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Author(s): Corey Devin Anderson, H. Lisle Gibbs, Michael E. Douglas, and Andrew T. Holycross
Source: Copeia, 2009(4):740-747.
Published By: The American Society of Ichthyologists and Herpetologists
https://doi.org/10.1643/CG-08-152
URL: http://www.bioone.org/doi/full/10.1643/CG-08-152

Copeia 2009, No. 4, 740–747

Conservation Genetics of the Desert Massasauga Rattlesnake (*Sistrurus catenatus edwardsii*)

Corey Devin Anderson[1], H. Lisle Gibbs[2], Michael E. Douglas[3], and Andrew T. Holycross[4,5]

Populations of the Desert Massasauga Rattlesnake (*Sistrurus catenatus edwardsii*) have declined rapidly as desert grassland communities have become reduced and fragmented. To provide information useful for management of remaining populations, the genetic characteristics (based on microsatellite DNA loci) of the last demonstrably extant population in the state of Arizona were compared to a population in the Rio Grande Valley of New Mexico. Results indicated that genetic diversity was relatively high in both populations, with statistically significant heterozygote deficiencies detected at only one of six loci in each population. Contingency tests, Wright's F-statistics, and Bayesian clustering algorithms all indicated substantial subdivision between populations in Arizona and New Mexico, but only contingency tests supported differentiation within the Arizona population. A preliminary hierarchical analysis of variance (incorporating both our data and published microsatellite data for the Eastern Massasauga) indicated that 73% of the total molecular variance was explained by variation within populations, with variation between the two subspecies accounting for 15% of the total variance. Results support the high conservation value of individual populations, as well as the need for further population genetic studies of the Desert Massasauga Rattlesnake.

THE Desert Massasauga Rattlesnake (*Sistrurus catenatus edwardsii*) inhabits xeric grassland communities in the desert southwest of North America, with peripheral populations in Mexico, New Mexico, southeastern Arizona, and eastern Colorado (Stebbins, 1985; Holycross, 2003). While the geographic range of this subspecies is often depicted as continuous, it actually consists of a series of isolated populations, many of which are declining, extirpated, or perceived at risk (Lowe et al., 1986; Greene, 1997; Werler and Dixon, 2000). This patchy distribution is a consequence of both narrow ecological tolerances and Holocene climate changes that have fragmented suitable habitat (Greene, 1997). More recently, anthropogenic effects such as desertification, encroachment by agriculture, road mortality, and/or extermination have been identified as significant causes of the decline and extirpation of populations (Lowe et al., 1986; Greene, 1997; Werler and Dixon, 2000).

For example, specimens in Arizona have been taken from the San Bernardino, San Simon, San Pedro, and Sulphur Springs valleys in Cochise County. Historic vouchers suggest the Desert Massasauga Rattlesnake was once distributed throughout the San Bernardino and San Simon Valleys, yet recently collected specimens are restricted to a relict Tobosa grassland straddling the valleys (Holycross, 2003). A specimen described by Sinclair and Snell (1990) verified the persistence of the Desert Massasauga Rattlesnake in the southern Sulphur Springs Valley, but none have been found in this area subsequently. Although the Desert Massasauga Rattlesnake may persist at low density in the lower Rio Grande Valley of New Mexico (Degenhardt et al., 1996), only a handful of individuals have been observed or collected from this region in the last 20 years (D. Burkett, pers. comm; L. Kamees, pers. comm; C. Painter, pers. comm.). Larger populations are known from the shortgrass prairie of the central Rio Grande Valley, but these are approximately 400 kilometers from southeastern Arizona.

Management of remaining populations of *S. c. edwardsii* in the desert southwest would benefit from data on genetic variability within and among populations. Such information would offer insight into important ecological and evolutionary processes, and would provide an empirical basis for defining management units (Crandall et al., 2000; Holycross and Douglas, 2007). Previous population genetic studies (Gibbs et al., 1997; Prior et al., 1997) have focused exclusively on one of three named subspecies, the Eastern Massasauga Rattlesnake (*S. c. catenatus*), found in the northeastern United States and southeastern Canada. Although population genetic studies of the Eastern Massasauga Rattlesnake have been informative, it is unknown how well observed patterns (e.g., high levels of population genetic structure) apply to other subspecies. Indeed, Shine (2008) questioned whether results from regions with extreme cold temperatures accurately predict patterns in warmer regions, where climatic conditions do not enforce seasonal aggregation.

In the present study, we used microsatellite DNA loci to compare the genetic characteristics of a population of the Desert Massasauga Rattlesnake from the San Bernardino Valley of Arizona with a population from the central Rio Grande Valley of New Mexico (Fig. 1). Results were used to assess genetic diversity and exchangeability, and were compared to published data for the Eastern Massasauga Rattlesnake to clarify how genetic variation is apportioned within and between subspecies.

