Genetic Diversity of *Crotalus cerastes* in Southwestern North America

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Abstract

Crotalus cerastes is a species of rattlesnake native to the southwestern United States and northern Mexico. Three subspecies have been described, *Crotalus cerastes cerastes, Crotalus cerastes cercobombus, and Crotalus cerastes lateroropens*. However, subspecies are often classified based on color pattern and other superficial traits that may not represent the evolutionary history of the species (Burbrink et al 2000). By obtaining DNA from samples of *Crotalus cerastes* and barcoding them, the genetic diversity of populations at varying levels of genetic and geographic interconnectedness could be determined. Once the sequences came back, and were compared using a map and a phylogenetic tree, the diversity within these populations of differing relations was apparent. The findings suggested that both within the same subspecies and within the same general geographical area, there were significant enough genetic differences that distinct genetic groups could be identified. This implies that the way organisms are grouped into subspecies based on morphology may not be indicative of evolutionary history.

Introduction

Our project is to generate DNA barcodes from Sidewinder snakes, or *Crotalus cerastes*, from samples taken across the southwest US to better understand the phylogeographic history of this species. Our research will be adding to the data generated for a previous paper, this study focused on four different species of rattlesnake (*Crotalus mitchellii* [speckled rattlesnake], *Crotalus cerastes* [sidewinder], *Crotalus tigris* [tiger rattlesnake], and *Crotalus ruber* [red diamond rattlesnake]). They used mitochondrial DNA to determine species relationships, their relationships to the specific regions they were found in, and how genetically diverse they are. Our study is different in that it is going to focus on the three different subspecies of Sidewinders (*Crotalus cerastes cerastes* [Mojave Desert Sidewinder], *Crotalus cerastes lateroropens* [Colorado Desert Sidewinder] and *Crotalus cerastes cercobombus* [Sonoran Sidewinder]), as opposed to what was done in the previous study where four completely different species of snakes were analyzed.

One of our project objectives is to examine the three subspecies of these Sidewinders, and determine which of the three each sample belongs to. In determining whether there is significant genetic variation within populations of the same subspecies, we might give a more accurate phylogeographic map of the different subspecies of *Crotalus cerastes* in the southwest North America.

In order to understand our research, readers need to know that isolated populations of the same species can evolve differently. Initially, the species comprises one population in one certain area. But over time, as the species spreads out, different genetic mutations cause each population to adapt to better fit its new surroundings. These genetic mutations can be phenotypic or genotypic, and different subspecies are formed when populations differ significantly enough to be distinguished. Given ample time, it is possible that any of these subspecies could become an entirely new species. This could be determined when the individuals of different populations are unable to produce fertile offspring.

Materials & Methods

Our 29 samples were collected between 2013 and 2015. They are exclusively from *C. cerastes*, from the Mojave Desert, Sonoran Desert, and Colorado Desert. Each sample was either liver tissue or scales, because both are relatively easy to collect; A snake's liver can be easily located by cutting into a snake in the middle of its body, and scales can be extracted with nail clippers. Most our samples were taken from roadkill, because it's much easier to collect from a deceased animal than a live one. However, we also downloaded and georeferenced 11 samples from (Douglas et al 2006) to get a larger variety of data.

For our lab protocols, we used the Qiagen DNeasy Kit. Then, we amplified the selected DNA using PCR (a polymerase chain reaction) in order to prepare it to run a gel electrophoresis. The primers we used in the PCR were 9974L: 5'-AGC ACT AGC CTT TTA AGY T-3' and 10830H: 5'-AGA AAC CCT ATT TTT AGT ACT AG-3' (Bryson et al 2011). We ran the samples alongside a 1,000 base pair ladder so we could easily compare them. Then, we sequenced our PCR products on an ABI 3730 DNA sequencer housed in the molecular lab at the American Museum of Natural History. The resulting DNA sequence data was sent to DNA Subway, which was used to reconstruct the phylogenetic tree based on the the gene that was amplified, ATPase subunits 8 and 6.

Results

The data gathered through genetic barcoding returned DNA fragments between 680 and 950 base pairs in length. In grouping the sequence data from the snake samples and using phylogenetic inference, we identified 5 distinct clades with significant enough genetic differences to establish singular populations (see Figure 1). We organized our data both on a map to classify unique geographic populations (see Figure 1) and phylogenetic tree to pinpoint divergences and common ancestors over time (see Figure 3). The only subspecies that we found to be monophyletic was *Crotalus cerastes lateroropens* suggesting that genetic diversity may represent species-level diversity.

Tables & Figures

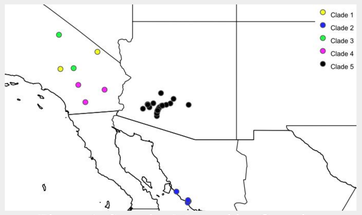

Figure 1: Samples Mapped by Genetic Similarity

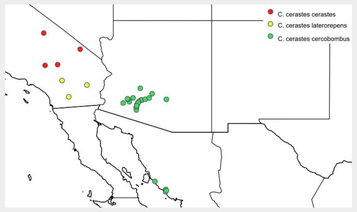

Figure 2: Samples Mapped by Subspecies

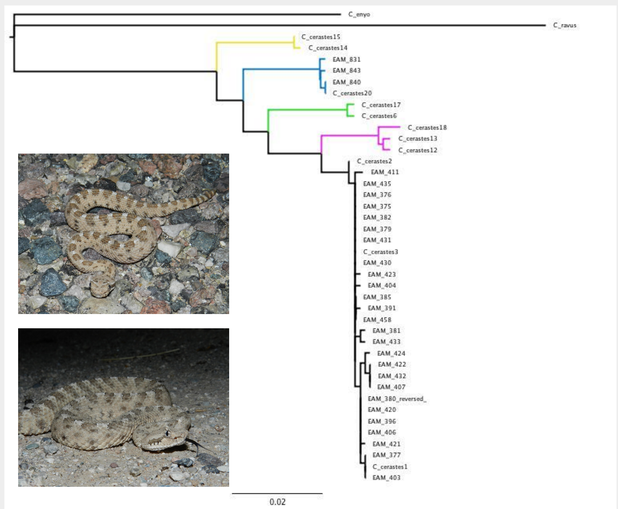

Figure 3: Phylogenetic Tree of *Crotalus cerastes* and photos of *C. c. cercobombus*

Discussion

Previous data has shown that there are three subspecies of *Crotalus cerastes*, which are *Crotalus cerastes cerastes, Crotalus cerastes cercobombus, and Crotalus cerastes lateroropens*, and that they are all unique to their own specific region of Arizona, California, and/or Mexico. In fact, *Crotalus cerastes cercobombus* is isolated from the other two subspecies by the Colorado River, which geographically isolates the population and may act as a barrier preventing inter-population breeding. As a goal for the project, we sought to classify our samples with each subspecies they belonged to, and see if those subspecies had significant effects on the genetic differences between population. However, while these subspecies manifested vaguely in our phylogenetic tree, their classifications are based on color pattern and other physical attributes as opposed to the evolutionary history of the species. That corresponds to our data, which showed no significant correlation between subspecies and genetic variation, but rather a stronger connection between subspecies and geographical separation. In our data, even samples within the same subspecies were significantly genetically varied when they were separated by large enough distance; as shown in Figure 1, clades 2 and 5 belong to the same subspecies, but are genetically different. Furthermore, as demonstrated with clades 1 and 3, populations of the same subspecies and in the same geographical area can also be genetically different.

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