

Conservation Genetics of Tiger Rattlesnakes in Saguaro National Park and the Surrounding Tucson Basin

FINAL REPORT

Submitted To:

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Introduction

Tiger rattlesnakes (*Crotalus tigris*) inhabit foothill areas in both the Rincon Mountain and Tucson Mountain Districts of Saguaro National Park (SAGU), and other natural areas (e.g., US Forest Service, Pima County Parks) in the surrounding Tucson Basin. Foothill areas are considered prime real estate and are being developed at an alarming rate in the mountains surrounding Tucson and Phoenix. As urban sprawl encroaches on park boundaries and other natural areas, increased threats to wildlife are inevitable. This is especially true for rattlesnakes, which are often perceived as dangerous pests that should be killed or relocated to less populated areas. Several fire departments in the Tucson area relocate thousands of rattlesnakes each year, often moving them long distances from where they were found (typically private residences adjacent to uninhabited foothill areas). Relocating rattlesnakes, especially out of established home ranges, is of great concern to wildlife managers, because studies have shown that relocated snakes often exhibit aberrant movement patterns, may cease feeding, and may even die (Reinert and Rupert 1999, Nowak and van Riper 1999).

I studied tiger rattlesnakes in the Rincon Mountain District of SAGU, and on adjacent private land, from 1997-2001, and in the Tortolita Mountains at, and adjacent to, the Stone Canyon development in the town of Oro Valley, from 2002-2006. In addition, I obtained numerous tiger rattlesnakes from the Santa Catalina Mountains, which were removed from residences by the Rural Metro Fire Department. My research has relied on traditional methods of field study, including radiotelemetry, mark-recapture, and behavioral observations, to gain greater insight into the natural history and ecology of this poorly understood species. This approach has led to several important findings about tiger rattlesnake spatial ecology (movement patterns and home range characteristics; Goode et al. in press) and mating system dynamics (mating histories of individual snakes and temporal patterns; Goode and Wall 2002). Although traditional methods have yielded critical information, they only provide a snapshot of the natural history of a species. Genetic analyses enables us to ask questions that cannot be answered using demographic techniques, and allow for greater understanding of how populations were structured in the past, before the advent of anthropogenic influences (Frankham et al. 2002).

Survival of wildlife populations is determined both by demographic and genetic processes, including the extent of genetic variation (i.e., heterozygosity) within, and the flow of genetic material among, populations (Lande 1988). Gene flow represents dispersal between habitat islands, and measurements of gene flow can tell us the extent to which populations are connected, or were connected in the past. Restricted dispersal among local populations may be due to natural barriers, such as valleys and ridges, or may be anthropogenic in nature, such as roads, canals, and housing developments. Whatever the underlying cause, decreased gene flow may lead to increased levels of inbreeding, smaller effective population sizes, and ultimately, increased risk of extirpation (Hedrick 2001). Tiger rattlesnakes are a good model to study effects of fragmentation on genetic structure of populations, because they are representative of an array of wildlife species that primarily live in increasingly isolated foothill habitats, but make infrequent movements among mountain ranges that presumably function to maintain genetic diversity. The primary goal is to gain a better understanding of the extent to which human-dominated landscapes may decrease or eliminate historic levels of gene flow.

Conservation of a species also requires an understanding of patterns of genetic variation within populations. Because of habitat variability, apparently continuous populations of some species may be genetically structured on extremely fine scales due to limited dispersal, genetic drift, or behavior. This is especially true for less vagile species, such as snakes. Recent studies on

reptiles have indicated that populations separated by relatively short distances (i.e., < 50 km) may differ significantly, thereby constituting genetically distinct “subpopulations”. Management of very small populations may require concurrent consideration of genetic relatedness and the impact of underlying landscape structure (Manel et al. 2003). For example, research on timber rattlesnakes (*Crotalus horridus*) using genetic markers, has shown that even within a small geographic area, individuals with shared winter hibernacula were significantly more closely related than individuals from different hibernacula (Bushar et al. 1998). Movement patterns of these snakes, as determined by a simultaneous study using radiotelemetry, suggested that physical barriers such as ridges were primarily responsible for restricting local gene flow.

