5)	

	SFW			
	N_e	O-18	O-26	O-02
O-18	83 (31, 119)		1.4 (0.4, 2.7)	3.1 (1.0, 5.0)
O-26	95 (44, 144)	0.8 (0.3, 1.7)		2.0 (0.7, 3.9)
O-02	217 (156, 256)	0.7 (0.3, 1.7)	0.8 (0.3, 1.5)	

	SFE		
	N_e	O-01	O-39
O-01	95 (44, 143)		1.1 (0.3, 2.0)
O-39	47 (6, 81)	0.9 (0.1, 2.0)	

	BMN		
	N_e	O-05	O-32
O-05	208 (131, 269)		0.6 (0.1, 1.4)
O-32	193 (93, 269)	0.8 (0.2, 1.7)	

	BMS		
	N_e	R-10	R-11
R-10	223 (156, 269)		0.9 (0.3, 1.7)
R-11	249 (168, 306)	0.6 (0, 1.3)	

effective migration (confidence intervals overlap for both directions in every pair), W-01 in the AD region and O-02 in the SFW region both appear to be important source populations with qualitatively high effective population sizes and emigration rates.

Landscape genetics analysis

We found no significant correlation between F_{ST} and the Euclidean distance between pairs of populations, indicating no isolation by distance using simple straight-line distances between hibernacula ($r = 0.1$, $P = 0.2$, Fig. 2). However, we detected a highly significant positive correlation between F_{ST} and least-cost distance, a metric which takes into account the amount of potential shared basking habitat between hibernacula ($r = 0.63$, $P = 0.01$, Fig. 2). Therefore, appropriate habitat and the aggregative behaviour of individuals at these patchy sites plays an important role in maintaining connectivity between hibernacula.

Parentage analysis

Low levels of differentiation among most pairs of hibernacula in this study suggest historical admixture among populations, but do not offer information about the mechanism of effective dispersal. Identification of parent–offspring relationships offers a complimentary method to examine gene flow in the form of interhibernacular mating. If gene flow is mediated primarily through male mating dispersal, we expect most mothers and offspring to be found within the same hibernaculum but a significant portion of fathers to be found at hibernacula other than that of their offspring. Our test of the power of our markers using individuals of known parentage showed that all 11 offspring

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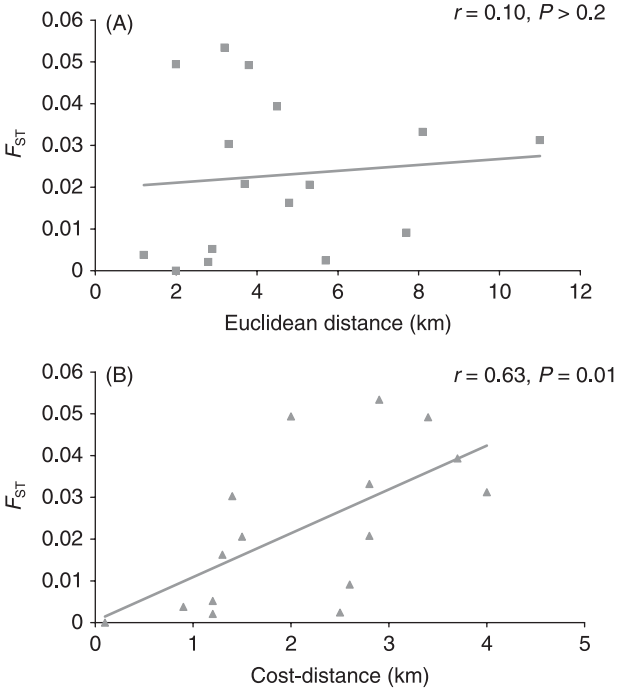

Fig. 2 Patterns of isolation by distance for *Crotalus horridus* hibernacula not separated by anthropogenic barriers (A) with geographical distance as Euclidean distance ($r = 0.10$, $P > 0.2$), and (B) with geographical distance taking into account the amount of potential basking habitat between hibernacula ($r = 0.63$, $P = 0.01$). The availability of appropriate habitat in the landscape has a significant effect on population connectivity in this species.

were compatible with their putative mother, but only five of the 11 were significant at the 0.05 level. Thus, our parentage assignment appears to be accurate, but very conservative. Over all regions, three of the 33 (9%) mother–offspring pairs were collected at different hibernacula, whereas 15 of the 46 (33%) father-offspring pairs were from different hibernacula (Table 4). The proportion of mother-offspring pairs collected at the same hibernaculum was significantly different from that of father–offspring pairs ($\chi^2 = 4.39$, DF = 1, $P = 0.03$).

