Genetic Diversity and Population Structure of Armenian Vipers, *Montivipera raddei*, in Two Landscapes: Implications for Conservation

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Abstract.—Armenian Vipers, *Montivipera raddei*, have a fragmented distribution in portions of eastern Turkey, Azerbaijan, Armenia, and northwestern Iran. They are restricted to mountain habitat above 1300 m and have allopatric populations resulting from climatic oscillations of the Plio-Pleistocene periods. Anthropogenic landscape changes and over-collection for the pet trade have resulted in dramatic population declines over the past four decades. While we have some insights into spatial ecology and habitat use of this species, we know nothing about their population structure. We examined the genetic diversity and population structure of Armenian Vipers inhabiting an agricultural landscape and a recovered-natural landscape separated by 397 km. We used seven microsatellite loci to genotype 63 individuals representing two sampling locations in each of the two sites. There were no indications of population bottlenecks within any of the sampling locations. While we found evidence of inbreeding at one of the locations in the agricultural landscape, the F_{ST} value indicates that individuals can still make contact with one another. We found no significant differentiation between sampling locations at the local scale (F_{ST} values of 0.03 to 0.006), but highly significant differentiation between the geographically separated populations (F_{ST} ranged from 0.14 to 0.20). The Bayesian clustering algorithm STRUCTURE also identified two distinct population clusters, one consisting of the two agricultural sites and the other the two recovered-natural sites. Conservation efforts should focus on maintaining high quality habitat corridors that allow for gene flow and the management of the geographically separated populations as independent genetic units.

Key Words.—agricultural landscape; geographically separated populations; microsatellites; recovered-natural landscape

Introduction

The survival of species depends on access to suitable habitat, and habitat loss is considered to be a major contributing factor in species loss/extinction around the world (Fahrig 1997; Thomas et al. 2004). An associated concern in conservation biology is habitat fragmentation (Meffe and Carroll 1997), a landscape-scale process that involves both the loss and subdivision of habitat (Fahrig 2003). There is inherent patchiness in nature (landscape heterogeneity) that results from ecological and geological processes, as well as spatial and temporal variation in the abundance and distribution of resources (Wiens 1997). However, in conservation biology habitat fragmentation typically focuses on anthropogenic landscape heterogeneity and the associated patterns and outcomes (Collinge 2009). Human-fragmented landscapes most likely have altered functional qualities, such as reduced connectivity or greater edge effects, due to differences in landscape structure and their contrast with adjoining habitat types (Forman 1995; Collinge 2009). Examining the genetic structure of a population in relation to landscape structure is a particularly powerful method to look at the impact of fragmentation on movement of individuals within a population (Storfer et al. 2007).

The degree of genetic structure at large spatial scales is often much higher for reptiles, such as snakes, than for most birds and mammals (Ward et al. 1993). Beyond the inherent differences in mobility, snakes require hibernacula and thermoregulation microhabitats that may be heterogeneously distributed in the landscape and this can contribute to restricted gene flow even at local scales (Reinert 1993). Genetic analyses may provide the best approach for assessing whether these ecological factors have impacted dispersal and subsequent mating behaviors (Gibbs and Weatherhead 2001).

Eastern Massasauga Rattlesnakes (*Sistrurus c. catenatus*; Gibbs et al. 1997) and Adders (*Vipera berus*; Ursenbacher et al. 2009) both showed significant differentiation between geographically separated populations as well as between neighboring populations (separated < 3.5 km) where landscape structure did not impede dispersal. These studies suggested that there is very limited dispersal, minimal mate-searching behavior, or both. By contrast, Timber Rattlesnakes (*Crotalus*

FIGURE 1. Map depicting the fragmented distribution of *Montivipera raddei* in Armenia. The two study site populations (Abovian: Study Site 1 and Shikahogh: Study Site 2) from the current study have been identified on the map. (The map was used with permission from Aghasyan and Aghasyan 2014).

horridus) showed only modest differentiation (average $F_{ST} = 0.02$) between neighboring populations, indicating that there is regular gene flow between them (Clark et al. 2008). There was also a significant correlation between genetic differentiation and the availability of thermoregulation sites, suggesting that gene flow between adjacent populations may be increased through shared basking sites providing males with easy access to females during the breeding season (Bushar et al. 1998; Clark et al. 2008). Similar patterns of either significant or modest genetic differentiation at the local level have been noted in other reptiles, including Blue Mountain Water Skinks (*Eulamprus leuraensis*; Dubey and Shine 2010) and Ornate Box Turtles (*Terrapene ornata ornata*; Richtsmeier et al. 2008), respectively. The results of all the aforementioned studies underscore the importance of integrating landscape features and individual behaviors into population genetic analyses (Clark et al. 2008).

