5-6-2015

Spatial Ecology, Genetic Diversity and Population Structure of Armenian Vipers, Montivipera raddei, in Two Different Landscapes

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Spatial Ecology, Genetic Diversity and Population Structure of Armenian Vipers, *Montivipera raddei*, in Two Different Landscapes

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A dissertation submitted to the Graduate School of the
University of Missouri – St. Louis
in partial fulfillment of the requirements for the degree
Doctor of Philosophy in Biology
with an emphasis in Ecology, Evolution and Systematics

April 2013

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Dissertation abstract

Habitat fragmentation is a worldwide conservation concern that results in habitat loss and subdivision. Species survival depends upon the availability of suitable habitat, in terms of both quantity and quality. Anthropogenic changes to the landscape not only subdivide habitat, but can also impact the resources that are available to a species. Ultimately these landscape changes can directly influence a species spatial ecology and gene flow. In an effort to gain a better understanding of the impact that human-modified landscapes are having on snake populations, and to develop conservation management strategies to mitigate these changes, I studied the spatial ecology, gene diversity and population structure of the Armenian Viper, *Montivipera raddei* in two different landscapes in Armenia. We first examined the spatial ecology and habitat use of Armenian Vipers in a human-modified landscape with a combination of agricultural fields and overgrazed native steppe habitat. We hypothesized that Armenian Vipers would move more rapidly through croplands compared to steppe and that their respective home ranges would be larger if they included a larger percentage of cropland. We also expected the vipers to prefer steppe habitat to cropland. While there were no differences in movement rates for either sex through croplands compared to steppe, we did find that males had larger home ranges during the spring if it included cropland. While the mosaic of steppe and croplands does not appear to impede seasonal movements in this human-modified landscape, vipers overwhelmingly prefer steppe to cropland. We were interested in how the spatial use of vipers in this heavily altered habitat compared to a population inhabiting a recovered-natural habitat. How resource availability (i.e. – prey) impacts home range

size was of particular interest. Our hypotheses were that prey abundance would be higher in a recovered-natural landscape compared to a human-modified landscape with overgrazing pressure, and that snakes with better body condition would have smaller home ranges. The home range size and mean movements were significantly smaller and the abundance of small mammals was significantly higher in the recovered-natural landscape. However, we found no correlation between body condition and home range size. In fact, snakes inhabiting the two landscapes had equivalent body condition. These data suggest that snakes in the human-modified landscape have larger home ranges in order to find enough prey over the course of an active season. While the radiotelemetry data provided insight into the spatial ecology of Armenian Vipers in these two landscapes, we were also interested in examining their genetic diversity and population structure. We collected genetic samples from two locations within each of the two landscapes. Based on our radiotelemetry data we hypothesized that there would not be any structure between the two sampling locations within either landscape. We further predicted that two landscapes, which were geographically separated, would show strong genetic differentiation. At the local scale there was no significant differentiation between sampling locations, but on the regional scale we found the two geographically separated populations to be significantly differentiated from one another. The focus of conservation efforts for the Armenian Viper in altered habitat should be on maintaining corridors with high quality habitat that allow for seasonal movements, shelter, foraging and gene flow. Due to the strong genetic differentiation between the two populations we also recommend that regional populations be managed as independent conservation units.

Acknowledgements

First of all, I wish to thank my dissertation advisor, Dr. Patty Parker for the guidance and support she has provided over the last seven and a half years. As a non-traditional graduate student I ended up splitting my time between my fulltime career and the university, which made things challenging at times. I truly appreciate Patty's understanding and patience during this entire process. Thanks to her direction and insights I have a heightened appreciation for developing key questions and hypotheses, which will prove invaluable for my involvement with future conservation research projects and programs. Her guidance has made me a stronger scientist.

I would like to thank the members of my dissertation committee: Dr. Robert Marquis, Dr. Patrick Osborne and Dr. Harry Greene, for their insightful questions, comments and suggestions which have strengthened my work. In addition, I thank Dr. Bette Loiselle and Dr. Amy Zanne for providing valuable input during the early stages of the dissertation. All of the members of the Parker lab group, past and present, have played a role in this dissertation. I have learned a tremendous amount from each and every member of our lab group and for that I extend my thanks and appreciation. For helping me learn the ropes during my initial attempts at lab work I thank Kelly Halbert. I am especially thankful to Cindee Rettke for her guidance, assistance and friendship. She was a tremendous asset during my core period of lab work. Special thanks go to: Dr. Karen DeMatteo for teaching me how to use GIS and for always being there to answer my questions, Robert Meile for guidance on statistical analyses, Dr. Iris Levin for her editorial comments and assistance with

statistical programs, and to Jose Luis Rivera for being a sounding board for my ideas and questions and for providing great feedback.

A special thank you to the Biology Department Office staff: Pat Hinton, Kathy Burney-Miller and especially Maryann Hempen who was always there to help with my tuition reimbursement forms, questions regarding policies and my multitude of other questions. Thank you Maryann!

I extend a very special thank you to the Saint Louis Zoo, especially Dr. Jeffrey Bonner, Dr. Eric Miller, Jack Grisham and the late Ron Goellner, for providing me with the opportunity to attend graduate school and pursue a doctorate degree. To the late E. Desmond Lee for establishing the Des Lee Collaborative Zoological Scholar Program that provided funding for my educational costs at UMSL. To my assistant, Mark Wanner, for keeping everything running smoothly at the Herpetarium during my periods of absence at the university and in the field….thank you! I also thank my curatorial colleagues and the Herpetarium staff for their support and understanding over the past seven plus years.

I would like to thank Dr. Natalia Ananjeva and Dr. Nikolai Orlov of the Russian Academy of Sciences for organizing my first field excursion to Armenia in 2004. Nine years later and after more than a dozen trips, some lasting for months, Armenia has become my second home. This work would have never been possible without the assistance of my dear friends and colleagues Dr. Aram Aghasyan and his son, Dr. Levon Aghasyan. I thank them for welcoming me into their home, for their help with permits, assorted logistics, radio tracking, data collection and for introducing me to Armenia's phenomenal cultural and natural history.

I am grateful to all the field assistants on our expeditions in Armenia: Alexander Malkhasyan (WWF – Armenia), Gor Kaloyan (Scientific Center of Zoology and Hydroecology of the National Academy of Sciences, Yerevan, Armenia), Ando Gyonjyan (Scientific Center of Zoology and Hydroecology of the National Academy of Sciences, Yerevan, Armenia), Alen Mkrtchyan (Director, Zangezur Sanctuary, Armenia), Ruben Mkrtchyan (Director, Shikahogh State Reserve, Armenia), Andy Snider (Fresno, CA), Tara Brooks, DVM (St. Louis, MO), Erika Travis, DVM (Hogle Zoo, Salt Lake City, UT), Matt Edgar (Belleville, IL), Ryan Turnquist (Saint Louis Zoo), Mark Wanner (Saint Louis Zoo), Vicki Brown (Beyond Motion Productions), Mike Favazza (Beyond Motion Productions), Jamie Ryan (Beyond Motion Productions), Dr. Natalia Ananjeva (Russian Academy of Sciences), Dr. Nikolai Orlov (Russian Academy of Sciences), Konstantin Shiryaev (Tula Exotarium, Russia), Roman Khalikov (Russian Academy of Sciences), Roman Nazarov (Russian Academy of Sciences), Boris Tuniyev (Deputy Director of Science, Caucasus Biosphere Reserve), Sako Tuniyev (Kuban State University, Russia) and Mehdi Rajabizadeh (International Center for Science, Iran).

Financial support from a number of organizations and individuals made this body of research possible. For their support I thank: American Zoo and Aquarium Association Conservation Endowment Fund, Saint Louis Zoo Field Research for Conservation Grant Program, Cleveland Metroparks Zoo Conservation Grant Program, St. Louis Herpetological Society, Kansas City Herpetological Society, Dale Belcher, Mike and Ann Case, John and Denise Hall, Phil Hinson and Kaye Campbell-Hinson and Nancy Suelflow.

To my lifelong friends and mentors, Terry and Jan Primas, thank you for all of your support and encouragement over the years. Above all else thanks for believing in me.

For their unconditional love and support I thank my family. They have always been there to support me and for that I am eternally grateful. A very special thank you to my parents for tolerating my snake collection in the basement during my youth, see, it did pay off! Finally, I thank the love of my life, Holly, for her unconditional love, support and encouragement during my pursuit of the doctorate degree.

Chapter 1: Spatial Ecology of Armenian Vipers, *Montivipera raddei*, in a Human-Modified Landscape

Published as: Ettling, J. E., L. A. Aghasyan, A. L. Aghasyan and P. G. Parker. 2013. Spatial Ecology of Armenian Vipers, *Montivipera raddei*, in a Human-Modified Landscape. Copeia 2013: 64-71.

ABSTRACT: Armenian Vipers (*Montivipera raddei*) have a restricted and fragmented distribution throughout portions of Armenia, eastern Turkey and northwestern Iran. Over the past 40 years their population numbers have dropped by nearly 88% due to a combination of over-collection for the pet trade, conversion of habitat to agriculture and overgrazing by livestock. While a few studies have examined aspects of their reproductive biology, we know very little about the spatial ecology of this species. We used radiotelemetry to study the spatial ecology and habitat use of Armenian Vipers inhabiting a landscape modified by human use in Kotayk Province, Armenia during the spring 2007 - 2009 (17 males, 11 females) and for complete active seasons 2008 - 2009 (8 males, 6 females). We found no significant difference between sexes for home range size, average movements or movement rates through areas involving cropland versus strictly steppe. Home ranges were significantly larger for males whose spring core area included some cropland. Both sexes showed significant preference for mountain steppe over cropland. Despite these differences, the interspersing of cropland among steppe habitat does not appear to impede the snakes' movements and seasonal use of the available habitat. While conservation of intact mountain steppe habitat is the ultimate goal, providing

corridors of habitat in areas of agricultural development should be considered a high priority for managing this viper population into the future.

INTRODUCTION

How individual animals use space has a strong influence on the growth, spatial extent and persistence of their populations. Access to adequate food sources, avoidance of predators and survival during environmental extremes depends upon individuals making appropriate selections of foraging sites, refuges and movement pathways, respectively. Our understanding of these essential requirements for survival has resulted from studying the habitat selection of animals (Manly et al., 2002). Understanding habitat selection is critical to identifying the factors that affect abundance or occurrence at a given site as well as those that contribute to changes in population size (Blouin-Demers and Weatherhead, 2001; Pringle et al., 2003; Waldron et al., 2006).

Habitat selection by snakes can be influenced by several factors. The seasonal movements and habitat usage of some snake species (e.g., *Liasis fuscus*) have been directly linked to prey abundance (Madsen and Shine, 1996); however, other species (e.g., *Pantherophis obsoletus*) show no such relationship (Blouin-Demers and Weatherhead, 2001). The selection or avoidance of particular habitats by ectothermic animals, such as snakes, can be due to the presence or absence of thermoregulation sites (Huey et al., 1989; Blouin-Demers and Weatherhead, 2001; Row and Blouin-Demers, 2006a). Lastly, structural components, like canopy cover and retreats, can determine whether one habitat is selected over another (Pringle et al., 2003).

The home range size of snakes, like habitat selection, is also influenced by a number of factors. For snakes we know that sex, reproductive status, age, size, habitat structure and resource availability all have an impact on spatial ecology (Gregory et al., 1987; Weatherhead and Prior, 1992; Johnson, 2000). In addition, spatial ecology may also vary among populations of the same species (Shine, 1987; Macartney et al., 1988). Wildfires (Santos and Poquet, 2010) and hurricanes (Wunderle et al., 2004) can affect spatial ecology and habitat use in snakes, and such natural disturbances are part of the evolutionary history of a species. Land conversion for livestock grazing and agricultural crops, however, are human disturbances that typically result in permanent land transformations. Studies of the impact of agricultural development on vertebrate populations have primarily focused on amphibians (Babbit et al., 2009), birds (Dallimer et al., 2010) and mammals (Fischer et al., 2011), whereas snakes have largely been ignored (Durner and Gates, 1993; Shine and Fitzgerald, 1996; Reading and Jofré, 2009; Corey and Doody, 2010). With ongoing expansion of human populations into natural areas and the subsequent habitat conversion that follows, understanding how snakes use these modified landscapes is critical to the development of effective conservation management plans (Corey and Doody, 2010).

Many species of vipers (Family Viperidae) in the Caucasus and Transcaucasus regions are considered highly vulnerable to extinction due to restricted distributions, habitat alteration, over-collection, and unnaturally high mortality resulting from human persecution (Nilson and Andrén, 1999). These regions include portions of northern Iran, eastern Turkey, southwestern Russia, Republic of Georgia, and Republic of Armenia. There are approximately 15 taxa of vipers inhabiting these

regions, including members of the mountain viper (*Montivipera*) complex (Nilson and Andrén, 1986; Nilson et al., 1999a, 1999b). We have limited knowledge of their biology in nature, which is due in part to their isolated, restricted distributions (Nilson and Andrén, 1986).

The Armenian Viper (*Montivipera raddei*) is a medium-sized snake with a known range that includes easternmost Turkey, Armenia, and extreme northwestern Iran (Nilson and Andrén, 1986). In Armenia it occurs at elevations between 1100 and 2400 meters in rocky habitat covered by thin oak forests and bushes (Darevsky, 1966). In eastern Turkey it most often occurs on ridges composed of volcanic lava blocks with little or no vegetation (Flardh, 1983; Sochurek, 1984). It is listed as Vulnerable by the Republic of Armenia (Aghasyan and Kalashyan, 2010) and Near Threatened by the IUCN (Nilson et al., 2008). Throughout Armenia the habitat of the Armenian Viper has been subjected to considerable modification for agricultural activities (Nilson et al., 2008). Due to its communal sharing of hibernation sites it is particularly vulnerable to overexploitation for the pet trade. This has been the case for Turkish populations along the Aras River where it has been heavily impacted by over-collection (Nilson et al., 2008). Armenian Viper populations have shown a steady decline with 20 – 50 specimens/ha in the mid-1960s (Darevsky, 1966), 10 – 25 specimens/ha in the 1980s, and current estimates indicate densities of 4 – 10 specimens/ha (Mallow et al., 2003; Nilson et al., 2008).