Discussion

The timber rattlesnake hibernacula in this study showed only modest levels of genetic differentiation. This result is somewhat surprising given previous evidence from demographic mark–recapture studies indicating that less than 1% of individuals ever disperse from their natal hibernaculum (W.S. Brown, R. Stechert, unpublished data). Our results from analyses based on F_{ST}, assignment tests, exclusion tests, and Bayesian clustering analyses all corroborate the findings of low levels of differentiation among neighbouring hibernacula at the scale of 2–8 km in relatively undisturbed habitat. Although hibernacula show modest differentiation,

Table 4 The number of genetically inferred parent–offspring pairs of *C. horridus* where both individuals were collected at the same hibernaculum (same), vs. those where parent and offspring were collected at different hibernacula (diff.). M–O, mother–offspring pairs; F–O, father–offspring pairs; sampled hibernacula region abbreviations are as defined in Table 1

Region	M–O same	M–O diff.	F–O same	F–O diff.
AD	6	1	1	6
BMN	4	0	6	2
BMS	5	2	8	3
SFE	1	0	2	0
SFW	14	0	14	4
Total	30	3	31	15

in most regions gene flow among hibernacula is sufficiently high that they should be considered a single panmictic deme.

Our data emphasize the importance of corridors of appropriate habitat among population cores, even in undisturbed populations, and underscore how species-typical requirements and habitat specialization result in complex patterns of population connectivity. This landscape genetic approach to population connectivity has been useful in elucidating how habitat structure affects genetic structure in a variety of species (reviewed in Manel *et al*. 2003) Many more recent examples have been reported as well, including the findings that population connectivity in the Pacific jumping mouse is correlated with the amount of habitat suitable for dispersal movements (Vignieri 2005), and that forested overpass structures may increase genetic connectivity among roe deer populations fragmented by roads (Kuehn *et al*. 2007). For timber rattlesnakes, differentiation among hibernacula is a function of both geographical distance and the availability of thermoregulation sites in the habitat matrix. Hibernacula connected by areas with steep, south-facing slopes show less genetic differentiation (Figs 1 and 2). Furthermore, this pattern does not appear to be skewed by unusually large differences in any given region, given that the three hibernacula in Fig. 2 with the greatest cost-distance separating them came from three different regions (AD, SFW, and SFE).

Because access to basking sites may be particularly critical at more northerly latitudes, timber rattlesnake reproduction is strongly correlated with climatic conditions (Martin 2002). Shared basking sites may lead to increased gene flow among adjacent hibernacula because they provide a means by which males can locate females during the mating season (Bushar *et al*. 1998). During late summer, males begin moving long distances, often at the periphery of their home ranges, and exhibit behaviours indicative of scent-trailing (Reinert & Zappalorti 1988b; Waldron *et al*. 2006). Males most likely use chemoreception of skin lipid pheromones to track receptive females (Mason 1992), and these signals may be deposited reliably at basking sites by females that have

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visited them for thermoregulation. Therefore, connectivity among timber rattlesnake hibernacula is determined by mate-searching behaviours and dependence on particular habitat features.

Because field studies show that individuals rarely disperse permanently among hibernacula (W.S. Brown, R. Stechert, unpublished data), we hypothesized that gene flow must occur as a result of mating between individuals from different hibernacula, rather than direct individual migration. Furthermore, given the observations of increased male movements during the mating season, we predicted this gene flow would be male-mediated. Results from both STRUCTURE and GENECLASS revealed a significant degree of admixture among hibernacula, indicating high levels of contemporary gene flow. The long-term estimates of migration obtained from MIGRATE show that, on average, hibernacula exchange about one effective migrant per generation — a sufficiently high rate to maintain genetic connectivity over the long-term and prevent differentiation. Our parentage analyses also indicate a significant level of contemporary gene flow, and further illustrate the mechanism by which gene flow occurs. The high level of mother–offspring pairs found within hibernacula (91%) confirms that most individuals recruit to their maternal hibernaculum and do not migrate. Fidelity to natal hibernacula persists despite the occurrence of other hibernacula within the seasonal migratory range of individuals; adult males from the AD region have been observed to move up to 7 km from their hibernaculum over the course of an active season (Brown 1993). Field and laboratory studies of timber rattlesnakes show that newborns follow scent trails of adults to locate suitable hibernacula (Brown & MacLean 1983; Reinert & Zappalorti 1988a; Cobb *et al*. 2005). Because gestation and birthing usually occur in basking habitats adjacent to the female's hibernaculum, newborns are most likely to adopt their maternal hibernaculum as their own, leading to the high degree of hibernaculum sharing between mothers and their offspring. In contrast, a high proportion (33%) of individuals has fathers from neighbouring hibernacula. Thus, our genetic results corroborate the field results that high levels of gene flow among hibernacula can be maintained through summer mating movements, despite a lack of direct dispersal.