Prior to this study, we isolated six microsatellite DNA markers that were informative for tiger rattlesnakes (Goldberg et al. 2003). I used these markers to conduct preliminary analyses on tiger rattlesnake population genetics for this report. Microsatellite loci have become popular genetic markers for defining population structure and revealing differentiation among populations (Avice 2004). Microsatellites, or short tandem repeats (STRs), are non-coding repetitive DNA sequences composed of a variable number of tandemly repeating motifs. On average, STRs have mutation rates between 10^{-2} and 10^{-5} per gamete, per generation and vary between individuals within and among populations. Additionally, microsatellites are codominant, thereby allowing both alleles at a locus to be identified in heterozygotes, allowing heterozygosity to be measured directly. Unlike maternally inherited mitochondrial DNA, which may evolve too slowly to discern recent genetic structuring in small populations, microsatellites are considered selectively neutral and their concordance with simple Mendelian inheritance makes them useful for determining genetic diversity over relatively short time scales.

During this phase of the study, I examined genetic variation among Rincon, Santa Catalina and Tortolita Mountain tiger rattlesnake populations, including four long-term study sites at SAGU (Rocking K, Tanque Verde Ridge, Expansion Area, and Loop Road) and one site in the Tortolita Mountains (Stone Canyon). The Rincon and Santa Catalina Mountains remain connected at higher elevations, however, the sprawling city of Tucson has effectively isolated them from each other at lower elevations. The Tortolita Mountains are isolated geographically, and separated from the other two ranges by the Tucson Basin (i.e., Oro Valley). Distances between these mountain ranges are not so great as to preclude possible intermixing of rattlesnakes, especially in the past when the city of Tucson did not act as a presumably impenetrable barrier to movement. By comparing genetic distance (variation among populations) to geographic distance, and by calculating past migration rates among populations, I estimated historic rates of gene flow and assessed the degree of current isolation. In addition, I simultaneously gathered information on tiger rattlesnake movements, home ranges, and reproduction using radiotelemetry (Goode and Wall 2002). I used data on movement patterns to provide a modern context for observed genetic relationships among populations.

An additional objective of this study was to use genetic markers to conduct paternity analyses on neonate tiger rattlesnakes born to known mothers. My goal was to assign paternity to known males in order to make inferences about mating system dynamics, relative contributions to future genetic representation, and relative fitness of breeding adults. Unfortunately, I was unable to obtain a sufficient sample size of neonates, because tiger rattlesnakes typically only reproduce every 2-3 years, and females gave birth under large boulders, making it extremely difficult to capture offspring for paternity analyses.

Finally, using a combination of radiotelemetry data and genetic analysis, I make recommendations that will hopefully have management implications for species in fragmented

habitats, including translocation strategies, and reserve design. Both districts of SAGU face a difficult challenge in their efforts to mitigate against human encroachment; I hope that this research will lead to a greater understanding of potential effects of increasing isolation of these important protected areas.

Methods

To date, I have obtained nearly 700 blood samples from tiger rattlesnakes from throughout the Tucson Basin. This grant, and other NPS funding, supported the collecting and sampling of a large number of these snakes. However, as originally proposed, this report only contains preliminary analyses of a subset of sampled snakes.

I collected approximately 50 μ L of blood from the caudal vein of rattlesnakes using a 25 gauge, $\frac{3}{4}$ inch butterfly blood collection set, and I stored blood in Vacutainer[®] vials buffered with EDTA. I anesthetized all animals in a closed chamber using 1 cc Isoflurane[®] (inhalant agent) before collecting blood, to minimize stress and to enhance safety of researchers. This method was approved by the University of Arizona Institutional Animal Use and Care Committee (IACUC Protocol #03-023), and has proven to be safe and effective for snakes and researchers alike. Field collection of rattlesnakes was authorized by scientific collecting and research permits from SAGU - NPS, Coronado National Forest - United States Forest Service, Department of Parks and Recreation - Pima County, and the Arizona Game and Fish Department.