The Armenian Viper (*Montivipera raddei*) has a fragmented distribution that includes eastern Turkey, Armenia (Fig.1), Azerbaijan, and northwestern Iran (Nilson and Andrén 1986). Gene flow and range expansion are restricted due to their allopatric populations in isolated mountain habitat above 1300 m and unsuitable habitat in the intervening areas (Nilson and Andrén 1986; Stümpel et al. 2016). Habitat alteration due to land conversion for agricultural croplands and livestock overgrazing and overexploitation of populations for the pet trade are the major threats impacting Armenian Viper populations (International Union for the Conservation of Nature [IUCN] 2009. The IUCN Red List of Threatened Species 2009: Available at http://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T22993A9406370.en. [Accessed 11 October 2015]). Over the past 40 y there has been a steady decline in population numbers: 20–50 specimens/ha in the 1960s to current estimates of 4–10 specimens/ha (Darevsky 1966; Mallow et al. 2003; IUCN. 2009. *op. cit*.). As a result of this decline the Republic of Armenia has listed the species as Vulnerable (Aghasyan and Kalashyan 2010) and IUCN has listed it as Near Threatened (IUCN. 2009. *op. cit*.).

To date, genetic studies have focused on the phylogenetic relationships of the nine species comprising *Montivipera* and the taxonomic position of the genus
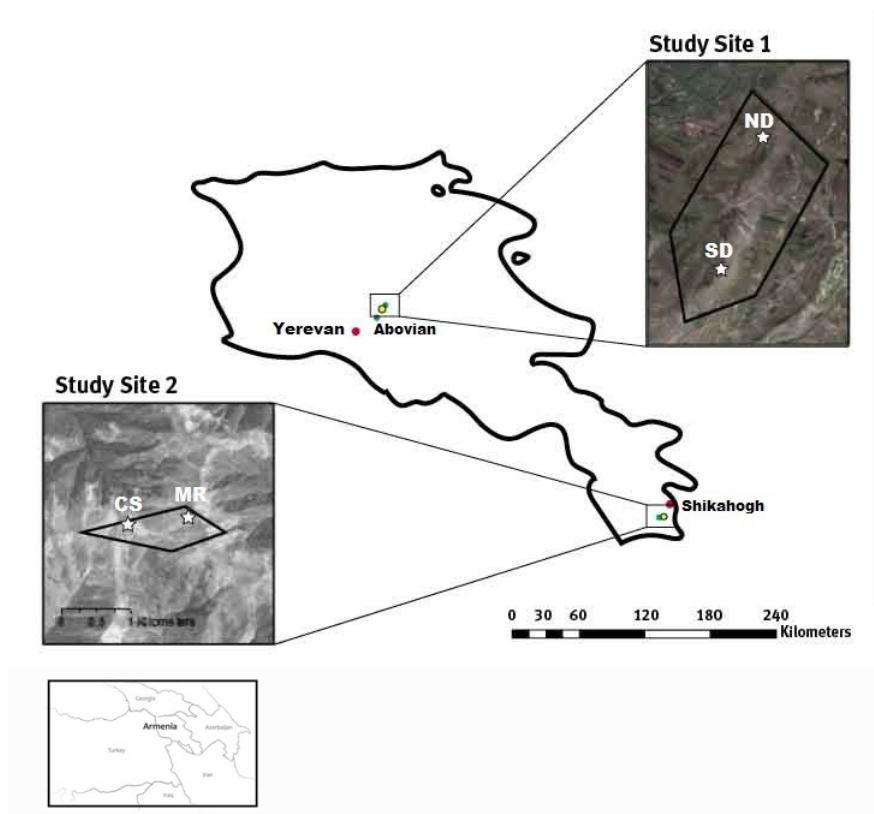
FIGURE 2. Map of Armenia with locations of two study sites. The "stars" on study site maps denote sampling locations. Study Site 1 = Abovian (human-modified landscape) and Study Site 2 = Shikahogh (recovered-natural landscape). Inset map showing Armenia in relation to the surrounding countries in western Asia. SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite.