Timber rattlesnakes court and mate from late July through mid-September, when individuals of both sexes are dispersed across the landscape (Aldridge & Brown 1995; Reinert 1984; Brown 1991) and when the probability of mating with an individual from a different hibernaculum is highest. It is still unknown whether males or females actively discriminate against kin when mating and whether this mating dispersal evolved specifically to reduce inbreeding in this highly philopatric species. However, it seems likely that kin recognition does play a role, given that recent studies show surprising levels of social complexity in timber rattlesnakes, including conspecific attraction in a foraging context (Clark 2007) and kin recognition among females (Clark 2004). Thus, the high degree of extra-hibernacular paternity may reflect active discrimination in mate choice, as well as seasonal migrations away from hibernacula. Future experimental studies will focus on the possibility that discrimination against kin occurs in a mate-searching or courtship context.

Few other studies on snakes have examined population differentiation at this geographical scale. Bushar *et al*. (1998) examined genetic structure in a cluster of five *Crotalus horridus* hibernacula in Pennsylvania that occurred within a 2-km radius and found a moderate degree of differentiation. Average F_{ST} between hiberanacula was relatively low (0.05). Those results were based on three microsatellite loci, and relatively low sample sizes per hibernaculum (average $N = 6$); therefore, it is difficult to compare their results directly to ours. The only other fine-scale population genetic study in a rattlesnake that has been published examined populations of the massasauga (*Sistrurus catenatus*), a smaller species that inhabits wetlands throughout northeastern North America (Gibbs *et al*. 1997). Massasaugas differ from timber rattlesnakes in that the former hibernate singly; nonetheless, massasaugas also exhibit population genetic structure over very short distances (1–2 km), indicating either extremely limited natal dispersal, reduced movement associated with mating, or both.

Lougheed *et al*. (1999) examined population genetic structure in black ratsnakes (*Elaphe obsoleta*), a large colubrid sympatric with *C. horridus* over much of the northeastern USA that also uses communal hibernacula. They found less differentiation among hibernacula (average $F_{ST} = 0.012$) than we found here for *C. horridus* (average $F_{ST} = 0.023$). The studies are comparable in terms of sampling distances and sample sizes, and thus underscore how species-typical characteristics can mediate differences in genetic variation in common landscapes. There are several behavioural differences between the two species that may account for lower genetic structure in *E. obsoleta*. Unlike timber rattlesnakes, black ratsnakes do not usually oviposit near their own hibernaculum and this likely increases the probability that juvenile black ratsnakes will join a hibernaculum different from their mother's (Blouin-Demers *et al*. 2004). Additionally, a higher percentage of *E. obsoleta* matings (72%) involve snakes from different hibernacula (Blouin-Demers *et al*. 2005) — perhaps a result of the increased movements of receptive females (Blouin-Demers *et al*. 2004). In contrast, timber rattlesnake females do not apparently exhibit increased movements during the mating season compared to their nonmating season movements (Reinert & Zappalorti 1988b; Waldron *et al*. 2006).

Despite these differences, we also find commonality in the genetic structure of massassaugas, timber rattlesnakes, and black ratsnakes: all three species show significant genetic

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structure at a geographical scale that is small relative to individual movements (Gibbs & Weatherhead 2001). This fine-scale structure may result from philopatry and dependence on habitat features that are patchily distributed in the landscape. Water snakes (*Nerodia sipedon*) and garter snakes (*Thamnophis sirtalis* and *Thamnophis elegans*), three species associated with patchily distributed water bodies, also exhibit fine-scale population differentiation (Prosser *et al*. 1999; Manier & Arnold 2005). Thus, although it is possible that fine-scale structure is a characteristic of snakes in general, it is also possible that the populations examined to date have been subjected to strong ecological constraints on dispersal. In support of this hypothesis, Keogh *et al*. (2006) recently found a lack of fine-scale population differentiation in the small-eyed snake (*Rhinoplocephalus nigrescens*), an Australian elapid that is a habitat generalist with male-biased dispersal. The generality of ecological constraints resulting in fine-scale population structure in snakes will be tested as we accumulate more studies of ecologically divergent taxa at the appropriate geographical scales.