Previous work, also supported by NPS, Intermountain Region Office, produced six microsatellite DNA markers that were informative for the tiger rattlesnake (Goldberg et al. 2003). I used these markers to examine genetic relatedness among the six putative populations. This work was conducted in collaboration with the Laboratory of Molecular Systematics and Evolution (LMSE), University of Arizona. I used the software packages ARLEQUIN and GENEPOP to conduct genetic analyses. I tested each genetic marker, each population, and each marker at each population, and summarized estimates by calculating heterozygosity for each population and genetic distance (using F_{ST} estimates) among all possible pairs of populations.

At my two study sites in the Rincon Mountains, using radiotelemetry, I obtained 2740 locations of 35 individual snakes from 1997-2001, and in the Tortolita Mountains, I obtained 5181 locations of 61 individual snakes from 2002-2006. I displayed rattlesnake location data on high resolution, georeferenced aerial photographs using ArcView GIS software (v. 3.3, ESRI, Inc.). I examined snake movement patterns in the context of landscape structure. I calculated movement distances and home range characteristics using the ArcView extension Animal Movement Analyses (available on-line through USGS, Alaska BRD).

Results

For this preliminary analysis, I scored a total of 149 tiger rattlesnakes, using six microsatellite loci. Using GENEPOP, I generated expected versus observed heterozygosities for all six putative populations as categorized by mountain range (Table 1). Using ARLEQUIN, I generated F_{ST} values for all six putative populations, including Tanque Verde Ridge, Rocking K, Loop Road and the Expansion Area within SAGU. This allowed me to examine among and within mountain range genetic relatedness (Table 2). I also produced a Nei's Diagram, which is

simply a cladogram representing among population relatedness of all six putative populations (Figure 1).

Table 1. Observed versus expected heterozygosities for 149 individuals from three tiger rattlesnake populations near Tucson, Arizona. Values in bold were significant in exact tests implemented in GENEPOP. The number of alleles for each locus and population is also given in red numbers. S5, S6, S8, S9, S10 and S12 are the codes given to identify each microsatellite locus.

Mountain Range	N	S5	S6	S8	S9	S10	S12
Rincon	103	0.81/0.85 18	0.25/0.36 5	0.94/0.93 22	0.97/0.95 39	0.76/0.91 21	0.21/0.27 5
Santa Catalina	13	0.85/0.90 11	0.08/0.08 2	1.00/0.97 17	0.85/0.97 18	0.69/0.86 10	0/0.15 2
Tortolita	33	0.79/0.89 14	0.03/0.03 2	0.97/0.93 18	0.88/0.95 22	0.88/0.90 11	0.03/0.36 3
Mean		0.82/0.88 14.33	0.12/0.16 3.00	0.97/0.94 19.00	0.90/0.96 26.33	0.78/0.89 14.00	0.08/0.26 3.33

Table 2. F_{ST} values for all six putative populations of tiger rattlesnakes near Tucson, Arizona. EA = Expansion Area, LR = Loop Road, RK = Rocking K, SCM = Santa Catalina Mountains, SC = Stone Canyon, TVR = Tanque Verde Ridge. Values in red were significant at the 5% nominal level using sequential Bonferroni correction.

Population	EA	LR	RK	SCM	SC	TVR
EA	----					
LR	-0.0029	----				
RK	-0.0054	0.0064	----			
SCM	0.0123	0.0281	0.0160	----		
SC	0.0385	0.0387	0.0334	0.0152	----	
TVR	0.0093	0.0240	0.0071	0.0150	0.0345	----