within Viperidae (Nilson et al. 1999; Lenk et al. 2001; Stümpel et al. 2016). No studies have examined the population structure of the Armenian Viper or any of the other *Montivipera* species. The objectives of this study were to quantify the genetic diversity of Armenian Vipers from sampling sites in two different landscapes (human-modified versus recovered-natural), examine the extent of structure within and between these populations, and delineate whether there were specific genetic units that require conservation efforts. While our radiotelemetry data showed movement between sampling locations in both landscapes (Ettling 2013; Ettling et al. 2016), we were interested to see if the population inhabiting the human-modified landscape showed any signs of inbreeding due to the effects of overgrazing and conversion of steppe habitat to agricultural crops. Based on the radiotelemetry data we predicted that there would be no structure among the two sampling locations within either of the two study sites. Recent data (Yousefi et al. 2015) suggests that the last Pleistocene glaciation 21,000 y ago shaped the fragmented highland distribution of *Montivipera raddei* we see today. Their current distribution has been restricted further by human-mediated habitat alterations. Due to the long-term isolation of the allopatric

populations of *M. raddei* (Yousefi et al. 2015; Stümpel et al. 2016) we also predicted that there would be strong genetic differentiation between the geographically separated Abovian and Shikahogh populations.

MATERIALS AND METHODS

Study sites and sample collection.—Our study was conducted at two sites with different landscape characteristics (Fig. 2). The distance between the two sites was 397 km. We collected genetic samples from two locations at both sites. We attempted to find sampling locations that were equidistant at both sites, but were unsuccessful. The first study site was located 23 km northeast of Yerevan, Armenia in Kotyak Province near the town of Abovian. The site has been subjected to considerable human alteration and is comprised of a mosaic of agricultural fields and remnant mountain steppe habitat (Fig. 3). The remaining tracts of mountain steppe have been heavily impacted by livestock overgrazing (Ettling et al. 2013). Mountain steppe occurs at elevations between 1,200–2,200 m with rocky outcrops interspersed with grasses and shrubs (Adamian and Klem 1997). The distance between the two Abovian sampling localities was 3.2 km.

FIGURE 3. Study Site 1 is human-modified landscape near the town of Abovian (left). It is comprised of a mosaic of agricultural fields and remnant mountain steppe habitat. The steppe habitat is overgrazed by livestock and farming roads crisscross the landscape. The location of the South Den is identified on the photograph. Study Site 2 is a recovered-natural landscape comprised of mountain steppe/meadow vegetation (right). The site is located in Shikahogh State Reserve and has been off limits to public use since 2006. (Photographed by Jeffery A. Ettling).

The second study site was located 52 km southeast of Shikahogh village, Syunik Province on Meghri Ridge in Shikahogh State Reserve. Meghri Ridge has an elevation of 2,200+ m and is classified as high mountain steppe/meadow (Fig. 3). Rocky outcrops are scattered along the ridgelines. Grasses and shrubs are the common ground cover with oaks (*Quercus* spp.), European Ash (*Fraxinus excelsior*) and Caucasian Hornbeam (*Amygdalus fenzeliana*) in the valleys (Adamian and Klem 1997; Aivazyan 2006). Grazing practices on Meghri Ridge were halted in 2006 which has allowed the plants to grow taller and denser (Aram Aghasyan, pers. comm.) and for unimpeded movement by the vipers. The two Shikahogh sampling localities were separated by a distance of 0.8 km.