MATERIALS AND METHODS

Study sites and sampling information.—Individuals were collected from April through October in Cochise County, Arizona (1993–1997) and Valencia County, New Mexico (1997–1998). The Arizona population inhabits a Tobosa (*Hilaria mutica*) grassland that blankets a volcanic cinder

[1] The Biodesign Institute, Center for Evolutionary Functional Genomics, Arizona State University, P.O. Box 875301, Tempe, Arizona 85287-5301; E-mail: corey.d.anderson@asu.edu. Send reprint requests to this address.
[2] Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, Ohio 43210-1293; E-mail: gibbs.128@osu.edu.
[3] Illinois Natural History Survey, 1816 South Oak Street, Champaign, Illinois 61820; E-mail: med@inhs.uiuc.edu.
[4] School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501; E-mail: andrewholycross@gmail.com.
[5] Red Mountain Campus, Mesa Community College, 7110 East McKellips Road, Mesa, Arizona 85207.
Submitted: 28 August 2008. Accepted: 25 August 2009. Associate Editor: J. M. Quattro.
 DOI: 10.1643/CG-08-152

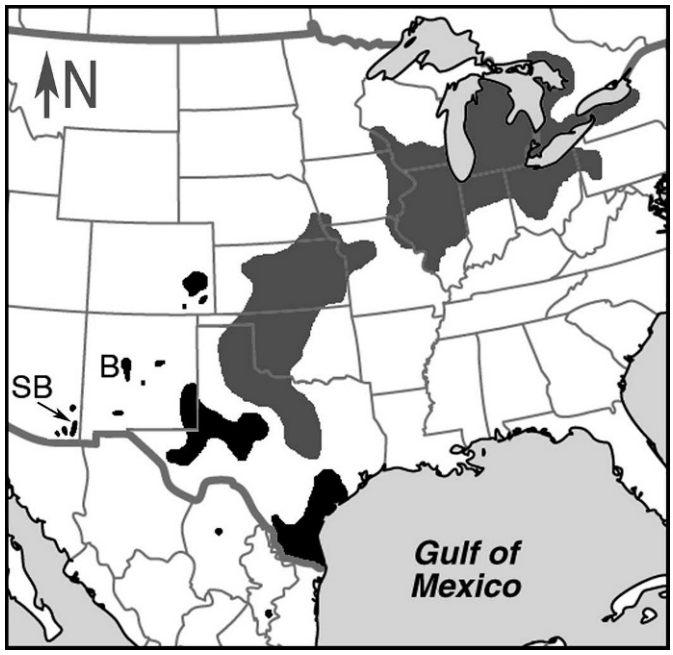

Fig. 1. Location of the San Bernardino (SB) and Belen (B) populations within the historic distribution of *Sistrurus catenatus edwardsi* (black). The historic distribution of *S. c. tergeminus* and *S. c. catenatus* are collectively illustrated in gray. Current distribution in Arizona is substantially reduced; SB is the last demonstrably extant population.

cone field in the San Bernardino Valley, located between the Chiricahua and Peloncillo mountains. The spatial extent and size of this population are unknown, as the species is notoriously difficult to observe and most potential habitat is located on private land. Most specimens are recorded from a stretch of US Highway 80 between Douglas, Arizona and Rodeo, New Mexico. Between 1993 and 1997, a 10.7-mile (17.1-km) transect of the highway was sampled, which included all sections where the subspecies was known to occur at the time of the study. Individuals were found while driving along the highway several hours after sunset (spending approximately equal time on all portions of the roadway). Posted mile markers and a car odometer were used to record the location of each captured individual to the nearest 0.1 mile (0.16 km). To test whether captures were nonrandomly clustered along the highway, a Geary's c correlogram based on capture frequency (with one-mile distance lags) was constructed using PASSaGE 2 (http://www.passagesoftware.net). Significant autocorrelation for a distance lag was inferred if the test statistic exceeded the expected value when no autocorrelation is present (c = 1), as determined by 999 permutations of the data. For Geary's c, values less than one indicate positive autocorrelation, while values greater than one indicate negative autocorrelation.

The New Mexico population occupies a shortgrass prairie in the Rio Grande Valley, east of Belen and west of the Manzano Mountains. The majority of this area (ca. 28 × 13 km) was under development for tract homes, and most specimens were sampled on roads created for the development; however, capture locations were only recorded approximately for some individuals via local landmarks.