Darevsky (1966) and Bozhanskii and Kudryavcev (1986) studied the ecology of the Armenian Viper, in the mountains of Armenia, and briefly described reproductive behavior and timing of mating; however, there are no published data on

the home range size, movement patterns or habitat use for the Armenian Viper or any other snakes in the genus *Montivipera*. The primary objective of this study was to gain a better understanding of the spatial ecology and habitat selection of Armenian Vipers inhabiting a landscape with agricultural croplands and overgrazing. Specifically, we describe the home ranges and movements for both spring activity and complete activity seasons, evaluate whether there is a correlation between home range size and proportion of cropland within snakes' activity ranges, examine whether there are differences in movements through steppe-only landscapes and those that involve croplands, and determine habitat preferences (whether use differs from availability). We expected that Armenian Vipers would prefer steppe habitat over cropland and that movements involving croplands would be more rapid than those through steppe. We also expected that snakes would have larger home ranges if they had a larger percentage of cropland within their home range. We made no specific hypotheses regarding the effect of sex on home range size or movements due to the amount of spatial use variation that has been documented to exist between sexes in snakes (Pearson et al., 2005; Marshall et al., 2006; Roth and Greene, 2006). We also address sample size in snake radiotelemetry studies and issues of sufficient statistical power.

MATERIALS AND METHODS

Study site

The study site was located 23 km northeast of Yerevan, Armenia in Kotayk Province. The boundaries of the study site (Fig. 1) were determined *post hoc* by using Geographical Information Systems (GIS; ArcMap 9.2, ESRI, Redlands, CA) and

Hawth's Tools Extension (Version 3.26, H.L. Beyer, 2006) to generate a minimum convex polygon (MCP) home range for the pooled locations of all radio tracked Armenian Vipers and overlaying it on a high resolution (2 m) satellite image of the region. Only locations of snakes tracked for an entire active season were used to calculate the study site MCP, which had an area of 480.2 ha.

The habitat within the study site consisted of a mosaic of mountain steppe and agriculture. Mountain steppe occurs at elevations between 1,200 – 2,200 m with rocky outcrops interspersed with grasses and shrubs (Adamian and Klem 1997). Predominant steppe vegetation consisted of common yarrow (*Achillea millefolium*), yellow starthistle (*Centaurea solstitialis*), common immortelle (*Xeranthemum squarrosum*), Siberian spurge (*Euphorbia seguieriana*), barrel medic (*Medicago coerulea*), long plantain (*Plantago lanceolata*), wheatgrass (*Agropyron sp.),* and narrow-leaved buckthorn *(Rhamnus pallasii*). The steppe areas are heavily overgrazed by the livestock of local farmers (Nilson et al., 2008; Ettling pers. obs.). The cropland areas are former steppe habitat cultivated predominantly for common wheat (*Triticum aestivum*), red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*). The study site consisted of 310.4 ha of steppe and 169.8 ha of croplands.

Capture techniques and data collection

Adult Armenian Vipers were captured in early May by searching the known den sites and the adjacent lower rocky slopes, which included the remnants of an abandoned concrete irrigation canal. Snakes were collected by hand with snake hooks and tongs. All captured snakes were marked for future identification with subcutaneous implanted passive integrated transponders (PIT) tags (Avid Identification Systems,

Inc., Norco, CA). Snakes were measured to the nearest 0.5 cm and both snout-vent length (SVL) and tail length (TL) were recorded. To provide safety to the handler as well as reduce stress on the snake, clear acrylic tubes were used during restraint procedures. Body weight was recorded to the nearest 0.5 g. Sex was determined by visual examination of tail length as well as by 'popping' which involves gently exerting pressure near the tip of the tail and rolling the thumb towards the cloaca. If the snake is a male, the pressure causes the hemipenes to evert. Manual palpation was used to check females for the presence of enlarged follicles (Fitch, 1987).

Radiotelemetry

Snakes to be implanted with transmitters were held in cloth sacks and kept at warm temperatures prior to and following surgery. Three different transmitters were used during the course of the study: Advanced Telemetry Systems (ATS), Inc. (Isanti, MN) model R1680 (4.1 g and 15.6 month average battery life) were used in 2007 and Holohil Systems, Ltd. (Ontario, Canada) models SB-2T (5.0 g and 12 month average battery life) and SI-2T (9.0 g and 18 month average battery life) were used in 2008 and 2009. None of the transmitters exceeded more than 5% of the snake's body mass. The transmitters were surgically implanted by a trained veterinarian and followed the procedure outlined by Reinert and Cundall (1982). Following surgery the snakes were held for 24 – 48 hours before being released at their original capture sites.

Over the three years that transmitters were implanted only one of 38 snakes (2.6%) died from what we believe were surgical complications. The number of snakes tracked varied by year (2007 = 13 males: 7 females; 2008 = 8 males: 7 females; 2009 = 2 males: 1 female). In 2007 all of the ATS transmitters failed prematurely at five

weeks post implantation and these data were only used for spring spatial and habitat analyses. As a result of mortality only a subset of the 2008 – 2009 snakes were used for statistical analysis (2008 = 6 males: 5 females; 2009 = 2 males: 1 female). Over the three years of the study 14 snakes (8 males: 6 females; Table 1) were tracked for one year. Collectively, Armenian Vipers with radio transmitters were located 195 times in 2008, and 41 times in 2009. Individual snakes were located an average of 16.9 (±0.8 SE) times over the course of an active season (May – October).

Following release we attempted to locate each snake at least one time weekly using a TRX-1000S radio receiver (Wildlife Materials, Inc., Murphysboro, IL) and a three-element, handheld Yagi antenna. More frequent locations were recorded during the first four weeks following release in an effort to observe reproductive behavior. Once a snake was located the geographic location was recorded with a hand-held global positioning system (GPS) (Meridian GPS, Magellan, Santa Clara, CA) and the type of macrohabitat (steppe or cropland) was noted as well. In order to not disturb or alter the behavior of the snakes we refrained from lifting rocks if we could not make visual contact following triangulation. Following the findings of Kapfer et al. (2008) we only included snakes in the spatial and habitat analyses if they had ten or more recorded locations within the year. To determine if home range sizes were affected by the number of locations recorded, a linear regression analysis was conducted to compare the home range estimates to the number of locations that were recorded for each snake (Seaman et al., 1999). When a snake was discovered dead we carefully examined the carcass in an effort to determine the cause of death. For analyses of spring spatial activity and habitat use we used data from 28 snakes (2007 = 9 males: 5

females; 2008 = 6 males: 5 females; 2009 = 2 males: 1 female). Complete active season spatial and habitat analyses used data from 14 snakes (2008 = 6 males: 5 females; 2009 = 2 males: 1 female).

Spatial and habitat analyses

We analyzed the spatial ecology and habitat use of Armenian Vipers by displaying the recorded geographic coordinates of each snake on a high resolution satellite image of the study site in Geographical Information Systems (GIS; ArcView 3.2 and ArcMap 9.2, ESRI, Redlands, CA). As suggested by Row and Blouin-Demers (2006b) we used a minimum convex polygon (MCP) to estimate the maximum home range for each snake and a combination of 95% fixed kernel home range and MCP to analyze habitat use. The latter was accomplished by adjusting the smoothing factor of the 95% fixed kernel until the area equaled that of each snake's MCP home range. This method emphasizes the core areas of habitat being utilized within the MCP. Hawth's Tools Extension (Version 3.26, H.L. Beyer, 2006) was used to calculate MCP home ranges and the Animal Movement Extension (Vers 1.1, P.N. Hooge and B. Eichenlaub, Alaska Biological Science Center, U.S. Geological Survey, Anchorage, AK, 1997) was used to calculate 95% fixed kernel home ranges.

The straight line distance (m) between successive movements was calculated using Hawth's Tools Extension (Version 3.26, H.L. Beyer, 2006). The total distance moved was calculated as the sum of all movements for an individual snake over the course of an entire active season. Mean distance moved per day and mean distance per move were calculated as in Roth and Greene (2006). We compared the mean

movement rates through steppe versus areas involving croplands to see if there were differences between sexes in movement rate through the two habitats.

To assess whether there was a correlation between home range size and the proportion of cropland within a given snake's home range we determined the relative proportions of steppe and cropland within each snake's MCP. These were calculated using ArcMap 9.2 (ESRI, Redlands, CA) and the Spatial Analyst Tools extraction option to 'extract by mask' the cells that corresponded to steppe and cropland within each snake's MCP home range.

To determine habitat preference of Armenian Vipers we compared observed habitat use to expected values based on the proportions of habitat types available [310.4 ha (64.6%) steppe and 169.8 ha (35.4%) cropland]. We evaluated the difference in habitat preference betweens sexes and years.

Since our data were often non-normally distributed we employed nonparametric tests to evaluate sexual differences in spatial patterns and habitat use. Mann-Whitney tests were used to assess differences in home range size and mean distance movement patterns and a Wilcoxon Paired-Sample test was used to evaluate differences in movements through steppe versus movements through cropland. Spearman Rank Correlation was used to assess the relationship between the proportion of cropland within an individual snake's home range and MCP size. Linear regression analysis was used to compare home range size to number of recorded locations. Chi-squared analyses were conducted to determine if habitat use differed from habitat availability. Statistical tests were conducted using StatistiXL 1.8 (StatistiXL, Nedlands, Western Australia) software.

To reduce the risk of committing a Type I error, resulting from the multiple comparisons that were made, we controlled the positive false discovery rate (pFDR) by utilizing the multiple hypothesis testing correction program QVALUE Version 1.0 (Dabney and Storey, 2004, http//faculty.washington.edu/~jstorey/qvalue) for R (Ihaka and Gentleman, 1996). In comparison to the overly conservative Bonferroni-type correction, pFDR not only reduces the probability of committing a Type I error, but also maintains higher levels of power (Storey, 2002). The Q-value, unlike the P-value, considers all of multiple test comparisons that were made. Q-values are interpreted like a P-value. Anything ≤ 0.050 is considered to be significant. We have reported the individual Q-values adjacent to their respective P-values in the manuscript and interpret results based on Q-values.

RESULTS

Home range and movements

Minimum convex polygon home range for 2007 – 2009 spring core areas averaged 2.1 ha ± 0.6 SE for males (n = 17) and 1.3 ha ± 0.5 SE for females (n = 11). The MCP for 2008 – 2009 complete active seasons averaged 18.8 ha ± 4.7 SE for males ([Fig. 2] [n = 8]) and 32.3 ha ± 13.8 SE for females ([Fig. 3] [n = 6]). Mann-Whitney tests revealed no significant difference in home range size between males and females for spring core areas ($U = 73.5$, $P = 0.358$, $Q = 0.511$) or for complete active seasons ($U = 22.0$, $P = 0.847$, $Q = 0.730$). The number of locations recorded per individual snake was not correlated with MCP size ($R^2 = 0.04$, $F_{1,12}$, $P = 0.491$, $Q = 0.614$).

The mean distance moved per day in 2007 – 2009 spring core areas averaged 17.2 m/day ± 3.3 SE for males (n = 17) and 14.9 m/day ± 2.1 SE for females (n = 11).

Mean distance per move in 2007 - 2009 spring core areas averaged 22.3 m/move ± 4.6 SE for males and 17.6 m/move ± 3.2 SE for females. There were no significant differences between males and females for either mean distance moved per day (*U = 94.0, P = 0.840, Q = 0.730*) or mean distance per move (*U = 88.5, P = 0.653, Q = 0.726*).

Mean distance moved per day in 2008 – 2009 complete active seasons averaged 17.2 m/day ± 2.2 SE for males (*n* = 8) and 18.6 m/day ± 4.2 SE for females (*n* = 6). Mean distance per move in 2008 – 2009 complete active seasons averaged 17.8 m/move ± 2.4 SE for males and 19.0 m/move ± 4.1 SE for females. No significant differences were detected between males and females for either mean distance moved per day (*U = 24.0, P = 0.949, Q = 0.730*) or mean distance per move (*U = 24.0, P = 0.949, Q = 0.730*).

For 2007 – 2009 spring core areas we found no support for our hypothesis that snakes would move more rapidly through areas involving cropland than those containing only steppe. For 2007 – 2009 spring core areas the mean distance moved per day for males (*n* = 17) averaged 12.7 m/day ± 1.5 SE through steppe and 27.4 m/day ± 15.9 SE through areas with cropland. Wilcoxon Paired-Sample test revealed no significant difference between movements through steppe versus those through cropland area (*t = 47.0, P = 0.174, Q = 0.348*). Average mean distance moved per day for females (*n* = 11) was 14.3 m/day ± 2.0 SE through steppe and 7.0 m/day ± 5.6 SE through cropland. While the p-value suggests that movements for females were significantly (*t = 11.0, P = 0.054, Q = 0.183*) more rapid through steppe than through

cropland, which is the opposite of what we expected, the multiple hypothesis test correction indicates that there is no statistical difference.