We collected 12–18 genetic samples from each of two locations within both study sites over a 5-y period (June 2006, May-June 2009, October 2009, June-August 2010, June 2011, and September-October 2011) representing 63 individuals. The sampling locations were labeled as North Den and South Den at Abovian and as Meghri Ridge and Campsite at Shikahogh State Reserve. We captured snakes by hand using snake hooks and tongs. We used clear acrylic tubes to restrain the snakes during data collection. We collected 30–50 µL of blood from the caudal vein of each snake using an insulin syringe and preserved it in 500–700 µL of lysis buffer (Longmire et al. 1998). We then took snout-vent length (SVL) and tail length (TL) measurements to the nearest 0.5 cm. Body mass was recorded to the nearest 0.5 g. We implanted passive integrated transponders (PIT) tags (Avid Identification Systems, Inc., Norco, California, USA) to permanently mark snakes.

Microsatellite genotyping.—We added proteinase K to all blood samples prior to incubation overnight and DNA was extracted using standard phenol/chloroform procedures (Sambrook et al., 1989) followed by dialysis in 1×TNE2 (10 mM Tris-HCl, 10 mM NaCl, 2 mM EDTA). We estimated the concentration of DNA in each sample using a spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) and adjusted them to 20 ng/µl working concentrations for use in polymerase chain reactions (PCR). We screened 13 microsatellite loci; 10 were polymorphic, and amplified seven in 63 individuals. The microsatellites were isolated from *Montivipera raddei* samples at the laboratory of Travis Glenn located at the Savannah River Ecology Laboratory (Aiken, South Carolina, USA) and primer pairs were designed. We amplified loci MoRa02, MoRa03, MoRa05, MoRa06, MoRa17, MoRa18, and MoRa21 using a My Cycler thermal cycler (Bio Rad) using the following method: PCR reaction conditions (12.5 µl) contained 10 mM Tris pH 8.4, 50.0 mM KCL, 25.0 µg/ml BSA, 0.4 µM unlabeled primer, 0.08 µM tag labeled primer, 0.36 µM universal dye-labeled primer, 2.0 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma-Aldrich, St. Louis, Missouri, USA), and 20-40 ng DNA template. Amplification of PCR products used the touchdown thermal cycling program (Don et al. 1991). The protocol was as follows: the touchdown cycles were 20 cycles of 95° C for 20 s, 55° C (decreased 0.5° C per cycle) for 20 s, 72° C for 30 s; 20 cycles of 95° C for 20 s, lowest annealing temperature for 20 s, and 72° C for 30 s followed by 7–10 min final extension. An Applied Biosystems (ABI) 3130xl sequencer was used to resolve

TABLE 1. Microsatellite characteristics in Armenian Vipers (*Montivipera raddei*) estimated from seven microsatellite loci. Site abbreviations are SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite.

Locus	Size Range (bp)	No. Alleles	Number of Alleles Per Site			
			SD	ND	MR	CS
MoRa02	249–269	9	6	4	4	5
MoRa03	216–248	10	9	7	4	5
MoRa05	165–197	13	5	10	5	7
MoRa06	268–276	15	6	8	11	9
MoRa17	261–273	10	8	4	4	6
MoRa18	179–232	10	9	6	7	5
MoRa21	230–288	18	10	9	10	9

the PCR products for all seven loci and GeneMapper (ABI) software version 4.01 was used to score allele sizes.

Statistical analyses.—We used ARLEQUIN 3.5 (Excoffier and Lischer 2010) to evaluate microsatellite genotypes for deviations from Hardy-Weinberg Equilibrium (HWE) within each of the four sample locations. A Markov Chain Monte Carlo (MCMC) algorithm, similar to Fisher's exact test but utilizing a contingency table of arbitrary size, is used by ARLEQUIN to calculate P values. For this analysis we used a chain length of 1,000,000 with 100,000 dememorization steps. We used GENEPOP 4.0.10 (Raymond and Rousset 1995) to evaluate linkage disequilibrium between loci pairs with Markov chain parameters of 1,000 dememorization steps, 100 batches, and 1000 iterations per batch.

