Analysis of Intra-Population Genetic Variation in an Isolated Population of the Eastern Massasauga Rattlesnake (*Sistrurus catenatus catenatus)*

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Wartburg College Spring 2008

Abstract

The eastern massasauga rattlesnake (*Sistrurus catenatus catenatus*) is listed as being endangered in the state of Iowa. Its once widespread distribution across the state has been reduced dramatically and the massasauga may be limited to only four discrete populations within Iowa, one of which is found in east-central Bremer County. Previous studies have found small, isolated populations to be more susceptible to reductions in genetic variation rendering them more susceptible to extinction. The present study examines existing genetic variation within the Bremer county population using mtDNA sequencing. It was hypothesized that the population would possess low levels of diversity due to its relative isolation and small size.

Introduction

The eastern massasauga rattlesnake (*Sistrurus catenatus catenatus*) had a widespread distribution across Iowa and the central-northeast portions of the U.S. However, habitat disruption and destruction has limited its range to only a few isolated populations (Figure 2). The massasauga is listed as an endangered species in the state of Iowa due to the known presence of only four remaining populations within the state. One such population is located in in east-central Bremer county (Figure 3). This population has been studied extensively since 2001 through a multi-year mark and recapture study by T. J. VanDeWalle. The results from this study indicate that the population is of relatively small size, but appears to be stable (VanDeWalle, 2005). Although stable, population genetic theory suggests that small isolated populations are subject to reductions in genetic variation due to the combined effects of inbreeding and genetic drift (Bushar et al., 1998). This reduction in genetic variation can render a population more susceptible to extinction (Templeton et al., 1990). In this study the cytochrome b gene of mtDNA, a rapidly evolving molecule, was used in order to assess the genetic variation in the Bremer county population. It was hypothesized that due to small population size and relative isolation the population will show low haplotype diversity indicative of low genetic variation within the snake population. Genetic variation was analyzed in terms of phylogenetic clustering patterns on cladograms constructed from the cytochrome b sequence data. The information collected should provide insight to the degree of genetic variation still present in the population. From this information the Iowa DNR will be able to assess the risk of extinction facing the Bremer county eastern massasauga population and can then determine the best management strategies to preserve the population and devise an appropriate conservation plan for this threatened population.

Figure 1. The eastern massasauga rattlesnake (*Sistrurus catenatus catenatus*).

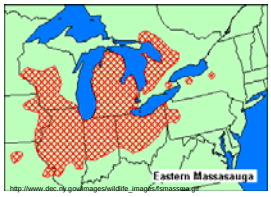

Figure 2. The eastern massasauga habitat range (highlighted in red) currently spans the central north-east portion of the U.S.

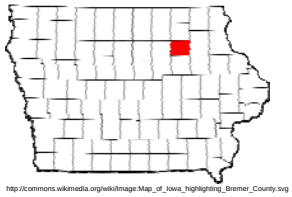

Figure 3. Bremer county (highlighted in red) is the location of the population of interest in this study.

Results

Due to complications with the DNA sequence analysis software, sequences could not generated for the entire Bremer county population. This prevented the construction of cladograms showing intra-population relationships. However, a sequence was generated from one sample. Amplification of the cytochrome b gene was confirmed by a NCBI BLAST database search. Results indicate that the sequenced fragment shared a 90% identity with *Sistrurus catenatus* mitochondrial cytochrome b-like gene, and an 85% homology with *Crotalus adamanteus* (related rattlesnake species) mitochrondrial cytochrome b. From this homology data a cladogram was constructed displaying the genetic similarity of the Bremer county sample to the *Sistrurus catenatus* sequence filed in the NCBI database (Figure 6).