Using data from the 2008 – 2009 compete active season we found no support for our hypothesis that snakes would move more rapidly through areas involving cropland than through areas comprised of only steppe. The average mean distance moved per day for males (n = 8) was 11.4 m/day ± 2.3 SE through steppe and 30.8 m/day ± 8.7 SE through cropland. A Wilcoxon Paired-Sample test revealed no significant difference in the movements of male vipers through areas with cropland versus strictly steppe habitat (*$t = 4.0, P = 0.055, Q = 0.183$*). Average mean distance moved per day for females (n = 6) was 12.9 m/day ± 2.2 SE through steppe and 22.6 m/day ± 8.6 SE through cropland. No significant difference was detected between the two movements (*$t = 5.0, P = 0.313, Q = 0.511$*) for females.

For data from the 2007 – 2009 spring core area the Spearman Rank Correlation revealed partial support for our hypothesis that snakes would have larger home ranges if cropland encompassed a larger proportion of their respective home range. The spring MCP home ranges of males (Fig. 4) were significantly larger when a proportion of the range involved cropland (*$r_s = 0.73, df = 15, P = 0.001, Q = 0.008$*). For females no correlation between MCP and the proportion of cropland was detected (*$r_s = 0.54, df = 9, P = 0.087, Q = 0.218$*).

We found no support for our hypothesis concerning larger home range size and proportion of cropland for 2008 – 2009 complete active season MCP home ranges for males (*$r_s = -0.03, df = 6, P > 0.100$*) or females (*$r_s = 0.67, df = 4, P > 0.050$*).

Habitat preference

We found strong support for our hypothesis that Armenian Vipers would prefer steppe habitat over croplands. Chi-squared analysis revealed that habitat use differed from expected based on the relative availability of the two habitat types in 2007 – 2009 spring core area use for males ($x^2 = 79.15$, $df = 1$, $P < 0.050$) and females ($x^2 = 55.31$, $df = 1$, $P < 0.050$) and also in 2008 – 2009 complete active seasons for males ($x^2 = 33.69$, $df = 1$, $P < 0.050$) and females ($x^2 = 44.77$, $df = 1$, $P < 0.050$). In 2007 – 2009 spring core areas 97.5% of the male locations and 99.1% of the female locations were in steppe habitat. During the 2008 – 2009 complete seasons 88.7% of the male locations and 97% of the female locations were in steppe habitat.

While agricultural croplands were largely avoided, they were occasionally used by the snakes. In 2007 – 2009 spring core areas, 1.9% of all snake locations, 2.5% of male locations, and 0.9% of female locations were in croplands. In 2008 - 2009 complete active seasons, 8.1% of all locations, 11.3% of male locations, and 3.4% of female locations were in croplands. Males in our study utilized cropland more often than females. Other studies (Kapfer et al., 2008) have noted that snakes that frequented agricultural fields were typically killed either due to encounters with agricultural machinery in the fields or vehicles on nearby roads. At our study site no snake mortalities resulted from either of these causes.

DISCUSSION

Armenian Vipers inhabiting Koytak Province did not exhibit any significant sexual difference in MCP home range size. For many snake species males often have much larger home ranges than females. This size difference has been attributed to the fact

that males often make extensive movements during the breeding season in search of mates (Roth, 2005; Kapfer et al., 2008). Home range sizes for Eastern Massasauga Rattlesnakes, *Sistrurus catenatus catenatus* (Johnson, 2000; Marshall et al., 2006; Degregorio et al., 2011), Blacksnakes, *Pseudechis porphryiacus* (Shine, 1987) and Southwestern Carpet Pythons, *Morelia spilota imbricata* (Pearson et al., 2005) can vary among habitats and between sexes. While males in one population may have larger home ranges than females, it may be the reverse situation in another population, and in a third population sexes may have home ranges of similar size. These observed differences in spatial patterns between sexes indicate that there may be a host of factors, other than reproduction, driving home ranges size. These may include prey availability, population density and interactions with both congeners and other species (e.g. – predators) (Roth, 2005).

The similar sized home ranges for male and female Armenian Vipers at our study site may be the result of the agricultural fields that are interspersed within the steppe habitat. Both sexes of a Western Rat Snake, *Pantherophis obsoletus* population inhabiting Remington Farms, Maryland had equivalent size home ranges compared with congeners from other areas of the species range that showed extreme sexual differences in home range size (Durner and Gates, 1993). The major difference between Durner and Gates (1993) study site and those of other studies was the large areas of crop fields that occurred within the home ranges of their snakes. While the row crops planted at Remington Farms may not have provided the proper vegetation structure to support a prey base for the Western Rat Snakes (Durner and Gates, 1993), the agricultural fields at our study site were planted with wheat, clover and alfalfa that

theoretically should provide good foraging habitat for *Microtus spp.* and other rodents that constitute a major dietary item for Armenian Vipers. Further study involving prey base surveys are ongoing to confirm this hypothesis.

Marshall et al. (2006) noted that 50 individuals of each sex would need to be radio tracked to have sufficient statistical power (0.8) to detect differences. While no significant difference between male and female home range size was detected in our study, we acknowledge that with increased sample size there is the possibility that a detectable difference may exist. With rare and vulnerable species, such as the Armenian Viper, surgically implanting transmitters in large numbers of snakes is not feasible largely due to the small, localized populations. More importantly there are ethical considerations as well as the challenges of acquiring the required permission to use such a large number of specimens.

While we hypothesized that home range size of Armenian Vipers would be larger if they contained a larger proportion of agricultural croplands, only the spring activity range for males supported this hypothesis. Based on the fact that Armenian Vipers have a spring breeding system and mate in the weeks following emergence from hibernation, their spring activity ranges may be influenced by agriculture due to the concentrated areas they are actively patrolling in search of females and that cropland makes up a significant proportion of these areas. During the course of an entire active season the snakes may be using large enough areas within the habitat mosaic that the proportion of agriculture shows no significant influence on overall MCP home range size.

Armenian Vipers at our study site overwhelmingly preferred steppe to cropland. This finding is not surprising for two reasons. First, steppe is the 'natural' habitat and it comprised over half (64.6%) of the study site compared to cropland (35.8%). Secondly, there were few refuges available to the snakes within the agriculture. Rocks which had once been scattered in areas now under cultivation have been piled up between the fields in the swaths of remaining steppe. While male vipers had more locations in agricultural croplands than females, their movements through croplands were not statistically different than those through steppe. In the early spring the plants in these fields are small and provide limited cover; however, in the fall these same fields are barren following the harvest and plowing of the fields. In the latter case, with little to no cover, the snakes would be vulnerable to aerial predators such as Common Buzzards (*Buteo buteo*) and Short-toed Snake Eagles (*Circaetus gallicus*) which frequent the area. Snakes would be expected to move rapidly across these fields in the spring and autumn when vegetation cover is limited, but that was not evident based on our data. Durner and Gates (1993) had a similar hypothesis to why Western Rat Snakes, *Pantherophis obsoletus* avoided crop fields.

Armenian Vipers utilize a narrow range of niches within the landscape that depend on the availability of rocky, steppe habitat. These areas are used for hibernating, breeding, thermoregulating and searching for food. The ability to move between these different areas is facilitated by habitat corridors. Although our study site was fragmented due to human activity, the interweaving of steppe and cropland does not seem to impede the movement of the snakes through this landscape. Wisler et al. (2008) and Reading and Jofré (2009) noted the importance of a mosaic of

habitats to the conservation of the Grass Snake, *Natrix natrix*. While the highest priority should be given to maintaining intact parcels of mountain steppe, the results of our study suggest that maintaining a mosaic of habitats should be considered a key element in developing conservation strategies for Armenian Vipers inhabiting areas of agricultural development in Kotayk Province.

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Table 1. For each of the 14 radio-tracked *Montivipera raddei* the 95% minimum convex polygon (MCP) home range estimations are displayed. The snake identification number (ID), sex (male: M, female: F), snout-vent length (SVL), mass, number of days monitored (Days) and total number of observations (Obs.) are also provided.

ID	Sex	SVL (cm)	Mass (g)	Days	Obs.	MCP 95%
26	M	75.0	235.0	118	18	38.7
27	M	72.9	205.0	101	13	16.6
29	M	64.8	160.0	185	20	7.3
30	M	78.7	220.0	185	20	19.1
31	M	61.5	110.0	185	19	38.5
32	F	62.0	112.0	185	21	41.9
33	M	82.6	205.0	185	14	17.6
34	F	72.6	155.0	185	20	5.0
36	F	66.5	111.0	185	14	84.8
38	F	73.9	156.0	185	18	54.8
39	F	57.3	111.0	185	18	5.5
72	F	65.6	164.0	140	14	1.7
73	M	68.4	166.0	138	13	8.9
74	M	65.1	160.0	140	14	3.9

FIGURE LEGENDS

Figure 1. Study site in Koytak Province, Armenia with an area of 480.2 ha.

Figure 2. Minimum convex polygon (MCP) home ranges for male Armenian Vipers tracked during 2008 – 2009 complete seasons.

Figure 3. Minimum convex polygon (MCP) home ranges for female Armenian Vipers tracked during 2008 – 2009 complete seasons.

Figure 4. Impact of the proportion of cropland on male 2007 – 2009 spring core area minimum convex polygon (MCP) home range size.

Figure 1

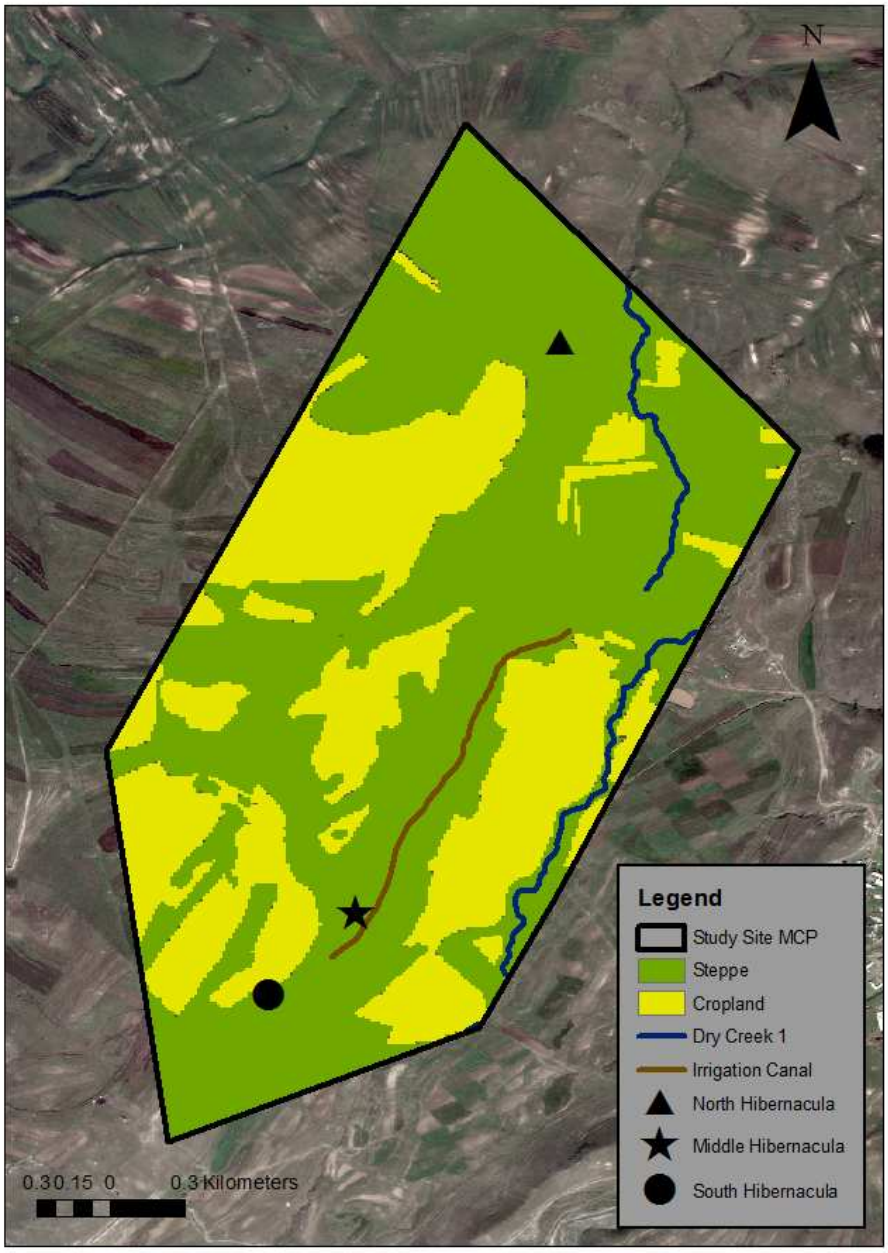
N
Legend
Study Site MCP
Steppe
Cropland
Dry Creek 1
Irrigation Canal
North Hibernacula
Middle Hibernacula
South Hibernacula
0.30.15 0
0.3 Kilometers