We evaluated the genetic diversity within each of the sample locations in a number of ways. We calculated mean expected (H_E) and observed (H_O) heterozygosities using ARLEQUIN. We used FSTAT 2.9.3.2 (Goudet 1995) to calculate fixation indices (F_{IS}). The significance of the deviations of F_{IS} values from zero were assessed using 95% confidence intervals generated through bootstrapping (1,000 replications). We used HP-RARE 1.0 (Kalinowski 2005) to calculate the mean number of alleles across all seven loci in each population as well as both total and private allelic richness. HP-RARE uses a rarefaction method to accommodate for sample size differences when calculating allelic richness. Using rarefaction our sample size was standardized to 18 per location. To test for null alleles at each locus as well as to look for evidence of scoring errors and large allele dropout we used MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). We evaluated recent reductions in effective population size using the program BOTTLENECK vl.2.02 (Piry et al. 1999) which tests for excess heterozygosity. To ascertain whether the number of loci with excess heterozygotes in a given

population were significant, we used the Wilcoxon's sign rank test under the two-phase model (TPM) with the variance among multiple steps set at 12 and single-step mutations set at 90%. Evidence that a bottleneck had occurred within a given location was based on the Bonferroni corrected P value of 0.002.

We used an analysis of molecular variation (AMOVA) in ARLEQUIN to examine genetic structure within and among populations. We were particularly interested in evaluating what impact agricultural practices may have on movements between sampling locations within the human-modified landscape compared to movements between sampling locations within the seemingly uninterrupted natural landscape. We made pairwise F_{ST} comparisons between all pairs of sample locations.

We also used STRUCTURE 2.3.3 (Pritchard et al. 2000) as an alternate means of examining genetic structure. STRUCTURE uses a Bayesian algorithm to cluster genetically distinct groups with or without *a priori* knowledge of the geographical location where the samples were collected. We selected to use the *a priori* setting (LOCPRIOR) because of its ability to recognize weak genetic structure and its sensitivity to detecting samples that truly have structure (Hubisz et al. 2009). We set the predicted number of populations (K) at 1-6 (two more than the number of sample locations). We ran 10 replicate runs for each K from one to six with 500,000 MCMC iterations following a burn-in of 50,000 iterations using the admixture model with correlated alleles and the LOCPRIOR setting. We used the delta K method (Evanno et al. 2005) to select the optimum number of genetic clusters from our dataset. To calculate probabilities for individual assignments to a given reference population or alternatively to the population where the data were collected, we used GENECLASS2 (Piry et al. 2004) with a Bayesian framework (Rannala and Mountain 1997) and $P < 0.05$ assignment threshold. Due to the multiple comparisons that were made, we employed a Bonferroni correction to reduce the chance of committing a Type I error (Lesack and Naugler 2011).

RESULTS

Alleles per locus ranged from 9–18 across all four sample locations (Table 1). Shared alleles between study sites ranged from 2–4 per locus (Table 2). Allelic richness was similar among the four sample locations and ranged from 5.43 in Campsite to 6.48 in North Den. Private alleles ranged from 0.68 in Meghri Ridge to 1.22 in South Den (Table 3).

Expected heterozygosities ranged from 0.69 (Campsite) to 0.81 (North Den), with an average of 0.76 across all four sample locations (Table 3). Five of 28 (17.9%) locus-population comparisons deviated

TABLE 2. Alleles shared between study sites. Study Site 1 = South Den and North Den. Study Site 2 = Meghri Ridge and Campsite.

MoRa02

	249	253	257	261	262	265	266	267	269
South Den	249	253	257	261	262		266		
North Den	249	253	257	261					
Meghri Ridge			257	261		265			269
Campsite			257	261		265		267	269

MoRa03

	211	215	219	223	227	231	235	239	243	247
South Den	211	215	219	223	227	231	235	239	243	
North Den	211	215	219	223	227	231	235			
Meghri Ridge				223		231	235	239		
Campsite						231	235	239	243	247

MoRa05

	173	177	180	181	184	185	188	189	192	193	196	197	200
South Den			180		184		188		192		196		
North Den		177	180		184	185	188	189	192		196	197	200
Meghri Ridge		177				185		189		193		197	
Campsite	173	177		181				189		193	196	197	