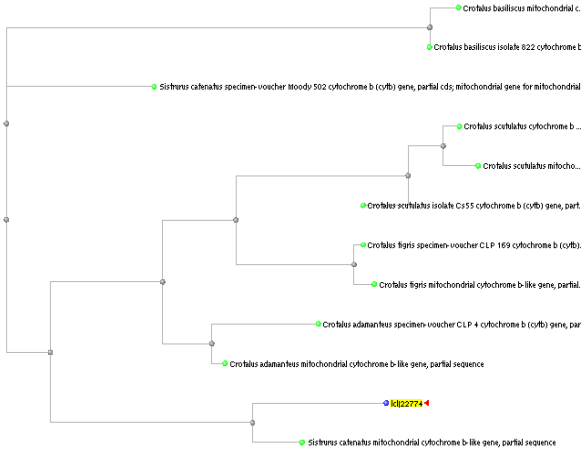

Figure 6. The figure above is a depiction of a cladogram that is generated by the NCBI BLAST. The Bremer county sample is indicated in yellow. The *S. catenatus* sample is from Ontario.

Materials and Methods

Twenty *S. c. catanatus* samples were examined from an Upper Wapsipinicon population located in Bremer County, Iowa. Samples were either subventral scale clippings or whole blood. DNA was isolated using standard phenol:chloroform:isoamyl methods following a SDS and proteinase K digestion (Howell and McCullough, 1990; Hedges et. al., 1991). MtDNA sequences were amplified with the polymerase chain reaction (PCR) using the following primer sequences flanking the target gene: L14841(5'AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3') and H15149(5'AAA CTGCAGCCCCTCAGAATGATATTTGTCCTCA-3')(Kocheret al., 1989). Thermocycling parameters included one cycle at 94ºC (3 min) followed by 40 cycles at 94ºC (1 min), 48ºC (1.5 min), 72ºC (2 min) and concluded with 5 minutes at 72ºC. Due to the low concentration of the initial PCR product, a second, identical PCR reaction was run on each sample. Following the second PCR, amplification was checked by looking for fragment bands on a 1% agarose electrophoresis gel (Figure 4). Upon verifying that the appropriate gene was amplified the PCR preps were purified using a Qiagen QIAquick PCR Purification Kit. After purification the samples were subject to another round of PCR in which fluorescent sequencing primers and dideoxynucleotides were incorporated into the amplified sequences, and were then analyzed using a LI-COR 4300 automated sequencer (Figure 5) (LICOR Biosciences, Lincoln, NE). Generated sequences were then analyzed using NCBI BLAST to verify the amplification of the cytochrome b gene and for phylogenetic alignment with related species.

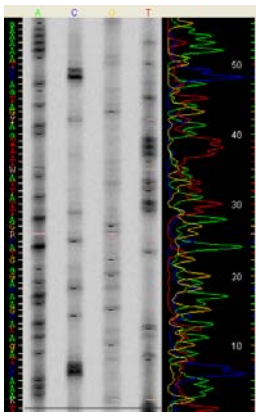

Figure 5. The figure above is a 1% agarose gel used to verify the amplification of cytochrome b. The first (top) lane displays the amplified cytb fragment in a sample of cow DNA acting as a control, while the successive lanes display the amplified cytb fragments *S. catenatus* samples.

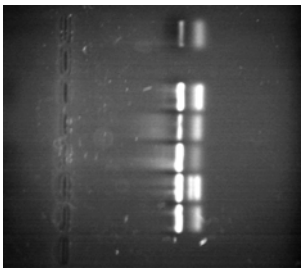

Figure 4. The figure above depicts the output from the Li-Cor DNA sequencer. This figure depicts the output from the Li-Cor DNA sequencer. The dark bands represent the various gene fragments on the gel. The histogram on the far right is used to determine the sequence of the gene.

Conclusions

- DNA was isolated successfully
- DNA sequencing confirmed the amplification of the species-specific cytochrome b gene which aligned most closely with a con-specific from S. Ontario
- Future work will be needed to resolve technical complications with sequence analyzing software

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Acknowledgements

We would like to thank T. J. VanDeWalle for his time and effort in collecting the tissue samples, as well as Dr. Shawn Ellerbroek for his support and assistance with DNA sequencing.