Figure 2

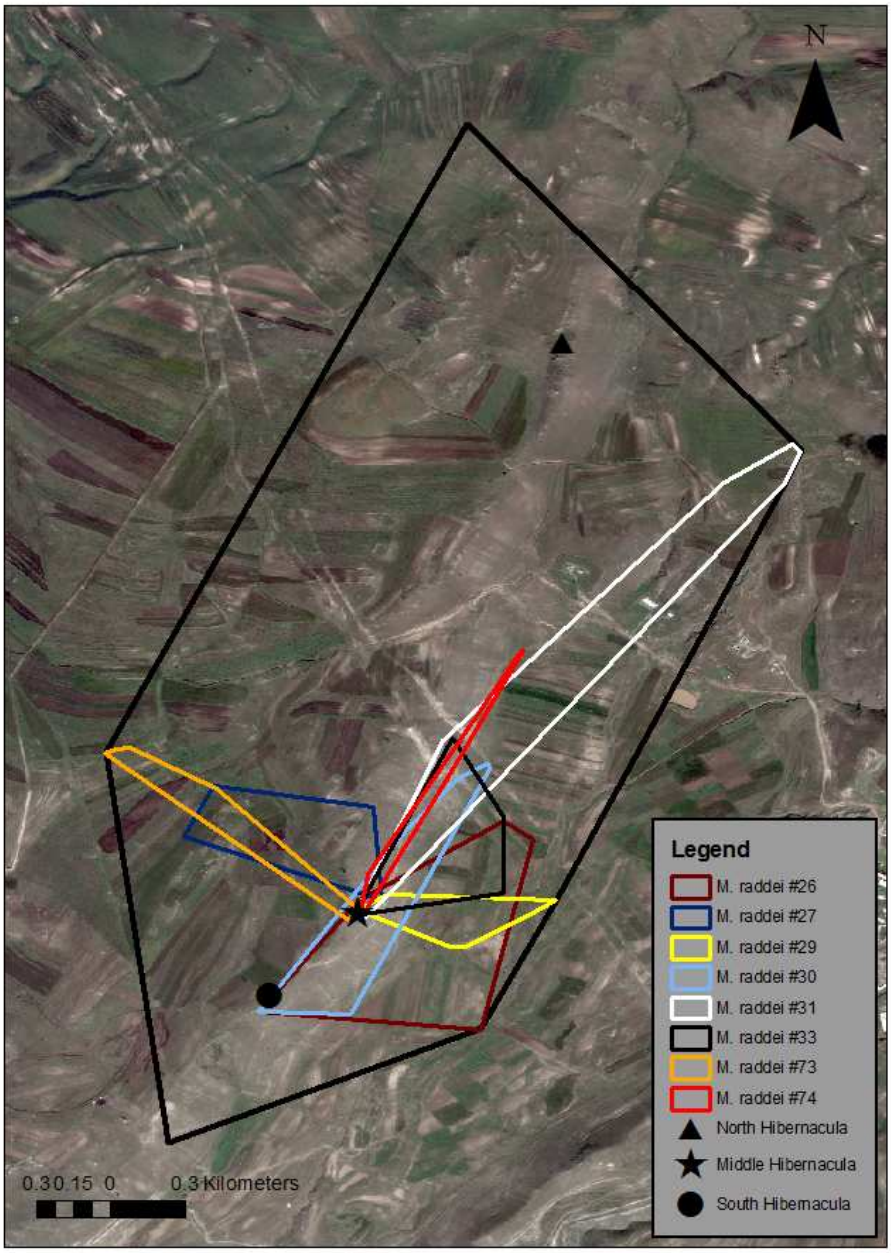
N
Legend
M. raddei #26
M. raddei #27
M. raddei #29
M. raddei #30
M. raddei #31
M. raddei #33
M. raddei #73
M. raddei #74
North Hibernacula
Middle Hibernacula
South Hibernacula
0.3 0 15 0
0.3 Kilometers

Figure 3

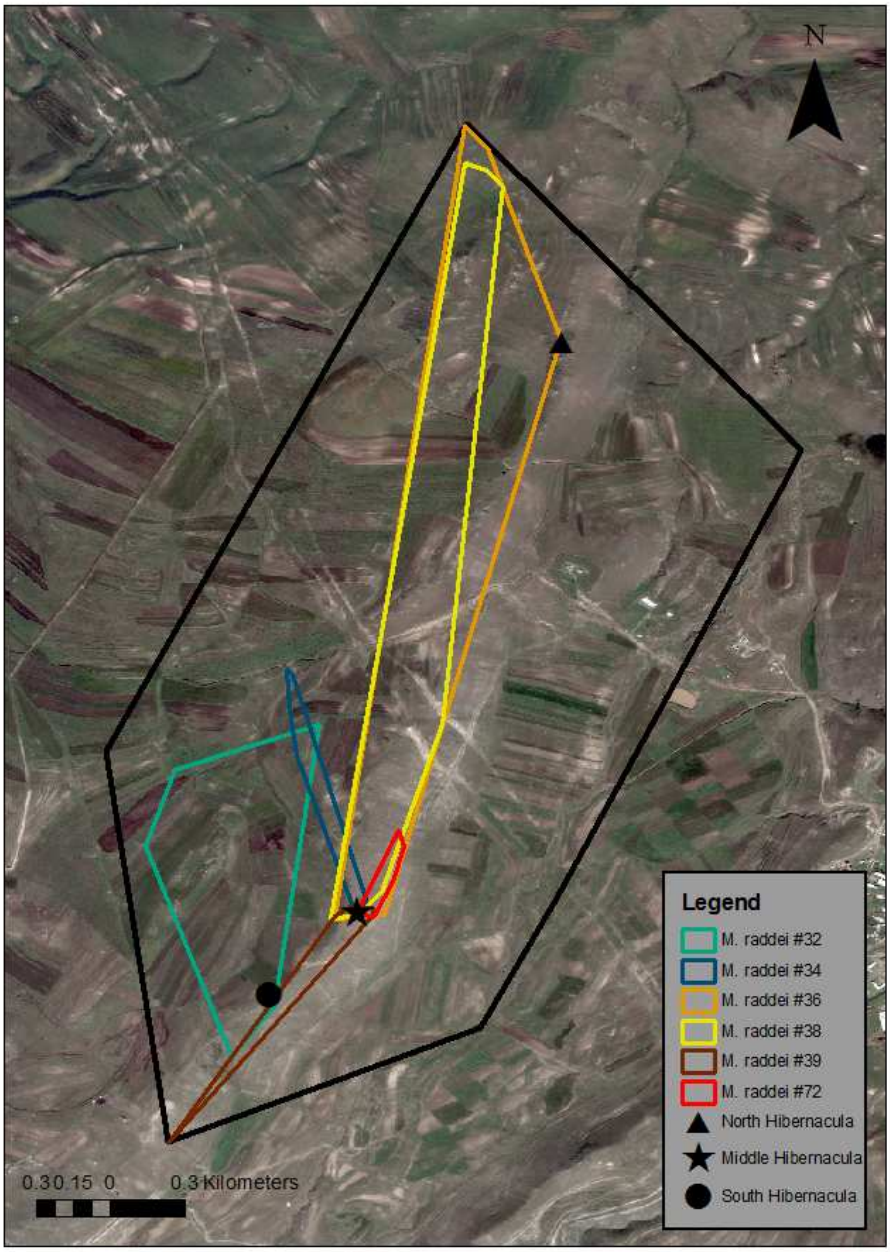
N
Legend
M. raddei #32
M. raddei #34
M. raddei #36
M. raddei #38
M. raddei #39
M. raddei #72
North Hibernacula
Middle Hibernacula
South Hibernacula
0.3 0 0.15 0.3 Kilometers

Figure 4

Chapter 2: Spatial Ecology of Armenian Vipers, *Montivipera raddei*, in Two Different Landscapes: Human-Modified vs. Recovered-Natural

Ettling, J. A., L. A. Aghasyan, A. L. Aghasyan and P. G. Parker, unpublished

ABSTRACT: Armenian Vipers, *Montivipera raddei*, have a range that includes Armenia and portions of eastern Turkey, Azerbaijan and northwestern Iran. They have a fragmented and restricted distribution that has been severely impacted by human activities, namely habitat alteration/degradation and over-collection for the pet trade, over the past 40 years. We used radiotelemetry to study and compare the spatial ecology of Armenian Vipers inhabiting a human-modified landscape near Abovian in Koytak Province and a recovered-natural landscape in Shikahogh State Reserve in Syunik Province. Radio-tracking at Abovian took place during 2008 – 2009 (8 males, 6 females) and during 2011 – 2012 at Shikahogh State Reserve (7 males, 4 females). Prey surveys were conducted at both sites to evaluate the abundance of small mammals. We found significant differences between the two populations in terms of home range size and mean movements. Home range and movements were significantly larger in the human-modified landscape. We found no difference in body condition between the two landscapes or a correlation between body condition and home range size. Prey abundance was significantly higher in the recovered-natural landscape. While the conservation of intact natural habitat should be given the highest priority, management strategies in agricultural landscapes should include both the inclusion of corridors to allow for movement between parcels of habitat and the

maintenance of high quality habitat within these landscapes to provide cover and food for both small mammals and vipers.

INTRODUCTION

The ability to effectively manage threatened and endangered species depends on having detailed knowledge of their spatial ecology (Durbian et al., 2008). Home range size and movement in snakes can be influenced by a number of different factors including sex, body size, age, season and reproductive condition (Gregory et al., 1987; Macartney et al., 1988). Additionally, the availability and distribution of resources including prey and refuges can influence spatial use in snakes (Madsen and Shine, 1996; Pringle et al., 2003; Roe et al. 2004). As ectotherms the behavior of snakes is directly associated with the ability to maintain an appropriate body temperature. The accessibility and spacing of thermoregulation sites can therefore have a profound effect on both spatial use and foraging ability (Blouin-Demers et al., 2003; Whitaker and Shine, 2002). Heterogeneous habitats have been shown to provide better opportunities for thermoregulation, shelter and prey abundance (Blouin-Demers and Weatherhead, 2001; Anderson et al., 2003; Pringle et al., 2003; Row and Blouin-Demers, 2006a; Wilgers and Horne, 2007).

When resources become less abundant and/or of reduced quality there is typically an increase in home range size (Harestad and Bunnel, 1979). For species with wide ranging geographic distributions (e.g., *Sistrurus catenatus catenatus*), home ranges have been shown to be larger at the northern edge of the range (Johnson, 2000; Degregorio et al., 2011). This increase in home range size has been attributed to the lower primary productivity of northern climate habitats (Steele et al., 1997). In

temperate snake species (e.g., *Thamnophis sirtalis, Crotalus viridis*) that utilize one habitat for hibernation and another for foraging, some populations have been noted to have relatively small home ranges while others have very large home ranges (Gregory, 1984; Macartney et al., 1988). These population differences in home range size and movements have been linked to resource distribution and quality (Gregory et al., 1987). Anthropogenic habitat changes most often negatively impact resources for snakes, notably shelter and prey (Weatherhead and Madsen, 2009). However, in some cases these alterations can enhance resource availability. European Adders, *Vipera berus*, in Sweden have benefitted from the mosaic landscape of natural habitat and cropland which supports increased populations of their primary prey item, small rodents (Weatherhead and Madsen, 2009).

Armenian Vipers, *Montivipera raddei*, are one of nine recognized species included in the mountain viper (*Montivipera*) complex of Western Asia (Nilson and Andrén, 1986; Nilson et al., 1999; Rajabizadeh et al., 2011). Due to their isolated and restricted distributions in remote, mountainous habitats we know very little about their natural history (Nilson and Andrén, 1986). Armenian Vipers have a distribution that includes eastern Turkey, Armenia, Azerbaijan, and northwestern Iran (Nilson and Andrén, 1986). They occur in sparsely vegetated rocky habitats at elevations of 1100 – 2400 meters (Darevsky, 1966; Flardh, 1983; Sochurek, 1984). A combination of threats including overgrazing, conversion of habitat to agricultural fields and over-collection for the pet trade has severely impacted Armenian Viper populations over the past 40 years (Nilson et al., 2008). Population numbers have dropped from 20 – 50 specimens/ha noted in the 1960s to current estimates of 4 – 10 specimens/ha

(Mallow et al., 2003; Nilson et al., 2008). The Armenian Viper has been classified as Vulnerable by the Republic of Armenia (Aghasyan and Kalashyan, 2010) and as Near Threatened by the International Union for the Conservation of Nature (IUCN) (Nilson et al., 2008).

Ettling et al. (2013) studied the spatial ecology and habitat selection of Armenian Vipers inhabiting a human-modified habitat consisting of a mosaic of overgrazed mountain steppe and agricultural fields. While no significant differences were found between sexes for home range size or movement rates through areas of strictly steppe versus areas with interspersed cropland during complete activity seasons, the spring home ranges of male vipers were considerably larger if they included a portion of cropland. Both sexes of vipers overwhelmingly preferred the natural steppe habitat to croplands (Ettling et al., 2013). The primary objective of the present study was to compare the spatial ecology of Armenian Vipers inhabiting a human-modified landscape to ones inhabiting a recovered-natural landscape. Specifically, we describe home range sizes and movements for complete activity seasons, determine whether there are differences in prey availability between the two landscapes, and evaluate whether body condition varies between a human-modified and a recovered-natural landscape. We expected that prey abundance would be higher in the recovered-natural landscape where overgrazing pressures were absent. We also expected that snakes with smaller home ranges would have better body condition. Due to the large amount of spatial use variation that exists between sexes in snakes we made no hypotheses concerning the effect of sex on movements or home range size (Pearson et al., 2005; Marshall et al., 2006; Roth and Greene, 2006). As a result

of the lack of difference in home range size between the sexes in Armenian Vipers (Ettling et al., 2013) we conducted home range and movement analyses between populations using combined data of males and females.

MATERIALS AND METHODS

Study sites

We studied the spatial ecology of the Armenian Viper in two different landscapes: human-modified (Study Site 1) and recovered-natural (Study Site 2). Geographical Information Systems (GIS; ArcMap 9.2, ESRI, Redlands, CA) and Hawth's Tools Extension (Version 3.26, H.L. Beyer, 2006) were used to calculate the boundaries of both study sites *post hoc* by generating minimum convex polygons (MCP) using the combined locality data of all radio-tracked vipers at each site. The area of Study Sites 1 and 2 were 480.2 ha and 41.2 ha, respectively. The distance between the two study sites was approximately 397.0 km.