MoRa06

	257	258	261	262	265	266	268	270	272	273	274	276	278	280	284
South Den		258					268		272		274	276		280	
North Den	257	258	261		265		268		272			276	278		
Meghri Ridge	257	258	261	262	265	266	268	270	272	273		276			
Campsite	257	258	261	262	265		268		272	273					284

MoRa17

	262	266	267	268	270	271	274	275	278	279
South Den	262	266	267		270	271		275	278	279
North Den		266		268	270					279
Meghri Ridge	262				270		274		278	
Campsite	262	266			270		274	275	278	

MoRa18

	198	202	206	210	214	215	218	222	226	238
South Den		202	206	210	214	215	218	222	226	238
North Den	198	202	206	210	214			222		
Meghri Ridge		202	206	210	214		218	222	226	
Campsite		202	206	210	214		218			

MoRa21

	229	250	254	258	259	262	263	266	270	271	274	275	278	279	282	283	286	287
South Den		250	254	258		262		266	270		274		278		282			287
North Den		250	254	258		262			270		274		278		282		286	
Meghri Ridge	229				259		263		270	271	274	275	278	279	282			
Campsite							263	266	270	271		275	278	279	282	283		

from Hardy-Weinberg equilibrium (HWE) following a Bonferroni corrected P value of 0.002 (Table 3). South Den had two loci not in equilibrium (MoRa06, MoRa17). North Den had a single locus (MoRa17) not in equilibrium. Meghri Ridge and Campsite each had one locus not in equilibrium (MoRa06). MICRO-CHECKER did not reveal any evidence of large allele dropout or scoring errors, but did suggest that MoRa17 had null alleles. We calculated the genetic distances and structure analysis with and without loci MoRa06 and MoRa17 and did not detect any discernable difference in the results, and therefore we included all seven loci. We only found evidence of linkage disequilibrium between one pair of loci (MoRa06 and MoRa17) in one location (Abovian–South Den) following a Bonferroni corrected P value < 0.001.

The inbreeding coefficient (F_{IS}) was calculated for each locus and revealed that over half (16/28 = 57%) of the F_{IS} values were greater than zero (Table 3). However, when all seven loci were pooled for each sampling location the overall F_{IS} value was significantly different from zero for only one of the four locations. These data

TABLE 3. Genetic diversity measures of four sample sites of Armenian Vipers (*Montivipera raddei*) estimated from seven microsatellite loci. Abbreviations are SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite; A = mean number of alleles per population; H_o = mean observed heterozygosity; H_E = mean expected heterozygosity; Ar = mean allelic richness; Ap = mean private allelic richness; Significant F_{IS} values and *P* value show in bold using 95% confidence intervals.

Site	Sample Size	A	H_O	H_E	Ar	Ap	F_{IS}	*P* value
SD	17	7.57	0.64	0.80	6.46	1.22	0.20	0.410
ND	12	6.86	0.74	0.81	6.48	0.99	0.10	0.004
MR	16	6.43	0.66	0.74	5.53	0.68	0.11	0.150
CS	18	6.71	0.69	0.69	5.43	0.74	-0.002	0.990

suggest that nonrandom mating is occurring within the Abovian South Den site. A excess of heterozygotes was detected under the BOTTLENECK TPM for the North Den site, but it was not significant (Table 3). The results for the other three sampling locations were also non-significant (Table 3).

We found strong support for our hypothesis that there would be strong genetic differentiation between the geographically separated Abovian and Shikahogh populations. Pairwise F_{ST} tests showed significant differentiation between four pairs following a Bonferroni corrected *P* value of 0.008 (Table 4). The Abovian sampling locations (North Den and South Den) differed significantly from the Shikahogh sampling locations (Meghri Ridge and Campsite). The pairwise F_{ST} values were: North Den/Meghri Ridge = 0.14, North Den/Campsite = 0.16, South Den/Meghri Ridge = 0.17, and South Den/Campsite = 0.20.