DNA extraction and microsatellite locus amplification.—Six microsatellite loci developed for the Eastern Massasauga Rattlesnake were used in this study: five are described by Gibbs et al. (1998; *Scu01*, *05*, *07*, *11*, and *26*), while another (*Scu140*) is based on unpublished information (available upon request from L. Gibbs). Procedures to extract DNA and characterize loci are provided in Gibbs et al. (1998). In brief, DNA was extracted from blood or muscle samples using DNAzol (Gibco, Carlsbad, CA) or standard phenol–chloroform procedures. Microsatellite loci were amplified in 10 μl PCR reactions, consisting of 50 ng of genomic DNA, 0.3 pmole of forward primer end-labeled with [^{33}P]-ATP (Dupont, Wilmington, DE), 0.4 pmole of unlabeled forward primer, 0.8 pmole of unlabeled reverse primer, 200 μM dNTPs, 0.5 U of AmpliTaq (Perkin Elmer, Waltham, MA), 0.1 M Tris-HCl (pH 8.3), 0.5 M KCl, and 1.5–2.0 mM $MgCl_2$. Amplifications were run in a Perkin Elmer 480 Thermocycler for 30 cycles, with each cycle consisting of 30 s at 94°C, 30 s at the appropriate annealing temperature (described in Gibbs et al. [1998]), and 30 s at 72°C. Amplification products (3 μl) were run on 6% denaturing polyacrylamide gels at 55 W for 2.5 h. Gels were dried and exposed to Biomax (Kodak, Rochester, NY) X-ray film overnight. Amplification products were sized by reference to sequencing reactions of a control template and amplifications from known-size clones for each locus. Some individuals from both populations (i.e., San Bernardino and Belen) were run on gels at the same time to crosscheck allele size differences. Individuals were not size referenced relative the Eastern Massasauga Rattlesnake, but all data were collected from the same laboratory where genotype data for the Eastern Massasauga were collected.

Population-genetic analyses.—Allele sizes for all microsatellite loci were entered into Microsoft Excel for preprocessing using the Excel Microsatellite Toolkit (Park, 2001). The Excel Microsatellite Toolkit was used to format text files and to calculate Nei's unbiased gene diversity (Nei, 1978). The computer program GENEPOP (Raymond and Rousset, 1995) was used to calculate the number of observed and expected heterozygotes, as well as to test for heterozygote deficiencies at each locus and across loci (via U tests). Fisher exact tests in GENEPOP were used to test for genotypic linkage disequilibrium between pairs of loci.

To estimate the frequency of null alleles for each locus, the computer program FreeNA (Chapuis and Estoup, 2007) was used to implement the Expectation Maximization (EM) algorithm of Dempster et al. (1977). According to Chapuis and Estoup (2007), this estimator performs better than those of Brookfield (1996) and Chakraborty et al. (1992). To correct for sample size differences when comparing the number of alleles observed at each locus (i.e., ''allelic richness''), the rarefaction method of Kalinowski (2004) was implemented using the computer program HP-Rare (Kalinowski, 2005). This method adjusts for size differences among samples by estimating how many alleles (or private alleles) are expected in a sample of a specified size, determined by the size of the smallest sample.

The computer program FSTAT (Goudet, 1995) was used to test whether populations were significantly differentiated in terms of allelic and genotypic frequencies (via G-based exact tests). Because tests of genic differentiation assume Hardy-Weinberg equilibrium, loci exhibiting significant heterozygote deficiencies were removed prior to testing. FSTAT was also used to calculate Wright's *F*-statistics (Weir and Cockerham, 1984). Ninety-five percent confidence intervals for the deviation from Hardy-Weinberg equilibrium within

the total population, ''F''($\approx F_{IT}$), the proportion of genetic variance in the total population due to variance among populations, ''θ'' ($\approx F_{ST}$), and the deviation from Hardy-Weinberg equilibrium within subpopulations, ''*f*'' ($\approx F_{IS}$), were estimated via bootstrapping over loci. Positive bias in estimation of ''θ'' ($\approx F_{ST}$) induced by the presence of null alleles was corrected using the *ENA* method in the computer program FreeNA (Chapuis and Estoup, 2007).

Population substructuring was also examined with the computer programs STRUCTURE 2.1 (Pritchard et al., 2000; Falush et al., 2003) and BAPS 5 (Corander et al., 2006). For both programs, population structure is evaluated relative to ''*K*'' hypothetical populations. STRUCTURE 2.1 uses a Markov Chain Monte Carlo (MCMC) algorithm for estimation, whereas BAPS 5 uses a stochastic optimization to infer the posterior mode of the genetic structure and does not simultaneously attempt to estimate admixture proportions for individuals. The latter approach has been recommended for cases wherein genetic differentiation is weak and population assignment is a secondary question.

For STRUCTURE 2.1, three different combinations of allele frequency and admixture models were used (no admixture with uncorrelated allele frequencies, no admixture with correlated allele frequencies, and admixture with correlated allele frequencies) for three different partitions of the data set (all individuals, Arizona only, and New Mexico only). When using the uncorrelated allele frequency model, the parameter ''λ'' (lambda) was estimated from the data with $K = 1$. For all runs, if the Markov Chain parameter ''α'' (alpha) failed to converge and if the proportion of the sample assigned to each population was symmetric (approx. $1/K$) then a lack of real structure was inferred from the data. In cases where program parameters indicated structure, estimated ln Pr(X|*K*) was averaged over the ten runs and plotted against *K*, and the maximum average estimate was used to determine the number of populations indicated by the data. For model combinations where the estimate of the likelihood function plateaued for larger *K*, the second order rate of change in the log probability of data between successive *K* values was used to determine the number of populations (Evanno et al., 2005).