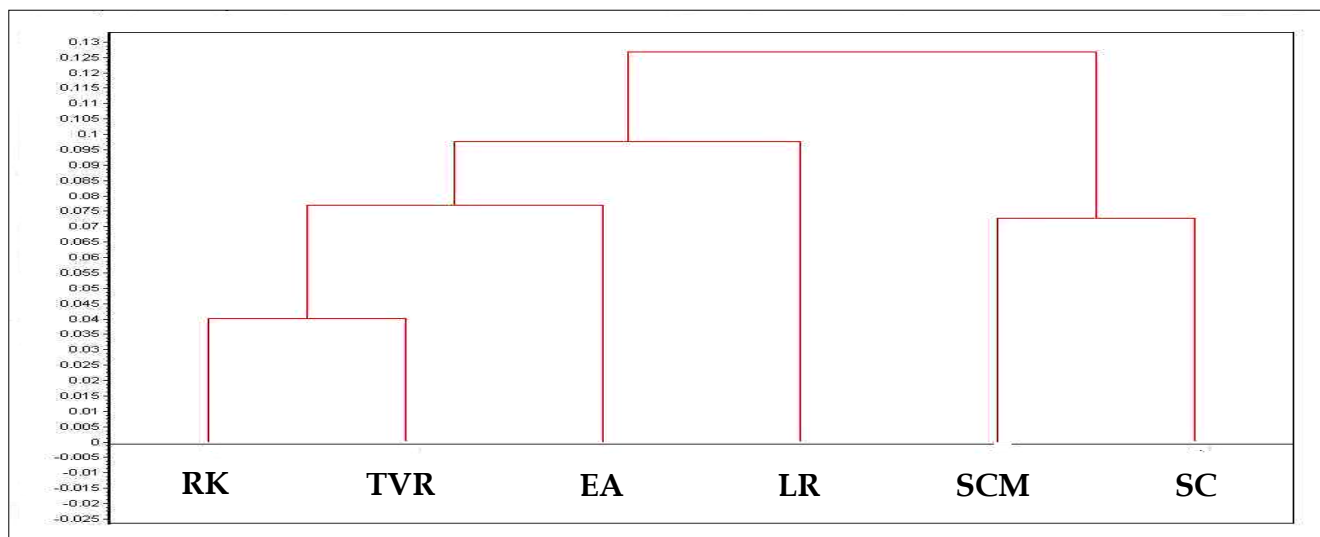


Figure 1. Nei's Diagram showing genetic relationships of six putative populations of tiger rattlesnakes near Tucson, Arizona based on 149 individuals scored using six novel microsatellite loci. Cladogram reveals an interpopulation relatedness that follows a relatedness by distance paradigm. EA = Expansion Area, LR = Loop Road, RK = Rocking K, SCM = Santa Catalina Mountains, SC = Stone Canyon, TVR = Tanque Verde Ridge.

Discussion

Tiger rattlesnake populations in the Tucson Basin exhibit considerable genetic structuring, even at relatively small spatial scales (i.e., 2-4 km). The pattern of genetic relatedness appears to conform to a relatedness-by-distance paradigm; the farther apart populations are geographically, the less genetic material they share in common. Future analyses will hopefully allow for closer inspection of differences in relatedness in relationship to potential barriers to gene flow, such as roads, housing developments and natural landscape features.

Interestingly, and not predicted by our research on tiger rattlesnake movement patterns, estimates indicate that at least a few individuals per generation have traveled between mountain ranges, crossing valley bottoms that are not typical of tiger rattlesnake habitat. For instance, computer analyses indicated that one to two individuals per generation contributed alleles to populations in neighboring mountain ranges. By way of example, Oro Valley and the large wash, Cañada del Oro, lies between the Santa Catalina and Tortolita Mountains. The minimum distance between rocky foothill habitats at the bases of both mountain ranges is approximately 4-5 km, a distance shorter than the longest known distance (i.e., 2.5 km) traversed by a male tiger rattlesnake during the course of an entire active season. Does this mean that observed sharing of genetic material between these two populations is historical, and if so, are recent anthropogenic barriers responsible for the lack of contemporary movements between mountain ranges? Perhaps the occasional snake does indeed travel the required distance, but we were just not fortunate enough to observe this kind of behavior in our radio tagged snakes. This seems unlikely given the large sample size of snakes we have followed over the last ten years.

Is it possible that resident populations of tiger rattlesnakes occupy valley bottom habitats between mountain ranges, and their offspring disperse to nearby foothill populations, thereby exchanging genetic materials? Again, our radiotelemetry data indicate that tiger rattlesnakes are unlikely to travel long distances away from rocky foothill habitat, and that valley bottom habitats are essentially unsuitable for long-term occupancy. However, tiger rattlesnakes do spend much of the active season in arroyos, which may allow the occasional snake to safely travel between mountain ranges. If we take the longest distance moved by male tiger rattlesnake (males move 4-5 times farther than females) during the course of an entire active season, the total maximum distance is over 6 km, which is greater than the distance separating the two mountain ranges. Of course, this would mean that the snake would have to move linearly, rather than within a polygon-shaped home range, where they often return to familiar sites within and among years. In addition, a snake would have to find a new den site, rather than returning to its previous den site. Although tiger rattlesnakes do exhibit linear movement patterns while using wash habitats, they typically return the way they came as they travel back to their dens, which they typically use year after year. However, male tiger rattlesnakes are known to switch den sites in successive years, including one male that traveled 2.5 km to a new den site.