The modest degree of differentiation among hibernacula and male-mediated effective gene flow in *C. horridus* has important conservation implications. Because population connectivity depends on males searching widely for mates and then returning to natal hibernacula, anthropogenic barriers to movements such as roads can have an especially strong impact on gene flow and population structure in this species. Increased mortality of males faced with anthropogenic barriers during the mating season can result not only in population declines, but also in reduced interhibernacular gene flow. Both population size and gene flow may be critical in maintaining viable populations, and, if interrupted, active management may be required to re-establish genetic connectivity. For example, an isolated population of another viperid (*Vipera berus*) exhibited inbreeding depression in the form of small litters, a high rate of deformed and stillborn offspring, and reduced heterozygosity (Madsen *et al*. 1996). Translocating individuals from another population reversed these trends (Madsen *et al*. 1999, 2004). However, wildlife management officials will need to balance the benefits of supplementing endangered populations with the potential costs of degrading local adaptation (Gibbs & Weatherhead 2001). Snake populations that exhibit fine-scale genetic structuring also have increased evolutionary potential for local adaptation. Even populations that are relatively close to each other can evolve significant specializations to the local biotic or abiotic environment (Daltry *et al*. 1996; Bronikowski 2000). Our data underscore the important point that active management of populations to maintain genetic viability should be guided by population genetic studies undertaken in natural populations in nonimpacted habitats. It is these studies that will reveal the relative roles of habitat, landscape, and individual behaviour in maintaining healthy genetic exchanges among populations.

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References

Aldridge RD, Brown WS (1995) Male reproductive cycle, age at maturity, and cost of reproduction in the timber rattlesnake (*Crotalus horridus*). *Journal of Herpetology*, **29**, 399–407.

Allendorf F, Luikart G (2006) *Conservation and the Genetics of Populations*. Blackwell Publishing, Malden, Massachusetts.

Andrews KM, Gibbons JW (2005) How do highways influence snake movement? Behavioral responses to roads and vehicles. *Copeia*, **2005**, 772–782.

Antolin MF, Savage LT, Eisen RJ (2006) Landscape features influence genetic structure of black-tailed prairie dogs (*Cynomys ludovicianus*). *Landscape Ecology*, **21**, 867–875.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences, USA*, **98**, 4563–4568.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.

Blouin-Demers G, Gibbs HL, Weatherhead PJ (2005) Genetic evidence for sexual selection in black ratsnakes, *Elaphe obsoleta*. *Animal Behaviour*, **69**, 225–234.

Blouin-Demers G, Weatherhead PJ, Row JR (2004) Phenotypic consequences of nest-site selection in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology*, **82**, 449–456.

Bonnet X, Shine R, Lourdais O (2002) Taxonomic chauvinism. *Trends in Ecology & Evolution*, **17**, 1–3.

Bronikowski AM (2000) Experimental evidence for the adaptive evolution of growth rate in the garter snake, *Thamnophis elegans*. *Evolution*, **54**, 1760–1767.

Brown WS (1991) Female reproductive ecology in a northern population of the timber rattlesnake, *Crotalus horridus*. *Herpetologica*, **47**, 101–115.

Brown WS (1993) Biology, status, and management of the timber rattlesnake (*Crotalus horridus*): a guide for conservation. *Herpetological Circular*, **22**, 1–78.

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Brown WS (in press) Sampling timber rattlesnakes (*Crotalus horridus*): phenology, growth, intimidation, survival, and a syndrome of undetermined origin in a northern population. In: *The Biology of Rattlesnakes* (eds Hayes WK, Beaman KR, Cardwell MD, Bush SP). Loma Linda University Press, Loma Linda, California.

Brown WS, Kéry M, Hines JE (2007) Survival of timber rattlesnakes (*Crotalus horridus*) estimated by capture–recapture models in relation to age, sex, color morph, time, and birthplace. *Copeia*, **2007**, 656–671.

Brown WS, MacLean FM (1983) Conspecific scent-trailing by newborn timber rattlesnakes *Crotalus horridus*. *Herpetologica*, **1983**, 430–436.

Brown WS, Parker WS (1976) A ventral scale-clipping system for permanently marking snakes (Reptilia, Serpentes). *Journal of Herpetology*, **10**, 247–249.