BAPS 5 runs were conducted for the Arizona population sample only, using the ''clustering of groups of individuals'' mode, with group assignment based on association with particular cinder cone slopes (discussed below). We used a vector of replicate maximum *K* values: ten times for each *K* (from $K = 2$ to $K = 3$). For each set of runs, the software reports the set of estimates with the ''best'' partition. If estimates are consistent across increasing values for *K*, then the estimated number of populations is indicative of the number of discrete clusters estimated by the data. In the ''clustering of groups of individuals'' mode, if BAPS 5 gives an error message and the graphical output yields the same color for all groups, then this is an indication that no structure exists in the data set (that is, $K = 1$; J. Corander, pers. comm.).

Lastly, microsatellite data for the Desert Massasauga was combined with published data for the Eastern Massasauga Rattlesnake (Gibbs et al., 1997) and an analysis of molecular variance (AMOVA) was conducted using Arlequin (Excoffier et al., 2005). The total molecular variance was partitioned into covariance components due to variation within populations, variation among populations within subspecies, and variation among subspecies.

RESULTS

Genotypic data were collected for 56 individuals from Arizona and 22 from New Mexico. In Arizona, capture frequency along the highway (Fig. 2) was positively autocorrelated (Fig. 3) for locations up to one mile apart ($P < 0.001$), with significant negative autocorrelation detected between one and two miles ($P = 0.007$), and between four and five miles ($P = 0.009$). Captures were associated with sections of the highway that intersected cinder cone bajadas (i.e., volcanic slopes associated with alluvial fans). For analyses that required *a priori* categorization of individuals, captures (*n*) were assigned to one of three bajadas (Fig. 2): Cinder Pit ($n = 21$), Red Hill ($n = 28$), or Price Road ($n = 7$).

All six microsatellite loci exhibited high levels of variation (Table 1). Alleles at each locus numbered 11 (*Scu26*), 12 (*Scu07*), 14 (*Scu01*), 17 (*Scu140*), 18 (*Scu11*), and 19 (*Scu05*). The average number of alleles per locus was 8.5 for New Mexico and 13 for Arizona. The number of alleles in the Arizona sample exceeded those identified from the New Mexico sample for all loci; when sample size was controlled for via rarefaction, the number of alleles was greater for the Arizona sample at all loci except *Scu140* (Table 2).

Both population samples contained a high proportion of unique (''private'') alleles. Over all loci, 25% (NM) and 51.2% (AZ) of alleles were unique to each population. Furthermore, 7.7% (NM) and 19.0% (AZ) of these occurred at frequencies ≥5%. The number of private alleles observed in the Arizona sample exceeded those identified from the New Mexico sample for all loci; when sample size was controlled for via rarefaction, the number of private alleles was greater for the Arizona sample, except *Scu140* (Table 2). Within the Arizona sample, 10.7% (Cinder Pit), 17.9% (Red Hill), and 5.4% (Price Road) of alleles were unique to each bajada subsample, with 5.4% (Cinder Pit), 6.0% (Red Hill), and 5.4% (Price Road) unique to each bajada at frequencies ≥5%.

Mean observed heterozygosity and Nei's unbiased gene diversity were greater for the Arizona population (0.764 and 0.800, respectively) than for New Mexico (0.695 and 0.7411). However, a deficit was found in the observed number of heterozygotes in the total sample ($P = 0.004$), as well as within each sample population (AZ, $P = 0.01$; NM, $P = 0.05$). Within the total sample, a significant heterozygote deficit was found at *Scu05* ($P < 0.001$), and a significant heterozygote deficit also existed at *Scu05* within the Arizona population ($P < 0.001$). Within the New Mexico population, a significant heterozygote deficit was found at *Scu01* ($P = 0.01$). When samples from Arizona were partitioned into three subsamples based on association with particular cinder cone bajadas, a significant heterozygote deficit over all loci was found in the Price Road subsample ($P = 0.04$); however, the test was no longer significant when *Scu140* was removed from the analysis. Significant heterozygote deficiencies were detected in 21 of 30 locus-population comparisons for the Eastern Massasauga Rattlesnake (Table 4).

Significant heterozygote deficiencies may be the result of nonamplifying (''null'') alleles, and the latter were estimated at a frequency of 7.0% at *Scu05* in the Arizona sample and at a frequency of 11% at *Scu01* in the New Mexico sample. However, these estimates were not consistent across population samples, as the estimated frequency of null alleles was 1.5% at *Scu05* in the New Mexico sample and 0% at *Scu01* in the Arizona sample.