Study Site 1 was located near the village of Abovian in Kotyak Province, Armenia. Abovian is approximately 23 km northeast of the capital city of Yerevan. The landscape has been heavily impacted by human agricultural activities and consists of a mosaic of cropland and overgrazed mountain steppe. The primary cultivated crops are alfalfa, *Medicago sativa*, common wheat, *Triticum aestivum*, and red clover, *Trifolium pratense* (Ettling et al., 2013). The area of the croplands within the study site was 169.8 ha (Ettling et al., 2013). The overgrazed mountain steppe occurs at elevations of 1,200 – 2,200 m and is comprised of a mixture of rocky outcroppings and steppe vegetation including common yarrow, *Achillea millefolium*, Siberian spurge, *Euphorbia seguieriana*, yellow starthistle, *Centaurea solstitialis*,

barrel medic, *Medicago coerulea*, and common immortelle, *Xeranthemum squarrosum* (Adamian and Klem 1997; Ettling et al., 2013). Mountain steppe within the study site had an area of 310.4 ha (Ettling et al., 2013). Study Site 1 will be denoted in the remainder of this paper as Abovian.

Study Site 2 was located on Meghri Ridge in Shikahogh State Reserve 52.0 km southeast of Shikahogh village in Syunik Province, Armenia. Meghri Ridge has an elevation of 2,200+ m and the habitat is considered high mountain steppe. The ridgelines are composed of scattered rocky outcrops and talus slopes. The ground vegetation consisted of grasses and shrubs with Caucasian hornbeam, *Amygdalus fenzeliana*, European ash, *Fraxinus excelsior*, and oaks, *Quercus* spp., in the valleys (Adamian and Klem 1997; Aivazyan 2006). While Shikahogh State Reserve was established as a protected area in 1958, the area where we conducted our research was not annexed as part of the Reserve until 7 September 2006. Prior to this date shepherds used these mountain meadows for livestock grazing during the summer months. This practice was halted after the inclusion of the area as part of the Reserve and the native plant community has since recovered to its natural state (Aghasyan, pers. comm.). Study Site 2 will be denoted as Shikahogh throughout the remainder of this paper.

Capture and data collection techniques

Armenian Vipers were collected between early May and late June at both sites by searching areas where snakes had previously been documented, including known hibernacula, rocky slopes and under human-generated debris (e.g., corrugated sheet metal, roofing tiles). Snake hooks and tongs were used to capture the snakes, and

clear acrylic tubes were used for safe restraint during data collection and surgical procedures. Each snake was weighed and measured to the nearest 0.5 g and 0.5 cm, respectively. Measurements recorded included both snout-vent length (SVL) and tail length (TL). The sex of each individual was determined by visually examining tail length and by using the 'popping' method to evert heimpenes in males. Females were evaluated for follicular development by manual palpation (Fitch, 1987). Passive integrated transponders (PIT) tags (Avid Identification Systems, Inc., Norco, CA) were implanted subcutaneously to provide a means of future identification.

Radiotelemetry

Snakes designated to receive a transmitter were kept in cloth sacks and maintained at warm temperatures, before and after surgery. Holohil Systems, Ltd. (Ontario, Canada) model SB-2 (3.8 and 5.0 g with 6 and 12 month average battery life, respectively) transmitters were used for this study. The transmitters were less than 5% of the body mass of the individual snakes. Surgical implantation of the transmitters followed the Reinert and Cundall (1982) methodology and was performed by a trained veterinarian. Snakes were typically held for 24 – 48 hours following surgery in order to monitor behavior and ensure that there were no surgical complications. Each snake was then released at the location where they were originally captured.

During the five years of the study a total of 37 snakes were implanted with transmitters. The number of radio-tracked snakes varied by year and site: Abovian (2008 = 8 males, 7 females; 2009 = 2 males, 1 female) and Shikahogh (2010 = 4 males, 2 females; 2011 = 3 males; 2012 = 5 males, 4 females). Due to mortality only a subset of snakes were tracked for a complete active season: Abovian (2008 = 6

males, 5 females; 2009 = 2 males, 1 female) and Shikahogh (2010 = 2 males, 1 female; 2011 = 3 males; 2012 = 4 males; 4 females). Only two of 37 snakes (5.4%) died from what were thought to be surgical complications. Over the five years 28 snakes (Abovian = 8 males, 6 females, Table 1; Shikahogh = 9 males, 5 females, Table 2) were tracked for a complete season (Abovian = May-October; Shikahogh = late June-early September). Radio-transmittered Armenian Vipers were collectively located a total of 366 times between 2008 and 2012, with each snake being located an average of 13.1 (±0.8 SE) times.

After release, at the point of capture, an attempt was made to locate each snake a minimum of one time weekly using a handheld TRX-1000S radio receiver (Wildlife Materials, Inc., Murphysboro, IL) and a Yagi antenna. A handheld geographic positioning system (GPS; Meridian GPS, Magellan, Santa Clara, CA) was used to record the geographic location of each snake. Notes on macrohabitat and behavior were also recorded. In an effort to avoid altering the snake's behavior we minimized the lifting of rocks and other debris if visual confirmation could not be made following triangulation of the transmitter signal. However, if the same location was noted on several subsequent visits the object was lifted to confirm that the snake was still alive. If discovered dead the carcass of the snake was thoroughly examined to attempt to ascertain the cause of death. Only snakes with nine or more locations during a complete active season were included in spatial analyses: 14 snakes for Abovian (2008 = 6 males, 5 females; 2009 = 2 males, 1 female) and 11 snakes for Shikahogh (2011 = 3males; 2012 = 4 males, 4 females).

Prey surveys

We conducted small mammal trapping to assess whether there were differences in prey abundance between the human-modified and the recovered-natural landscapes. Trapping was conducted at both study sites in June and September 2011 and in July 2012. The traps were set in locations where telemetered snakes had been found during the active season. We used 40 Sherman live traps set 10 meters apart to establish a 40 m x 100 m grid. Trapping was conducted for three consecutive nights at both sites. Traps were baited each evening with a small piece of an oatmeal bar and checked in the morning prior to rising temperatures. All captured small mammals were identified to species, weighed and marked for future identification before being released at the site of capture.

Analyses

We used Geographic Information Systems (GIS; ArcView 3.2 and ArcMap 9.2, ESRI, Redlands, CA) software to analyze the spatial ecology of Armenian Vipers. We followed the recommendation of Row and Blouin-Demers (2006b) and estimated the maximum home range for each snake using a minimum convex polygon (MCP) and a combination of fixed kernel density estimates (KDE) and MCP to quantify the core areas of use. To accomplish the latter we adjusted the smoothing factor of the 95% KDE until the area equaled that of the MCP home range for each snake. Using this method the 50% core areas of use were quantified within the MCP. The MCP home ranges were calculated using Hawth's Tool Extension (Version 3.26, H.L. Beyer, 2006) and 95%/50% KDE-MCP home ranges were calculated using the Animal

Movement Extension (Vers. 1.1, P.N. Hooge and B. Eichenlaub, Alaska Biological Science Center, U.S. Geological Survey, Anchorage, AK, 1997).

Hawth's Tool Extension (Version 3.26, H.L. Beyer, 2006) was used to calculate the straight line distance (m) between consecutive movements. The total distance that an individual moved over an entire active season was calculated as the summation of all movements. The methodology employed by Roth and Greene (2006) was used to calculate the mean distance moved per day and the mean distance moved per move.

We investigated variation in spatial use between individuals at both study sites by evaluating body condition. We generated a regression of the natural log (ln) mass on natural log (ln) SVL for each snake using body weight and SVL data from both radio tracked snakes and PIT tagged snakes from a mark-recapture study at both sites. The residuals resulting from these regressions were used as a body condition index (BCI) in a correlation analysis of BCI against the MCP home range for each snake (Roth and Green, 2006).

Due to our data often being non-normally distributed we employed nonparametric statistical tests to assess differences in spatial use. Mann-Whitney tests were used to evaluate differences in home range size, mean distance movements and body condition between snakes at both study sites. Pearson Correlation analyses were used to evaluate the possible relationship between body condition and MCP size. Chi-squared analyses were used to evaluate whether prey abundance (presence/absence) differed between the human-modified and recovered-natural landscapes. The Schnabel Index was used to estimate rodent population numbers in

the two landscapes. Statistical tests were conducted using StatistiXL 2013.1.02 (StatistiXL, Nedlands, Western Australia) and PopTools 3.2.3 (Hood, G. M. 2010. http://www.poptools.org) software.

RESULTS

Home range and movements

Minimum convex polygon home range for 2008 – 2009 complete active seasons averaged 24.6 ha ± 6.5 SE for Abovian ([Fig. 2] [Table 1] [*n* = 14]) and for the 2011 – 2012 active seasons averaged 4.6 ha ± 2.0 SE for Shikahogh ([Fig. 3] [Table 2] [*n* = 11]). Mann-Whitney tests revealed a significant difference in home range size between snakes inhabiting the human-modified landscape versus the recovered-natural landscape (*U = 133.0, P = 0.002*).

The 50% fixed kernel density estimates for 2008 – 2009 complete active seasons averaged 2.9 ha ± 0.8 SE for Abovian ([Fig. 4] [Table 1] [n = 14]) and for the 2011 – 2012 active seasons averaged 0.7 ha ± 0.4 SE for Shikahogh ([Fig. 5] [Table 2] [n = 11]). Mann-Whitney tests revealed a significant difference in the 50% KDE-MCP between snakes in the two different landscapes (*U = 125.0, P = 0.01*).

Mean distance moved per day in 2008 - 2009 complete active seasons averaged 17.8 m/day ± 2.1 SE for Abovian (*n* = 14) and for the 2011 – 2012 active seasons averaged 10.2 m/day ± 1.9 SE for Shikahogh (*n* = 11). Mean distance per move for 2008 – 2009 complete active seasons averaged 18.3 m/move ± 2.2 SE for Abovian and for the 2011 – 2012 active seasons averaged 10.3 m/move ± 1.9 SE for Shikahogh. A significant difference was detected between Abovian and Shikahogh

for both mean distance moved per day (*U = 120.5, P = 0.02*) and mean distance per move (*U = 125.5, P = 0.01*).

Body condition and correlation of BCI to MCP

Despite the significant difference in home range size between the Abovian and Shikahogh populations there was no significant difference in body condition between the two sites (*U = 2014.0, P = 0.89*). Mean mass for Abovian snakes averaged 172.8 g ± 8.4 SE (n = 73) and 178.5 g ± 12.3 SE (n = 56) for Shikahogh. We found no support for our hypothesis that snakes with better body condition would have smaller home ranges. There was no significant correlation between BCI and MCP home range for either the 2008 – 2009 complete active seasons for Abovian (*t = -1.51, P = 0.16*) or the 2011 – 2012 complete active seasons for Shikahogh (*t = -0.10, P = 0.93*).

Prey surveys

We found strong support for our hypothesis that prey abundance would be higher in the recovered-natural landscape which was not subjected to the pressures of livestock overgrazing. Using data from the 680 total trap nights, a significant difference (Table 3) in the abundance of small mammals was detected between the two study sites (x^2 = 34.8; df = 1, n = 48, P < 0.001). The combined data from the three survey periods was also used to estimate the small mammal populations in both landscapes. The population numbers of small mammals was significantly higher in the recovered-natural landscape (Shikahogh: 129) as compared to the human-modified landscape (Abovian: 10).

DISCUSSION

Armenian Vipers inhabiting Abovian had average MCP and 50% KDE-MCP home range sizes that were significantly different from the viper population at Shikahogh. In fact, there was a five fold difference in MCP home range size between the two the sites with the larger home ranges being recorded for snakes inhabiting the human-modified landscape. Significant differences in home range size have been documented between populations of other snake species. The home range sizes of Western Rat Snakes, *Pantherophis obsoletus,* on Remington Farms, Maryland were larger than congeners from Ontario, Canada. The large areas of soybean, *Glycine max*, sunflower, *Helianthus* spp., rye, *Secale cereale,* and corn, *Zea mays,* fields that were part of the home range of the population at Remington Farms were the primary factor attributed to the larger home range sizes (Durner and Gates, 1993). Similarly, Grass Snakes, *Natrix natrix,* in Switzerland (Wisler et al., 2008) had significantly larger (40 ha) home ranges compared to a Swedish population (25 ha) (Madsen, 1984). While the Swiss Grass Snake's had agriculture within their home ranges, the low density of nesting sites and prey were both considered factors contributing to the larger home range sizes (Wisler et al., 2008). The observed differences in home range size between viper populations in our study as well the other aforementioned studies suggests that snakes inhabiting high quality habitats where resources are more abundant often have smaller home ranges than snakes in low quality habitats (Stickel and Cope, 1947; Madsen, 1984; Durner and Gates, 1993; Wisler et al., 2008).

Prey surveys conducted to determine if the abundance of small mammals differed between a human-modified landscape (Abovian) and a recovered-natural

landscape (Shikahogh) indicated that small mammals were more abundant in the recovered-natural landscape. While the abundance of small mammal populations might be expected to be higher in areas with forage and cereal crops, such as alfalfa, *Medicago sativa*, and common wheat, *Triticum aestivum*, respectively, that was not the case at Abovian. The lower than expected abundance of small mammals at Abovian may be due to the lack of sufficient vegetation cover, resulting from overgrazing, in the steppe habitat surrounding the agricultural fields. This overgrazed habitat likely reduces the availability of both food and burrows for small mammals, and may increase their vulnerability to predation by raptors and small carnivores (Torre et al., 2007; Laidlaw et al., 2012). Fischer et al. (2011) noted that the abundance of small mammals, in terms of both diversity and richness, was higher in areas where agriculture was adjacent to complex landscapes that provided quality shelter and habitat connectivity. In contrast to the overgrazed steppe habitat at Abovian, the recovered vegetation at Shikahogh reached heights of 1 – 2 meters during summer months and provided good cover and food for the resident small mammals. Higher rodent densities have been directly correlated with vegetation that is taller and of thicker density (Smit et al., 2001; Torre et al., 2007; Ascensão et al., 2012). While radio-tracking vipers at Shikahogh small mammals were routinely seen scurrying about on the ground (Ettling, pers. obs.). Additionally, of the 73 Armenian Vipers that were collected at Abovian we never had a single snake regurgitate a small mammal while being held in cloth bags for data processing. On the other hand eight (14.3%) of the 56 vipers collected at Shikahogh regurgitated single or multiple small mammals (Ettling, unpub. data). The lack of vegetation complexity at Abovian is the

likely factor impacting the observed differences in small mammal abundance between the two sites.