In any case, if anthropogenic changes have blocked gene flow between the Santa Catalina and Tortolita Mountains, then the question remains whether there has been enough time to observe these changes. Based on observations of tiger rattlesnake reproductive behavior and periodicity, I estimate that their minimum generation time is approximately three years. At what point in time could anthropogenic changes in Oro Valley become a significant barrier to gene flow among populations on either side of the valley? Only scattered, low-density development was present in the area in the 1960's, which means that even if this limited human presence led to a significant reduction in gene flow, there would only be approximately 40 years, or 13

generations to see changes in allelic frequencies. Although microsatellite markers are known to evolve rapidly, 40 years is a relatively short period of time, and may not explain observed differences. We will refine our analysis in the future by quantifying the relative resistance of the landscape (e.g., number of roads and associated traffic volumes, housing developments, washes) and using this variable in an analysis of molecular variance that will allow for comparison of F_{ST} values relative to the resistance to movement stemming from the configuration of the landscape between populations.

Although connections between tiger rattlesnake populations can be inferred based on field data, indirect measures using genetics may provide better information in less time and at lower cost. With recent advances in genetics we may now address the issue of connectivity with a greater depth of understanding than was previously possible. Genetic analyses allow us to take a step back in time, so to speak, to reconstruct population dynamics in the past. It also allows us to peer into the future using data to construct predictive models. Because humans have relatively short life spans, researchers are unable to obtain this kind of information using traditional methods of ecological inquiry.

This preliminary analysis has been critical to formulation and refinement of future research questions, analytical techniques, and sampling strategies. In addition, we plan to use mitochondrial DNA markers to examine among-population relatedness at a much deeper time scale, which will hopefully shed light on historical connections among populations, and provide an important context for interpreting results from microsatellite data.

Management Implications

Managing the effects of habitat fragmentation and population isolation on wildlife populations is perhaps the greatest challenge for Arizona's land and wildlife managers. Currently, growth in the Tucson area (this holds true for Phoenix as well) is concentrated in areas of prime tiger rattlesnake habitat. As development proceeds, and natural areas such as SAGU become increasingly isolated, the need to maintain connections between wildlife populations becomes more acute. Although this research is preliminary, and we must use caution in applying the results to management, we feel that it has implications not only for SAGU in its efforts to manage the impact of human encroachment, but to other land and wildlife management agencies whose responsibility is to ensure that future generations can enjoy Arizona's incredible natural resources.

Based on this research, it seems likely that in order for tiger rattlesnake populations (and likely a variety of other species that travel to and from the park and surrounding areas) to persist in the mountains surrounding the Tucson Basin, connections that permit the occasional individual to travel from one mountain range to another will need to be maintained. This means that the most difficult challenge to SAGU, and other national parks and natural areas experiencing rapid development along their borders, will be not only to protect and promote wildlife populations within their boundaries, but to work with adjacent landowners to maintain connections between intact habitat fragments. To be effective, land management plans will have to take a regional approach in deciding which lands to protect and which lands to develop. Like the increasingly isolated wildlife populations they protect, national parks tend to operate in isolation, as they struggle to meet their mission of maintaining natural resources in a pristine condition. As threats to national parks become more and more externalized, park managers will need to become more involved in partnerships with neighboring land managers. This will require that park

managers engage not only other land management agencies with which they are used to dealing, but new and often difficult entities, such as private landowners that may have relatively little interest or knowledge of their surroundings, and the important role that they play in wildlife and habitat conservation. On the positive side, in the Tucson area, the Sonoran Desert Protection Plan is poised to provide a framework for long-term sustainability of the region, and protecting connections among mountain ranges has received considerable attention in the plan. It is worth noting that personnel at SAGU have played an important role in regional conservation planning, establishing effective relationships with the many and varied stakeholders involved.

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