The null hypothesis that genotypes at one locus are independent of those at another (i.e., linkage disequilibri-

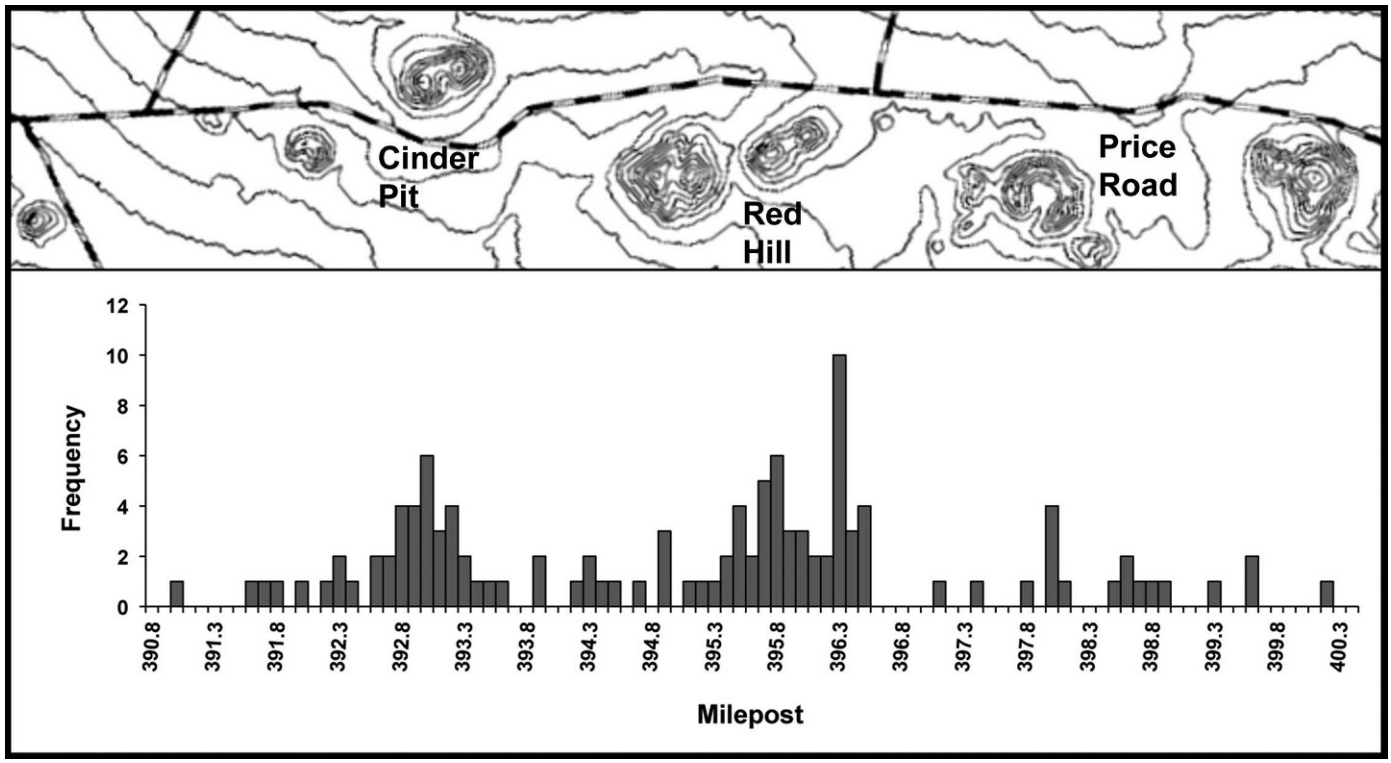

Fig. 2. Frequency of captures along a 17.1 km transect (US Highway 80) through *Sistrurus catenatus edwardsii* habitat in Cochise County, Arizona.

um) was rejected for *Scu01* and *Scu07* in the Arizona population ($P = 0.018$). G-based exact tests conducted in FSTAT indicated that allele frequencies in Arizona and New Mexico differed significantly ($P = 0.001$), as did genotype frequencies ($P = 0.001$). Likewise for the Arizona population sample, both allele and genotype frequencies differed significantly among clusters associated with different cinder cone bajadas ($P = 0.01$). Differentiation among bajadas was primarily due to pairwise differentiation between Cinder Pit and Red Hill ($P = 0.02$) and between Cinder Pit and Price Road ($P = 0.03$).

Hierarchical analysis of population structure using Weir and Cockerham (1984) *F*-statistics (Table 3) revealed a positive deviation from Hardy-Weinberg equilibrium within the total sample population (F = 0.168, 95% CI: 0.102–0.233), primarily attributable to variance among population samples ($\theta = 0.126$, 95% CI: 0.073–0.180). Correcting genotype data for positive bias induced by the potential presence of null alleles did not substantially impact θ (after correction, $\theta = 0.121$, 95% CI: 0.070–0.171). The deviation from Hardy-Weinberg equilibrium within populations was nonsignificant ($f = 0.049$, 95% CI: −0.002–0.100).