Energetics and the availability of prey are often suggested as the drivers of variation that we observe in movements and home range size in snakes (Madsen and Shine, 1996). Additionally, it has been hypothesized that larger snakes have larger energy requirements than smaller snakes and therefore should have larger home ranges to meet these demands (Shine, 1987). Alternatively, we hypothesized that snakes with better body condition would have smaller home ranges and shorter movements than snakes with poorer body condition. Having better body condition and smaller spatial activity was used to infer that resources (i.e. – prey species) were more abundant in a given habitat (Gregory et al., 1987). While there was a significant difference in terms of home range size, mean movements and prey abundance between the two study sites, there was no correlation in our study between BCI and MCP home range size for either population. In fact, there was no difference in body condition between the two study sites, suggesting that vipers inhabiting the Abovian site have larger home ranges than the Shikahogh population due to the scarcity of small mammals and the need to move further in order to find sufficient food. While small mammals constitute a large portion of the Armenian Viper's diet, nestling birds and orthopteran insects (Ettling, pers. obs.) are also consumed. Passerine birds nest in low shrubs as well as on rock ledges at Abovian and Armenian Vipers have been observed eating hatchlings (Hakobian and Martirosyan, pers. obs.). In addition, the steppe habitat supports a large diversity of orthopterans (Ettling, pers. obs.). Prey specialization has been documented in a large number of taxa and can be site-specific

depending on the abundance of a particular prey species (Bolnick et al., 2003). Whether diet specialization on birds or insects has occurred at Abovian due to the low abundance of small mammals is a question that requires further investigation.

Swaths of steppe habitat provide corridors between the agricultural fields at Abovian and allow for movement between hibernacula and foraging areas as well as gene flow between neighboring hibernacula (Ettling et al., 2013; Ettling et al., unpub. data). While the vipers inhabiting this human-modified landscape do not appear to have their movements impeded, the results of this current study indicate that overgrazing of the steppe surrounding the agriculture has dramatically impacted the abundance of small mammals suggesting that the vipers have larger home ranges in order to find enough food during their active season. The discontinuance of grazing at Shikahogh over a seven year period appeared to benefit the abundance of small mammals. While conserving intact native habitat should always be the highest priority, the development of conservation management strategies for agricultural areas, such as Abovian, should not only include the maintenance of adequate habitat corridors, but also the maintenance of high quality habitat that will provide good shelter and food for both predator and prey.

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Table 1. For each of the 14 radio-tracked *Montivipera raddei* at Abovian the 95% minimum convex polygon (MCP) and 50% kernel density estimation – minimum convex polygon (KDE-MCP 50%) home range estimations, in hectares, are displayed. The snake identification number (ID), sex (male: M, female: F), snout-vent length (SVL), mass, number of days monitored (Days) and total number of observations (Obs.) are also provided.

ID	Sex	SVL (cm)	Mass (g)	Days	Obs.	MCP 95%	KDE-MCP 50%
26	M	75.0	235.0	118	18	38.7	3.2
27	M	72.9	205.0	101	13	16.6	1.6
29	M	64.8	160.0	185	20	7.3	1.2
30	M	78.7	220.0	185	20	19.1	2.6
31	M	61.5	110.0	185	19	38.5	6.1
32	F	62.0	112.0	185	21	41.9	3.8
33	M	82.6	205.0	185	14	17.6	2.1
34	F	72.6	155.0	185	20	5.0	0.5
36	F	66.5	111.0	185	14	84.8	10.5
38	F	73.9	156.0	185	18	54.8	5.6
39	F	57.3	111.0	185	18	5.5	1.4
72	F	65.6	164.0	140	14	1.7	0.1
73	M	68.4	166.0	138	13	8.9	1.3
74	M	65.1	160.0	140	14	3.9	0.3

Table 2. For each of the 11 radio-tracked *Montivipera raddei* at Shikahogh the 95% minimum convex polygon (MCP) and 50% kernel density estimation – minimum convex polygon (KDE-MCP 50%) home range estimations, in hectares, are displayed. The snake identification number (ID), sex (male: M, female: F), snout-vent length (SVL), mass, number of days monitored (Days) and total number of observations (Obs.) are also provided.

ID	Sex	SVL (cm)	Mass (g)	Days	Obs.	MCP 95%	KDE-MCP 50%
92	M	78.6	331.0	81	9	1.4	0.2
97	M	82.0	329.0	103	10	11.0	1.6
103	F	57.8	156.0	91	10	1.1	0.2
106	M	58.5	154.0	103	10	5.7	0.5
107	M	66.2	219.0	104	10	22.5	4.0
117	F	54.7	131.0	81	9	2.5	0.4
118	F	56.6	116.0	81	9	0.6	0.07
120	M	66.3	241.0	81	9	1.4	0.1
121	F	65.9	280.0	91	10	1.3	0.4
122	M	83.2	365.0	81	9	2.5	0.3
123	M	72.8	289.0	91	10	1.0	0.2

Table 3. Small mammal relative abundance by Study Site (2011 – 2012). Based on 680 total trap nights. Numbers reflect unique individuals only.

Species	**Abovian**	**Shikahogh**
Meriones persicus	1	0
Sylvaemus uralensis	4	0
Apodemys uralensis	0	14
Microtus arvalis	0	28
Sorex volnuchini	0	1
Capture Totals	**5**	**43**

FIGURE LEGENDS

Figure 1. Study sites in Abovian, Koytak Province (Study Site 1) and Shikahogh State Reserve, Syunik Province (Study Site 2), Armenia.

Figure 2. Minimum convex polygon (MCP) home ranges for Armenian Vipers tracked during 2008 – 2009 at Abovian (Study Site 1).

Figure 3. Minimum convex polygon (MCP) home ranges for Armenian Vipers tracked during 2011 – 2012 at Shikahogh (Study Site 2).

Figure 4. Representative fixed kernel density estimate – minimum convex polygons (KDE-MCP) for Armenian Vipers #32 and #36 tracked during 2008 – 2009 at Abovian (Study Site 1). The 50% KDE-MCP core areas of use are highlighted in blue.

Figure 5. Representative fixed kernel density estimate – minimum convex polygons (KDE-MCP) for Armenian Vipers #107 and #118 tracked during 2011 – 2012 at Shikahogh (Study Site 2). The 50% KDE-MCP core areas of use are highlighted in blue.

Figure 1

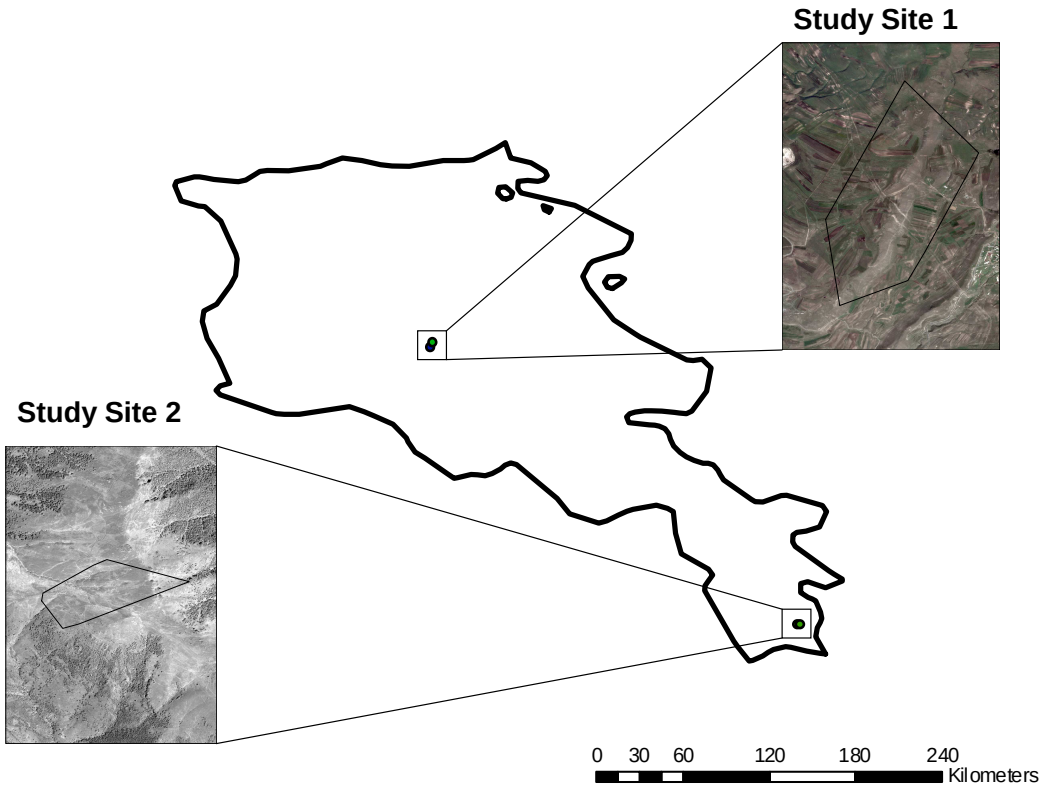
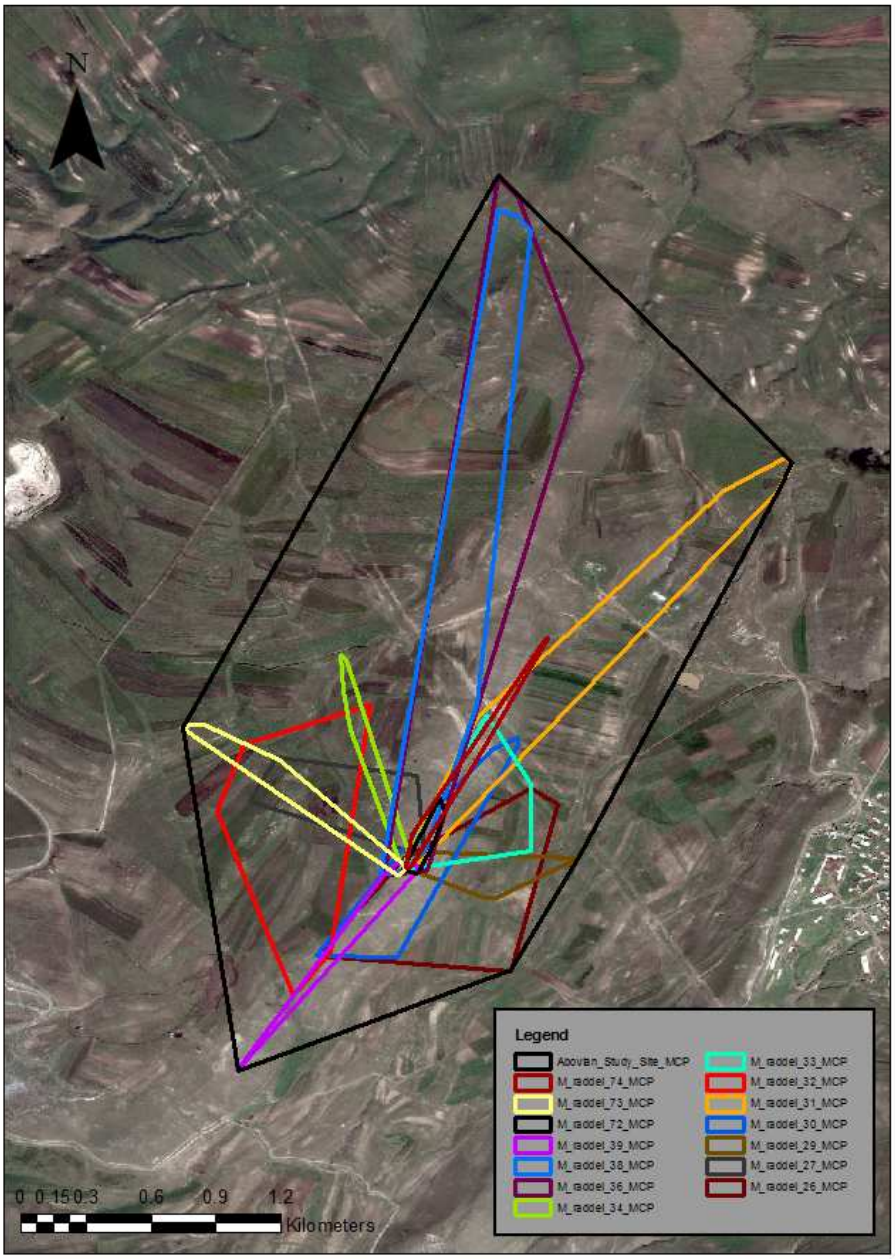