Brzustowski J (2002) Doh assignment test calculator. Online at: http://www2.biology.ualberta.ca/jbrzusto/Doh.php.

Burland TM, Barratt EM, Nichols RA, Racey PA (2001) Mating patterns, relatedness and the basis of natal philopatry in the brown long-eared bat, *Plecotus auritus*. *Molecular Ecology*, **10**, 1309–1321.

Bushar LM, Reinert HK, Gelbert L (1998) Genetic variation and gene flow within and between local populations of the timber rattlesnake, *Crotalus horridus*. *Copeia*, **1998**, 411–422.

Clark RW (2004) Kin recognition in rattlesnakes. *Proceedings of the Royal Society of London. Series B, Biology Letters*, **271**, S243–S245.

Clark RW (2006) Post-strike behavior of timber rattlesnakes (*Crotalus horridus*) during natural predation events. *Ethology*, **112**, 1089–1094.

Clark RW (2007) Public information for solitary foragers: timber rattlesnakes use conspecific chemical cues to select ambush sites. *Behavioral Ecology*, **18**, 487–490.

Cobb VA, Green JJ, Worrall T, Pruett J, Glorioso B (2005) Initial den location behavior in a litter of neonate *Crotalus horridus* (timber rattlesnakes). *Southeastern Naturalist*, **4**, 723–730.

Coulon A, Cosson JF, Angibault JM *et al*. (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**, 2841–2850.

Daltry JC, Wuester W, Thorpe RS (1996) Diet and snake venom evolution. *Nature*, **379**, 537–540.

Emlen ST (1982) The evolution of helping 1: an ecological constraints model. *American Naturalist*, **119**, 29–39.

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

Gardner MG, Bull CM, Cooper SJB, Duffield GA (2001) Genetic evidence for a family structure in stable social aggregations of the Australian lizard *Egernia stokesii*. *Molecular Ecology*, **10**, 175–183.

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

Gibbs HL, Weatherhead PJ (2001) Insights into population ecology and sexual selection in snakes through the application of DNA-based genetic markers. *Journal of Heredity*, **92**, 173–179.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Greene HW, May PG, Hardy DL, Sciturro JM, Farrell TM (2002) Parental behavior in vipers. In: *Biology of the Vipers* (eds Schuett GW, Höggren M, Douglas ME, Greene HW). Eagle Mountain Publishing, Eagle Mountain, Utah.

Guo SW, Thompson EA (1992) A Monte Carlo method for combined segregation and linkage analysis. *American Journal of Human Genetics*, **51**, 1111–1126.

Holycross AT, Douglas ME, Higbee JR, Bogden RH (2002) Isolation and characterization of microsatellite loci from a threatened rattlesnake (New Mexico ridge-nosed rattlesnake, *Crotalus willardi obscurus*). *Molecular Ecology Notes*, **2**, 537–539.

Keogh JS, Webb JK, Shine R (2006) Spatial genetic analysis and long-term mark-recapture data demonstrate male-biased dispersal in a snake. *Biology Letters*, **3**, 33–35.

Kuehn R, Hindenlang KE, Holzgang O *et al*. (2007) Genetic effect of transportation infrastructure on roe deer populations (*Capreolus capreolus*). *Journal of Heredity*, **98**, 13–22.

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

Madsen T, Shine R, Olsson M, Wittzell H (1999) Restoration of an inbred adder population. *Nature*, **402**, 34–35.

Madsen T, Stille B, Shine R (1996) Inbreeding depression in an isolated population of adders, *Vipera berus*. *Biological Conservation*, **75**, 113–118.

Madsen T, Ujvari B, Olsson M (2004) Novel genes continue to enhance population growth in adders (*Vipera berus*). *Biological Conservation*, **120**, 145–147.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.

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

Martin WH (1993) Reproduction of the timber rattlesnake (*Crotalus horridus*) in the Appalachian Mountains. *Journal of Herpetology*, **27**, 133–143.

Martin WH (2002) Life history constraints on the timber rattlesnake (*Crotalus horridus*) at its climatic limits. In: *Biology of the Vipers* (eds Schuett GW, Höggren M, Douglas ME, Greene HW), pp. 285–306. Eagle Mountain Publishing, Eagle Mountain, Utah.

Mason RT (1992) Reptilian pheromones. In: *Biology of the Reptilia*, Vol. 18 (eds Gans C, Crews D), pp. 114–228. University of Chicago Press, Chicago, IL.