For the Arizona population, a modest deviation from Hardy-Weinberg equilibrium was detected (F = 0.053, 95% CI: 0.005–0.096), but variance among bajadas did not account for a significant proportion of the total variance (θ

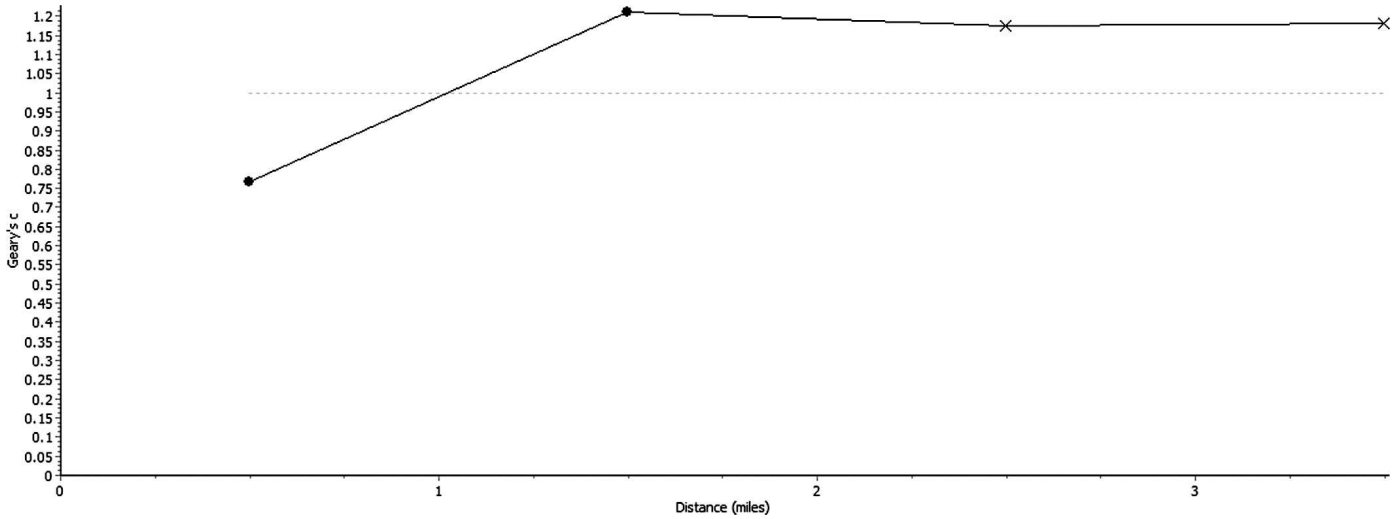

Fig. 3. Geary's c correlogram for capture frequency data partitioned into one-mile distance lags. Significant autocorrelation for an interval was inferred if the test statistic exceeded the expected value when no autocorrelation is present (c = 1), as determined by 999 permutations of the data. For Geary's c, values below one indicate positive autocorrelation and values above when indicate negative autocorrelation.

Table 1. Results of PCR Amplifications of Microsatellite DNA Loci for Sample Populations of the Massasauga Rattlesnake in Arizona and New Mexico. N_n = number of individuals sampled; HetExp = number of heterozygotes expected, HetObs = number of heterozygotes observed; *P*-values (H1 = heterozygote deficiency) were estimated in GENEPOP (Raymond and Rousset, 1995) with the Markov chain method (500 batches with 1000 iterations per batch); ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$.

	Locus	N_n	HetExp	HetObs	Allele sizes (bp)
Arizona	Scu140	55	41.67	37	*78*, *92*, 95, 96, 98, 100, 102, 106, 108, 110, 112, *114*, 116, *118*, 126
	Scu26	55	42.00	42	*167*, *175*, *183*, *185*, *199*, 265, 267, 269, *271*, 273, 275
	Scu11	56	47.50	48	70, 82, *94*, *98*, 106, *108*, *114*, *118*, 120, *122*, 124, 126, *128*, *132*, *134*
	Scu07	56	39.32	39	*166*, *172*, *176*, *188*, 190, *192*, 194, 196, 198, 200
	Scu05***	54	48.60	42	163, *165*, *167*, 179, 183, *187*, *215*, *223*, *225*, *229*, 233, 235, 237, 239, 241, *243*, *245*
	Scu01	54	44.79	44	*141*, *144*, 145, 149, *151*, *152*, *154*, *156*, 158, *160*
New Mexico	Scu140	20	18.26	16	95, 96, 98, 100, 102, *104*, 106, 108, 110, 112, 116, *120*, 126
	Scu26	22	14.91	13	265, 267, 269, 273, 275
	Scu11	21	17.32	19	*66*, *68*, 70, 82, *100*, 106, 120, 124, 126
	Scu07	19	13.22	15	*184*, 190, 194, 196, 198, 200, *202*
	Scu05	19	15.27	14	163, *177*, 179, *181*, 183, 233, 235, 237, 239, 241
	Scu01*	20	10.64	7	*133*, *137*, 145, 149, 158, *164*, *166*