We also found strong support for our hypothesis that there would be no structure between the two sampling locations within either population. The Evanno et al. (2005) delta K method identified two distinct population clusters (Fig. 4). One cluster consisted of the two Abovian sampling locations and the second cluster consisted of the two Shikahogh sampling locations (Fig. 5). The assignment values were high for each cluster, with scores averaging 0.99 for each site. The two clusters identified by STRUCTURE were confirmed by the results of the AMOVA tests. Within population variation explained 88% (*P* < 0.001) of the molecular variation and the remaining 12% (*P* < 0.001) was accounted for by among population variation.
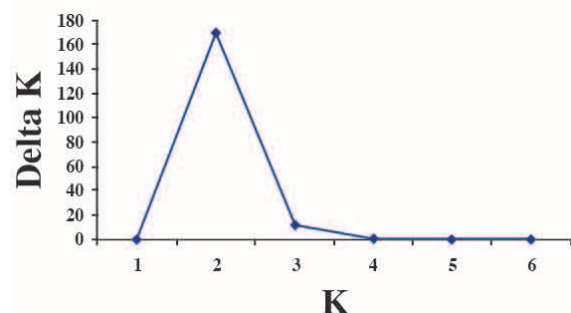
FIGURE 4. Plot of ΔK for the *Montivipera raddei* microsatellite data.

The probability scores for individual assignment using GENECLASS2 ranged from 56.5 to 100.0%, with a mean score of 90.5% (Table 5). Three of the 17 South Den (17.6%) and three of the 12 North Den (25.0 %) samples were misassigned. Combined there were 20.7% sample misassignments between the two Abovian sampling locations. For Meghri Ridge and Campsite, seven of the 16 samples (43.8%) and 10 of 18 samples (55.5%) were misassigned, respectively. Overall, 50.0% of the samples for the Shikahogh study site were misassigned. The high proportion of misassignments suggests a lack of subdivision and corroborates the genetic clusters indicated by both the AMOVA and STRUCTURE analyses.

DISCUSSION

Armenian Vipers have a fragmented distribution that was shaped by historical and environmental events/changes (Nilson and Andrén 1986; Yousefi et al. 2015; Stümpel et al. 2016). As noted earlier these allopatric populations have been severely impacted in recent years by anthropogenic activities including agricultural practices and over-collection for the pet trade (IUCN. 2009. *op. cit.*). Higher levels of allelic variation may suggest that the effective population size was historically large and has only recently undergone a population decline due to anthropogenic influences (Cornuet and Luikart 1996). The historical and current population estimates (Darevsky 1966; Mallow et al. 2003; IUCN. 2009. *op. cit.*) support this interpretation. Anderson et al. (2009) noted that Desert Massasauga Rattlesnakes (*Sistrurus catenatus edwardsii*) had higher gene diversity and allelic richness than other species

TABLE 4. Pairwise population F_{ST} comparisons among sample sites. SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite. Values in bold represent significant differences with a Bonferroni correction value of 0.008.

	SD	ND	MR	CS
SD	—			
ND	0.0281	—		
MR	**0.1682**	**0.1363**	—	
CS	**0.1971**	**0.1623**	0.0059	—

Figure 5. Results of genotype analysis in STRUCTURE 2.3.2. The chart depicts the observed population structure of *Montivipera raddei* within the four sampling locations.

of rattlesnakes from the region and attributed it to this same principle.

F_{IS} values greater than zero were exhibited by the South Den location at Abovian. King (2009) reviewed the results of 25 microsatellite studies of snakes comprising four families (Colubridae, Elapidae, Viperidae, and Boidae) and discovered that only one had an F_{IS} value less than zero, suggesting that inbreeding is common among snake populations. Whether this is typical among all snakes is unknown due to the lack of historical population data for most species. The mean F_{IS} value for the South Den (0.20) is considerably higher than that of either Adders (0.04; Ursenbacher et al. 2009) or Orsini's Vipers (0.04; Ferchaud et al. 2011). Although positive F_{IS} values can be indicative of inbreeding, they can also be caused by null alleles (Brookfield 1996) or unrecognized fine scale structure (Hartl and Clark 1987). Although MICROCHECKER suggested that one null (MoRa17) was present, the F_{IS} values did not vary significantly with or without its inclusion. Non-significant pairwise F_{ST} values between the North Den and South Den (0.03) were not indicative of any microgeographic fine scale genetic structuring. There were also no differences in allele frequencies between Abovian sample locations. Based on these results the positive F_{IS} values appear to be associated with inbreeding. However, despite anthropogenic landscape changes, over-collection pressure, and signs of inbreeding, our radiotelemetry data (Ettling 2013; Ettling et al. 2013) indicate that individuals from the

Table 5. Results of population assignment tests in GENECLASS2. SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite.