Figure 2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Kilometers
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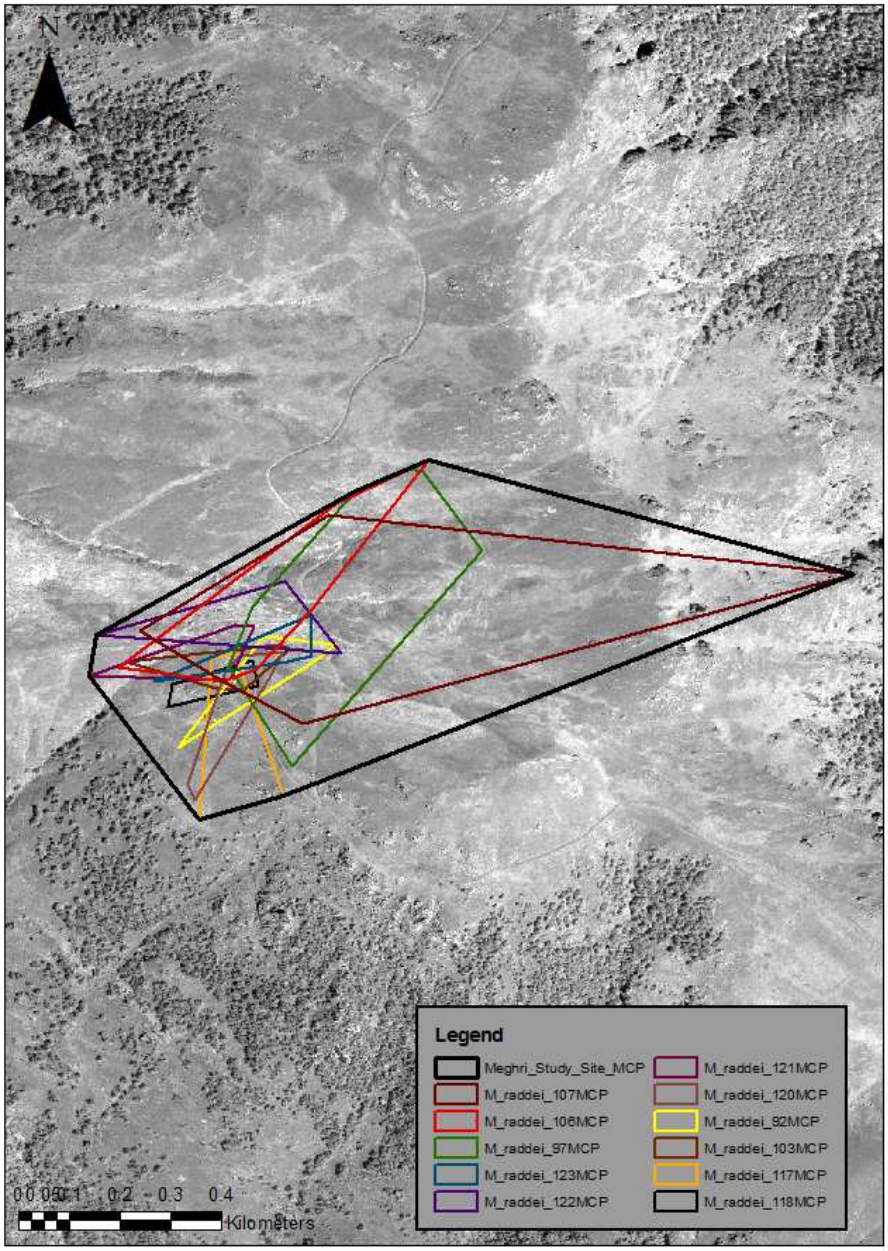
Figure 3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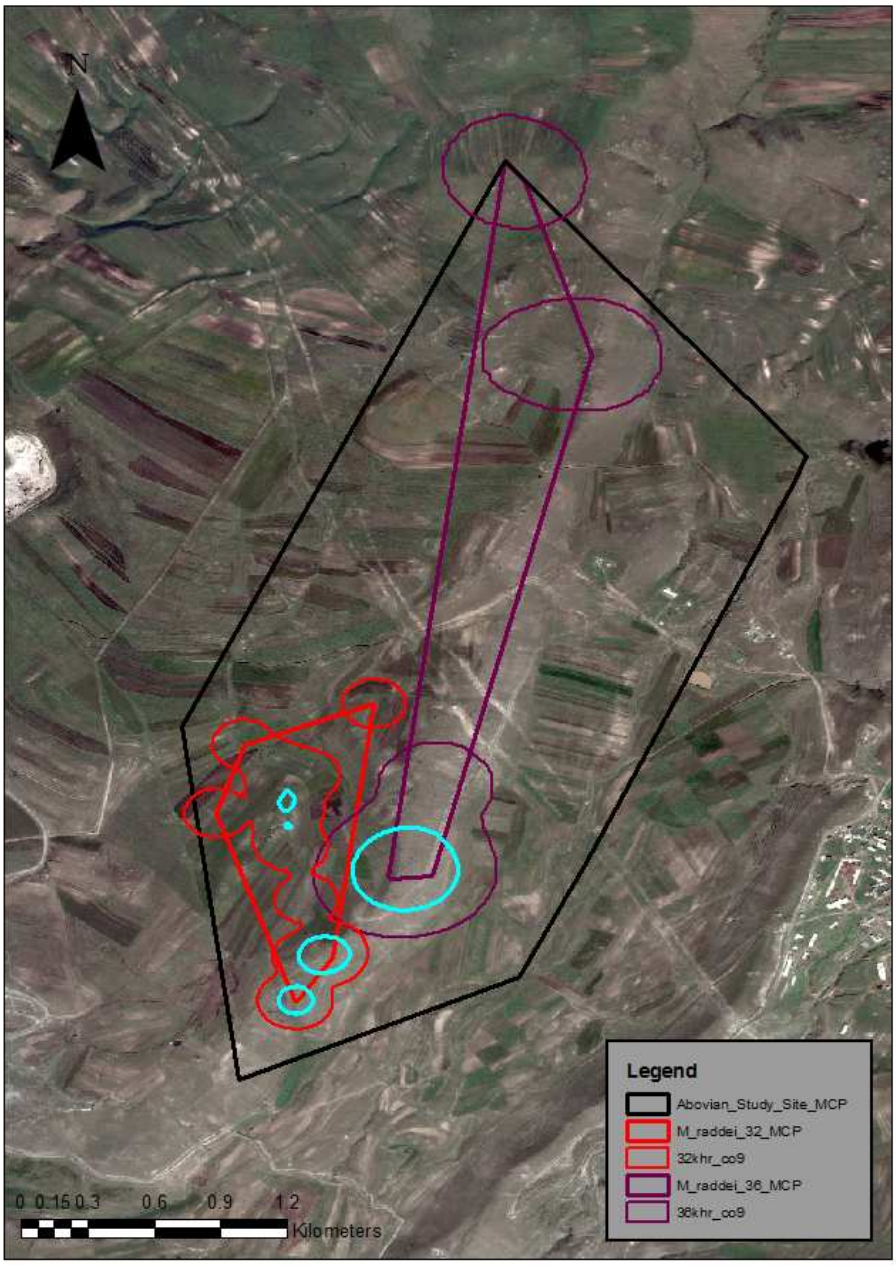
M_raddei_117MCP
M_raddei_118MCP

Figure 4


N
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32khr_co9
M_raddei_36_MCP
36khr_co9
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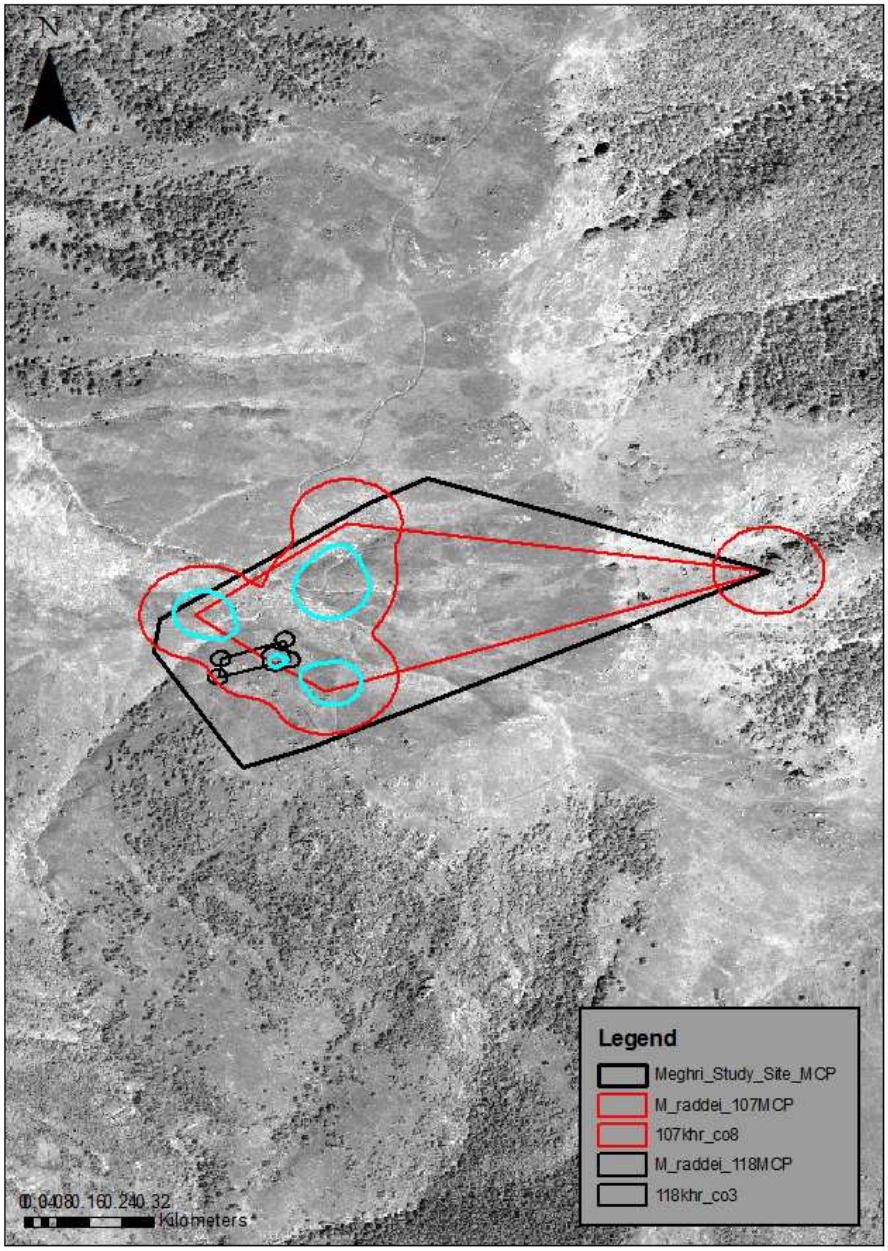
Kilometers

Figure 5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107khr_co8
M_raddei_118MCP
118khr_co3
0 0.04 0.08 0.16 0.24 0.32
Kilometers

Chapter 3: Genetic Diversity and Population Structure of Armenian Vipers, *Montivipera raddei*, in Two Different Landscapes

Ettling, J. A. and P. G. Parker, unpublished

ABSTRACT: Armenian Vipers, *Montivipera raddei* have a fragmented distribution in portions of eastern Turkey, Azerbaijan, Armenia, and northwestern Iran. Anthropogenic landscape changes and over-collection for the pet trade have resulted in drastic population declines over the past four decades. Recent radiotelemetry data demonstrated that Armenian Vipers are able to make seasonal movements in an agriculture-dominated landscape due to the availability of habitat corridors. While we have some insights into the spatial ecology and habitat use by the species, we know nothing about their population structure. We examined the genetic diversity and population structure of Armenian Vipers inhabiting an agricultural landscape as well as recoverd-natural landscape. Seven microsatellite loci were used to genotype 63 individuals representing two sampling locations in each of the two sites. There were no indications of population bottleneck 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 there is still genetic exchange between the two locations at this site. 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 locations within the agricultural landscape and the other consisting of the two locations within the recovered-natural landscape.

Conservation efforts should focus on the maintenance of habitat corridors that allow for gene flow and the management of the geographically separated populations as independent genetic units.

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 (i.e. – reduced connectivity, 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) showed significant differentiation not only between geographically separated populations, but also between neighboring populations 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 horridus* showed only modest differentiation (average $F_{ST} = 0.02$) between neighboring hibernacula, 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 hibernacula 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 distribution that includes eastern Turkey, Armenia, Azerbaijan, and northwestern Iran (Nilson and Andrén, 1986). 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 (Nilson et al., 2008). Over the past 40 years 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; Nilson et al., 2008). As a result of this decline the Republic of Armenia has listed the species as Vulnerable (Aghasyan and Kalashyan, 2010) and the International Union for the Conservation of Nature (IUCN) has listed it as Near Threatened (Nilson et al., 2008).

To date, genetic studies have focused on the phylogenetic relationships of the nine species comprising *Montivipera* and the taxonomic position of the genus within Viperidae (Nilson et al., 1999; Lenk et al., 2001). 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, examine the extent of structure within and between these populations, and delineate whether there were specific genetic units that require conservation efforts. Based on the results of our radiotelemetry data (Ettling et al., 2013; Ettling et al., unpub. data) we predicted that there would be no structure among the two sampling locations within either of the two study sites. We also predicted that there would be strong genetic differentiation between the geographically separated Abovian and Shikahogh populations.

METHODS

Study sites and sample collection

Our study was conducted at two sites (Fig.1) with different landscape characteristics. F1
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. 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 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. 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.

We collected 12 – 18 genetic samples from each of two locations within both study sites over a five year period (June 2006, May – June 2009, October 2009, June – August 2010, June 2011, and September – October 2011) representing a total of 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. Snakes were captured by hand using snake hooks and tongs. Clear acrylic tubes were used 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. Passive integrated transponders (PIT) tags (Avid Identification Systems, Inc., Norco, CA) were implanted subcutaneously to allow for future identification.

Microsatellite genotyping

Proteinase K was added to all blood samples prior to incubation overnight. Extraction of DNA used standard phenol/chloroform procedures (Sambrook et al., 1989) followed by dialysis in 1xTNE$_2$ (10mM Tris-HCl, 10 mM NaCl, 2 mM EDTA). We estimated the concentration of DNA in each sample using a spectrometer (Bio-Tek Instruments, Inc.) and adjusted them to 20 ng/μl working concentrations for use in polymerase chain reactions (PCR).