= 0.006, 95% CI: −0.004–0.016) and the deviation from Hardy-Weinberg equilibrium within bajadas was nonsignificant ($f = 0.043$, 95% CI: −0.006–0.091). Correcting genotype data for positive bias induced by the potential presence of null alleles did not have a substantial impact on estimates of θ among bajadas (before and after correction, $\theta = 0.006$).

When multilocus genotype data from population samples in Arizona and New Mexico were combined and evaluated using STRUCTURE 2.1, all model combinations indicated $K = 2$. For the model based on admixture with correlated allele frequencies and the model based on no admixture with correlated allele frequencies, the maximum average estimate was for $K = 2$. For the model based on no admixture and uncorrelated allele frequencies, the likelihood function plateaued for $K > 2$, and the delta K statistic (based on the maximum rate of change in the log probability of data between successive K-values) was used to determine K (Evanno et al., 2005). Delta K measured 125.8, 4.4, 15.7, and 9.4 for $K = 2$, 3, 4, and 5, respectively, indicating that $K = 2$. For all model combinations, most individuals were assigned strongly to one of the two clusters when $K = 2$, and cluster membership corresponded closely with population of origin. Subsequent runs conducted on each population (i.e., Arizona or New Mexico) failed to detect substructure. Likewise, runs conducted on the Arizona population using BAPS 5 indicated that bajada samples were the same deme.

A hierarchical analysis of variance with combined genotype data for the Eastern Massasauga Rattlesnake indicated that most (73%) molecular variance was attributable to individual populations. Variation between the two subspecies accounted for 15% of the total variance ($F_{CT} = 0.147$, $P < 0.0001$), and variation among populations within subspecies accounting for the remaining 12% ($F_{SC} = 0.143$, $P < 0.0001$).

Table 2. Comparison of Allelic Richness (a_g) and Private Allelic Richness (π_g) between Sample Populations of the Massasauga Rattlesnake in Arizona and New Mexico as Calculated by HP-Rare (Kalinowski, 2005).

	Arizona		New Mexico	
Locus	a_g	π_g	a_g	π_g
Scu01	8.3	5.3	6.9	3.9
Scu05	14.0	7.6	11.0	4.6
Scu07	8.3	4.5	7.0	3.2
Scu11	11.4	6.6	8.9	4.1
Scu26	8.6	4.3	4.9	0.6
Scu140	11.1	2.8	12.9	4.6

DISCUSSION

Genetic diversity.—The higher proportion of unique alleles in the Arizona population may be partly accounted for by disparity in sample size. However, trends in the number of alleles per locus remained higher in Arizona at five of six loci despite correction for sample size, suggesting that allelic richness may indeed be higher in this population.

Interestingly, allelic richness and gene diversity in both populations were higher than that reported for many threatened taxa (Frankham et al., 2002), including several montane rattlesnake species from the desert southwest (Holycross, 2002; Holycross and Douglas, 2007). Such high

Table 3. Weir and Cockerham F-Statistics and Queller and Goodnight's Version of Hamilton's Relatedness Coefficient (R) for Populations of the Massasauga Rattlesnake in Arizona and New Mexico. Confidence intervals (95%) were obtained with the computer program FSTAT (Goudet, 1995) by bootstrapping over loci; L = lower limit, U = upper limit, avg = mean; $F \approx F_{IT}$, $\theta \approx F_{ST}$, $f \approx F_{IS}$. The sample from Arizona was divided into three subsamples based on association with particular cinder cone bajadas (see Fig. 2).

		F	θ	*f*	R
AZ and NM	L	0.102	0.072	−0.002	0.130
	U	0.233	0.180	0.100	0.296
	avg	0.168	0.126	0.049	0.296
AZ	L	0.005	−0.004	−0.006	−0.008
	U	0.096	0.016	0.091	0.031
	avg	0.051	0.006	0.043	0.012

Table 4. Statistical Significance of Hardy-Weinberg Exact Tests (U test, H1 = heterozygote deficit) for Five Populations of the Eastern Massasauga Rattlesnake Examined by Gibbs et al. (1997). *P*-values were estimated in GENEPOP (Raymond and Rousset, 1995) with the Markov chain method (100 batches with 1000 iterations per batch). ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$.