Source Population	Assigned Population			
	SD	ND	MR	CS
SD	14	3		
ND	3	9		
MR			9	7
CS			10	8

North and South Dens at Abovian are still able to make contact with one another.

Armenian Viper populations in this study exhibited non-significant differentiation at the local regional scale, but significant levels of differentiation at the larger range-wide scale. While it may not seem surprising that there is significant genetic differentiation between populations separated by hundreds of kilometers, large distance between populations does not always equate to significant genetic differentiation in all snake species. Two populations of the Argentine Boa Constrictor (*Boa constrictor occidentalis*) in Argentina separated by a distance of 200 km had an F_{ST} value of less than 0.01 (Rivera et al. 2005; Cardozo et al. 2007). These data suggest that the original gene pool was recently fragmented due to human-mediated changes in the landscape including deforestation, overgrazing, and agricultural croplands (Rivera et al. 2005; Cardozo et al. 2007). Conversely, the allopatric populations of the Armenian Viper have been isolated since the last Glacial Maximum 21,000 y ago. Due to this long period of isolation, with limited to no gene flow, it is likely that these populations are now genetically unique (Stümpel et al. 2016).

The New Mexico Ridge-nosed Rattlesnake (*Crotalus willardi obsurus*) also has a fragmented distribution on mountain ranges in southwestern New Mexico, southeastern Arizona, and adjacent north-central Mexico (Holycross and Douglas 2007). This population exhibits similar patterns of genetic differentiation as noted for Armenian Vipers, with non-significant F_{ST} values at the local scale and highly divergent values ($F_{ST} = 0.16$) at range-wide scales (Holycross and Douglas 2007). Although Timber Rattlesnakes (*Crotalus horridus*) show high philopatry to their natal den sites, as well as limited dispersal, hibernacula separated at distances ranging from 2–8 km show little differentiation. Gene flow between widely separated hibernacula appears to be mediated through a combination of suitable corridors and thermoregulation sites (Clark et al. 2008). Male Timber Rattlesnakes typically have larger home ranges

and make larger movements searching for mates. Our radiotelemetry data demonstrate that both sexes in Armenian Vipers have similar home range size and that females will also make large movements during the spring breeding season (Ettling et al. 2013; Ettling et al. 2016). The aforementioned rattlesnake studies, together with our data, support the hypothesis that while temperate viper species exhibit high levels of philopatry and limited migration potential, uninterrupted habitat is vital to population connectivity and gene flow (Holycross and Douglas 2007; Clark et al. 2008; Ettling et al. 2013; Ettling et al. 2016).

Conservation implications.—The results of this study have identified two discrete populations that show high levels of genetic divergence. Because of the long-term geographic isolation and lack of gene flow between Abovian and Shikahogh we recommend that these two populations be managed separately in an effort to preserve their respective genetic uniqueness. Based on our data and that of Stümpel et al. (2016) it is likely that the other isolated populations of Armenian Viper within the country are also significantly differentiated; however, further research will be required to check this assumption. The intervening areas between isolated populations of Armenian Viper are in most cases comprised of unsuitable habitat (i.e., semi-desert) that has restricted gene flow and range expansion (Nilson and Andrén 1986; Stümpel et al. 2016). Our microsatellite data support the results of our radiotelemetry studies (Ettling 2013; Ettling et al. 2013), and indicate that individuals from dens 3.2 km apart can still make contact with one another despite considerable human-mediated landscape alterations (Study Site 1). While direct measures of connectivity between populations provide data on home range size and movements, they do not provide a means of evaluating genetic distinctiveness or lack thereof. Using a combination of connectivity measures such as radiotelemetry and genetic analyses is crucial for making informed management decisions. The focus of conservation management for the Armenian Viper should be on providing protection to regional allopatric populations and the maintenance of habitat corridors to allow for gene flow within each of these populations.

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