A total of 13 microsatellite loci were screened; ten were polymorphic, and seven were amplified in 63 individuals. The microsatellites were isolated from *Montivipera raddei* samples at Dr. Travis Glenn's laboratory located at the Savannah River Ecology Laboratory (Aiken, SC) 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), 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 the PCR products for all seven loci and GeneMapper (ABI) software version 4.01 was used to score allele sizes.

STATISTICAL ANALYSES

Genetic diversity

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. GENEPOP 4.0.10 (Raymond and Rousset, 1995) was used to evaluate linkage disequilibrium between loci pairs with Markov chain parameters of 1000 dememorization steps, 100 batches, and 1000 iterations per batch.

We evaluated the genetic diversity within each of the sample locations in a number of different ways. The mean expected (H_E) and observed (H_O) heterozygosities were calculated 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). HP-RARE 1.0 (Kalinowski, 2005) was used 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 utilizes 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). Recent reductions in effective population size were evaluated using the program BOTTLENECK vl.2.02 (Piry et al., 1999) which tests for excess heterozygosity. 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.

Population structure

An analysis of molecular variation (AMOVA) in ARLEQUIN was used 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 in the human-modified landscape compared to movements between sampling locations in the seemingly uninterrupted natural landscape. Pairwise F_{ST} comparisons were made 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. Unlike ARLEQUIN which requires a priori assignment of individuals to populations, STRUCTURE uses a Bayesian algorithm to

cluster genetically distinct groups without any a priori knowledge of the geographical location where the samples were collected. The predicted number of populations (K) was set at 1 – 6 (two more than the number of sample locations). We ran ten replicate runs for each K from 1 to 6 with 500,000 MCMC iterations following a burn-in of 50,000 iterations using the admixture model with correlated alleles and localities as priors. The delta K method (Evanno et al., 2005) was used to select the optimum number of genetic clusters from our dataset.

To calculate probabilities for individual assignments we used GENECLASS2 (Piry et al., 2004) with a Bayesian framework (Rannala and Mountain, 1997) and $P < 0.05$ assignment threshold. From the data the program generates 10,000 random genotypes and assigns individuals, using maximum likelihood, to a specific reference population.

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

Analyses of genetic data

Seven microsatellite loci were used to genotype 63 individuals representing two sampling locations from each of two geographically separated sites varying in landscape composition. Alleles per locus ranged from 9 – 18 across all four sample locations (Table 1). 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 2).

Expected heterozygosities (Table 2) ranged from 0.69 (Campsite) to 0.81 (North Den), with an average of 0.76 across all four sample locations. Five of 28 (17.9%) locus-population comparisons deviated from Hardy-Weinberg equilibrium (HWE) following a Bonferroni corrected P-value of 0.002 (Table 2). 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 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 of 0.0006.

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 2). 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 suggest that nonrandom mating is occurring within the Abovian South Den site.

A significant excess of heterozygotes was initially detected under the BOTTLENECK TPM (P = 0.05) for the North Den site, but after correcting for multiple tests (P = 0.002) it was no longer significant. The results for the other three sampling locations were also non-significant.

Population structure

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 (Table 3) following a Bonferroni corrected P-value of 0.008. The Abovian sampling locations differed significantly from the Shikahogh sampling locations.

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. 2). One cluster consisted of the two Abovian sampling locations and the second cluster consisted of the two Shikahogh sampling locations (Fig. 3). 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.

The probability scores for individual assignment using GENECLASS2 ranged from 56.5 to 100.0%, with a mean score of 90.5% (Table 4). 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 ten 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

Genetic diversity

To date there have been limited studies of the genetic diversity involving members of the viperinae (Ursenbacher et al., 2009; Ferchaud et al., 2011), but our results indicate that Armenian Vipers have comparable mean heterozygosity and allelic richness to both Adders, *Vipera berus* (Ursenbacher et al., 2009) and Orsini's Viper, *Vipera ursinii ursinii* (Ferchaud et al., 2011). Armenian Viper combined sampling locations had a mean expected heterozygosity of 0.76 and mean allelic richness of 5.97. In comparison, Adders and Orsini's vipers had mean expected heterozygosities of 0.52 and 0.50 and mean allelic richness of 2.98 and 5.07, respectively.

Armenian Vipers have a fragmented distribution that was shaped by historical and environmental events/changes (Nilson and Andrén, 1986). As noted earlier these fragmented populations have been severely impacted in recent years by anthropogenic activities including agricultural practices and over-collection for the pet trade (Nilson et al., 2008). 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; Nilson et al., 2008) 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 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 (Table 2). King (2009) reviewed the results of 25 microsatellite studies of snakes 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 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) or Orsini's Vipers (0.04). 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. Despite anthropogenic landscape changes, over-collection pressure, and signs of inbreeding the F_{ST} values and radiotelemetry data (Ettling et al., 2013; Ettling et al., unpub. data) indicate that there is exchange of genetic material between the North and South Dens at Abovian. The data also indicate exchange of genetic material between the Meghri Ridge and Campsite locations at the Shikahogh.

Population structure

Armenian Viper populations in this study exhibited non-significant differentiation at the local regional scale, but highly significant levels (P = 0.0006) of differentiation at the larger range-wide scale. Sampling sites separated at distances ≤ 3.2 km had levels of differentiation that ranged from 0.006 (Meghri Ridge/Campsite) to 0.028 (North Den/South Den). At geographical scales > 200 km the pairwise F_{ST} values were greater than 0.14. STRUCTURE also identified two distinct clusters: 1) Abovian (North Den and South Den) and 2) Shikahogh (Meghri Ridge and Campsite). There is greater molecular variation within populations (87.7%) than among populations (12.3%) of Armenian Vipers.

The New Mexico Ridge-nosed Rattlesnake, *Crotalus willardi obsurus* has a fragmented distribution on mountain ranges in southwestern New Mexico, southeastern Arizona and adjacent north-central Mexico and 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). In the aforementioned rattlesnake species males typically have the larger home ranges and make large movements searching for mates. Our radiotelemetry data demonstrate that the sexes have similar home range size in Armenian Vipers and that females will also

make large movements during the spring breeding season (Ettling et al., 2013). These 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 (Clark et al., 2008; Holycross and Douglas, 2007; Ettling et al., 2013)

Conservation implications

The results of this study have identified two discrete populations that are highly divergent. Due to the geographic isolation and lack of gene flow between Abovian and Shikahogh we recommend that these two populations be managed separately. Based on our data 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. Our microsatellite data support the results of our radiotelemetry studies (Ettling et al., 2013; Ettling et al., unpub. data), and indicate that gene flow is occurring between locations even where there have been considerable human-mediated landscape alterations. While direct measures of connectivity between populations provide data on home range size and movements, they do not provide a means of quantifying gene flow. 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 populations and the maintenance of habitat corridors to allow for gene flow within each of these populations.

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Table 1. Microsatellite characteristics in Armenian Vipers estimated from 7 microsatellite loci

Locus	Size Range (bp)	Number of alleles
MoRa02	249 – 269	9
MoRa03	216 – 248	10
MoRa05	165 – 197	13
MoRa06	268 – 276	15
MoRa17	261 – 273	10
MoRa18	179 – 232	10
MoRa21	230 – 288	18

Table 2 . Genetic diversity measures of four sample sites of Armenian Vipers estimated from 7 microsatellite loci

Site	Sample Size	A	H_O	H_E	Ar	Ap	F_{IS}	*P*-value for Bottleneck test
SD	17	7.57	0.64	0.80	6.46	1.22	**0.20**	0.41
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	0.15
CS	18	6.71	0.69	0.69	5.43	0.74	-0.002	0.99

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 in bold using 95% confidence intervals

Table 3. Pairwise population F_{ST} comparisons among sample sites

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

SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite
Values in bold represent significant differences with a Bonferroni correction of 0.008

Table 4. Results of population assignment tests in GENECLASS2

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

SD = South Den; ND = North Den; MR = Meghri Ridge; CS = Campsite

FIGURE LEGEND

Figure 1. Map of Armenia with locations of two study sites. The “stars” on inset maps denote sampling locations.

Figure 2. Inference of “Best K” for the Abovian and Shikahogh populations of Armenian Vipers.

Figure 3. Results of genotype analysis in STRUCTURE 2.3.2. Sampling sites are as follows: 1 = South Den; 2 = North Den; 3 = Meghri Ridge; 4 = Campsite.

Figure 1

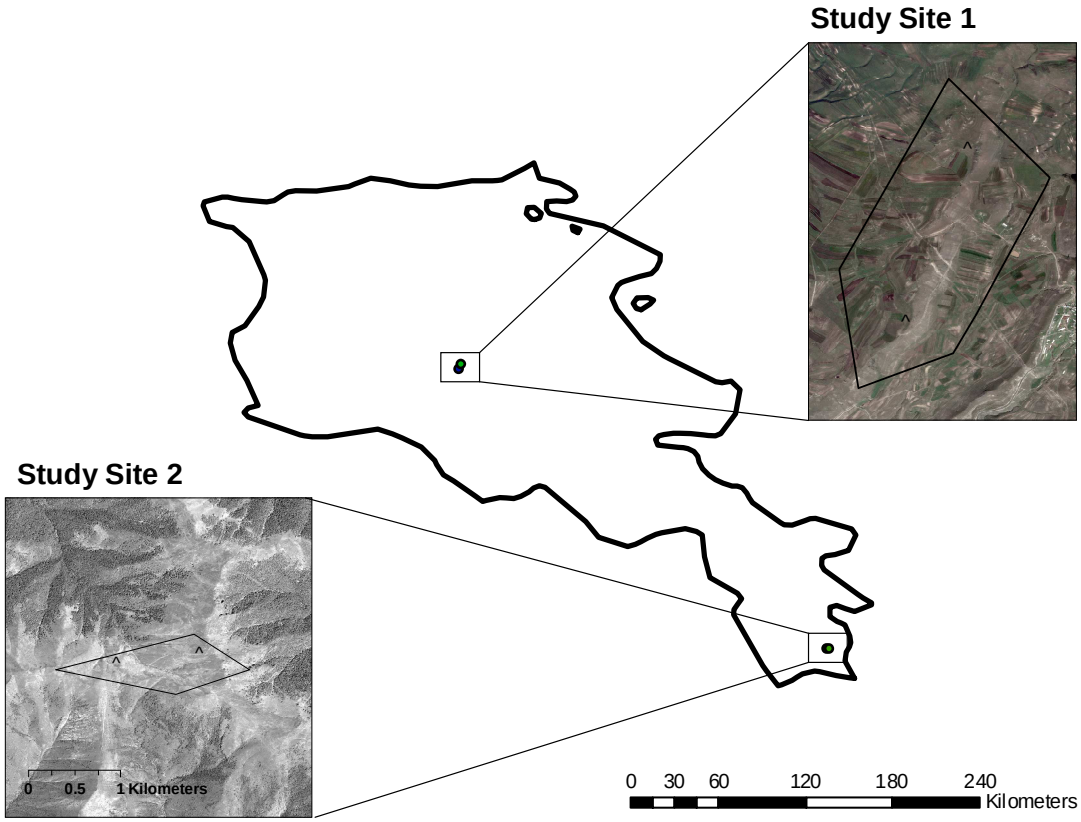
Study Site 1
Study Site 2
0 0.5 1 Kilometers
0 30 60 120 180 240
Kilometers

Figure 2

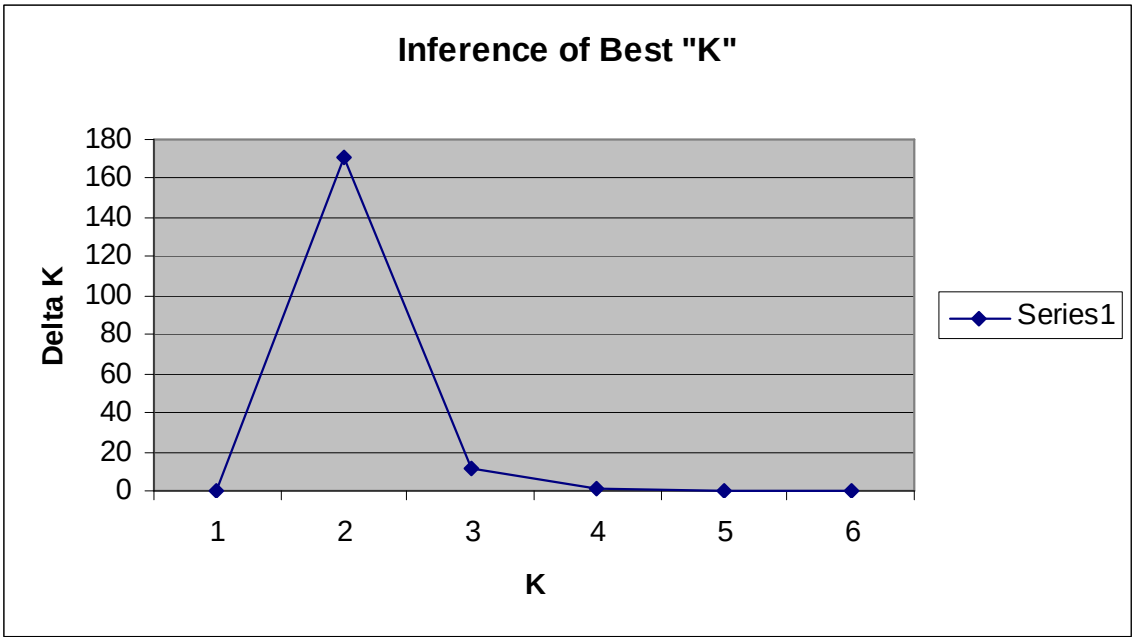
Inference of Best "K"
Delta K
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
K
Series1

Figure 3