Locus	BPNP	OHIO	CNY	GBINP	KPP
Scu01	*	***	**	***	***
Scu05	***	*	ns	ns	***
Scu07	***	**	**	*	***
Scu11	ns	ns	ns	***	***
Scu16	ns	***	*	ns	**
Scu26	ns	*	ns	**	***

variation could indicate that the historical effective population size was quite large for these populations and may have only been recently impacted by natural and/or anthropogenic habitat disturbance (Cornuet and Luikart, 1996).

Allelic richness and gene diversity were comparable to that reported for the Eastern Massasauga Rattlesnake by Gibbs et al. (1997), but for several loci the number of alleles was highest in the Desert Massasauga Rattlesnake in Arizona. While gene diversity was relatively high for both subspecies, significant heterozygote deficiencies were much more widespread for the Eastern Massasauga Rattlesnake. Gibbs et al. (1997) hypothesized that heterozygote deficiencies were probably due to null alleles combined with a Wahlund effect (Wahlund, 1928) associated with previously unrecognized structure within each population sample. While heterozygote deficiencies at some loci may be explained by the existence of non-amplifying alleles, this phenomenon is unlikely to explain the high percentage of heterozygote deficiencies across loci. On the other hand, widespread heterozygote deficiencies observed in the Eastern Massasauga Rattlesnake could reflect limited natal dispersal (Jellen and Kowalski, 2007), fidelity to hibernation areas (Harvey and Weatherhead, 2006; Marshall et al., 2006), increased inbreeding associated with declining census size (Gibbs et al., 1997), or colonization history (Boileau et al., 1992).

Population differentiation.—High proportions of private alleles, divergent allele frequencies, high F_{ST} values, and significant genetic clustering all suggest substantial divergence between populations from Arizona and New Mexico. It is unlikely that recolonizations or migration will ameliorate severe population reductions and extinctions, as these would require expansion of range and concomitant changes in climate and biotic communities. While natural recolonization is unlikely, experimental or restorative translocation of animals (i.e., assisted migration; Willis et al., 2009) should be considered with caution, since these two populations occur in different habitat settings and individual populations appear to be genetically distinct (Hedrick, 1995, 2005).

Within the Arizona population, results suggest that individuals from different bajadas are from the same deme or genetic neighborhood (Wright, 1969). Bajadas contained a high percentage of unique alleles, but sample sizes may not have been large enough to detect rare alleles, given the high level of genetic diversity observed. Non-significant F_{ST} and genetic clustering suggest that differentiation among bajadas (as detected by contingency tests) is weak and probably does not reflect subdivision due to genetic drift among bajadas.

While study results did not reveal significant subdivision between bajadas, study results underscore the importance of bajadas (and associated tobosa grassland habitat) to this population (Fig. 2), and strongly suggest that efforts should be made to minimize road mortality along these sections of the highway, specifically including construction of permanent barrier walls and safe crossing culverts (Dodd et al., 2004). These sections of the highway should also be monitored closely by wildlife officers to prevent continued illegal collection. Some form of population viability analysis (Beissinger, 2002) would also be useful in determining whether this population is sustainable given the high rate of mortality associated with vehicular traffic (48% of all captures were found dead on the road).

Conclusions.—Despite presumed declines in census size, high levels of genetic diversity were found in populations of the Desert Massasauga Rattlesnake in Arizona and New Mexico, indicating that the evolutionary potential of these populations remains high. Establishment of formal monitoring programs would be useful for determining species occupancy and testing hypotheses about local extinction (Solow, 2005; MacKenzie et al., 2006). Formal monitoring would also permit screening of genetic diversity over time, allowing estimation of important parameters such as effective population size (Waples, 1989). Further genetic screening with additional loci could also be used to better evaluate demographic phenomena such as genetic bottlenecks, while the inclusion of specimens from additional populations would better determine the scale at which fragmented populations are demographically independent. At the present time, our results suggest that populations should be managed as demographically independent units and that each has a high conservation value, encapsulating unique subsets of genetic variation.

MATERIAL EXAMINED

Arizona State University 30148–171, 30577–592, 30621–638, 30877, 30899–0906, 30985–86, 32947, 33151–57, 33260.

ACKNOWLEDGMENTS

We thank the late B. Krentz, the Krentz family, and B. Tomberlin for generous support and allowing us access to their lands and homes. T. Briton, B. Christman, C. Cole, P. Humphrey, B. Fedorko, M. Lee, J. Manzer, C. Painter, R. Reed, L. Smith, and F. Ward provided field assistance, and L. Collins and L. DeSouza assisted with lab work. Financial support for field and lab work was provided by the Wallace Foundation, Arizona Game and Fish Department (AGFD) Heritage Fund (I93043 and I97019), AGFD Nongame Branch, and the U.S. Forest Service Rocky Mountain Research Station. Specimens were collected under scientific permits (AGFD: HLYCR000038, SP605602, SP646832, SP711300. SP779370, SP841338; New Mexico Department of Game and Fish: 2834). The ASU Institutional Animal Care and Use Committees approved handling and marking methods (ASU: 93-280R).

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