McDonald DB, Potts WK, Fitzpatrick JW, Woolfenden GE (1999) Contrasting genetic structures in sister species of North American scrub-jays. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 1117–1117.

Nutt KJ (2005) Philopatry of both sexes leads to the formation of multimale, multifemale groups in *Ctenodactylus gundi* (Rodentia: Ctenodactylidae). *Journal of Mammalogy*, **86**, 961–968.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55–65.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

Painter JN, Crozier RH, Poiani A, Robertson RJ, Clarke MF (2000) Complex social organization reflects genetic structure and

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relatedness in the cooperatively breeding bell miner, *Manorina melanophrys*. *Molecular Ecology*, **9**, 1339–1347.

Parsons KN, Jones G, Davidson-Watts I, Greenaway F (2003) Swarming of bats at underground sites in Britain — implications for conservation. *Biological Conservation*, **111**, 63–70.

Pearse DE, Crandall KA (2004) Beyond F_{ST}: analysis of population genetic data for conservation. *Conservation Genetics*, **5**, 585–602.

Piry S, Alapetite A, Cornuet JM *et al*. (2004) GENECLASS 2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, **95**, 536–539.

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

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

Raymond M, Rousset F (1995) GENEPOP (version 1.2) — population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Reinert HK (1984) Habitat variation within sympatric snake populations. *Ecology*, **65**, 1673–1682.

Reinert HK, Zappalorti RT (1988a) Field observation of the association of adult and neonatal timber rattlesnakes, *Crotalus horridus*, with possible evidence for conspecific trailing. *Copeia*, **1998**, 1057–1059.

Reinert HK, Zappalorti RT (1988b) Timber rattlesnakes *Crotalus horridus* of the Pine Barrens, New Jersey, USA: their movement patterns and habitat preference. *Copeia*, **1988**, 964–978.

Rivers NM, Butlin RK, Altringham JD (2006) Autumn swarming behaviour of Natterer's bats in the UK: population size, catchment area and dispersal. *Biological Conservation*, **127**, 215–226.

Simon JL (1997) *Resampling: The New Statistics*, 2nd edn. Wadsworth, Belmont, California.

Stechert R (1982) Historical depletion of timber rattlesnake colonies in New York State. *Bulletin of the New York Herpetological Society*, **17**, 23–24.

Temple HJ, Hoffman JI, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher, *Ramphocinclus brachyurus*. *Molecular Ecology*, **15**, 3449–3458.

Valiere N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Van Bekkum M, Sagar PM, Stahl J-C, Chambers GK (2006) Natal philopatry does not lead to population genetic differentiation in Buller's albatross (*Thalassarche bulleri bulleri*). *Molecular Ecology*, **15**, 73–79.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Vignieri SN (2005) Streams over mountains: influence of riparian connectivity on gene flow in the Pacific jumping mouse (*Zapus trinotatus*). *Molecular Ecology*, **14**, 1925–1937.

Villarreal X, Bricker J, Reinert HK, Gelbert L, Bushar LM (1996) Isolation and characterization of microsatellite loci for use in population genetic analysis in the timber rattlesnake, *Crotalus horridus*. *Journal of Heredity*, **87**, 152–155.

Waldman B, Rice JE, Honeycutt RL (1992) Kin recognition and incest avoidance in toads. *American Zoologist*, **32**, 18–30.

Waldron JL, Lanham JD, Bennett SH (2006) Using behaviorally-based seasons to investigate canebrake rattlesnake (*Crotalus horridus*) movement patterns and habitat selection. *Herpetologica*, **62**, 389–398.

Wilmer JW, Allen PJ, Pomeroy PP, Twiss SD, Amos W (1999) Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). *Molecular Ecology*, **8**, 1417–1429.

Zaidan F, Beaupre SJ (2003) Effects of body mass, meal size, fast length, and temperature on specific dynamic action in the timber rattlesnake (*Crotalus horridus*). *Physiological and Biochemical Zoology*, **76**, 447–458.

This paper is part of R.W. Clark's postdoctoral research that aims to understand how behaviour and the environment shape patterns of genetic structure in timber rattlesnakes. The Zamudio laboratory studies mating system biology, population structure, and phylogeography of reptiles and amphibians with a focus on species inhabiting patchy landscapes. W.S. Brown and R. Stechert have conducted long-term surveys and mark–recapture field studies of this species in regions AD of northeastern New York, and regions BM and SF in southeastern New York, respectively. Our collaboration stems from a common interest in the population ecology and conservation of threatened species and underscores the benefits of combining long-term field research with molecular genetics.

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