

NORTHERN ILLINOIS UNIVERSITY

**NEUTRAL AND ADAPTIVE GENETIC VARIATION IN THE EASTERN
MASSASAUGA (*SISTRURUS CATENATUS*)**

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ABSTRACT

Genetic diversity is fundamentally important for the long-term viability of populations, yet reduced genetic variation is often associated with small, isolated populations. The Eastern Massasauga (*Sistrurus catenatus*) is a small rattlesnake native to the Midwest and Great Lakes regions of the United States and Canada. Several factors, including habitat loss and fragmentation, have contributed to the decline of this species throughout most of its range. Although neutral genetic variation has been previously examined in this species, the levels and patterns of adaptive genetic variation within and among existing populations remain undescribed. The fundamental goals of this project were to (i) characterize variation in genes of the major histocompatibility complex (MHC) in the Eastern Massasauga, (ii) compare neutral and adaptive genetic variation in wild Eastern Massasauga populations in Illinois, and (iii) provide a comprehensive assessment of neutral and genetic variation in a captive Eastern Massasauga population.

First, I developed a molecular tool with which to assess adaptive genetic variation in the Eastern Massasauga. I cloned and sequenced a 166-bp portion of major histocompatibility complex (MHC) IIB exon 2 in this species and found evidence of 3–4 loci (copies of the gene). Analyses of substitution rates revealed the effects of strong positive selection, especially within the functionally important antigen-binding residues. These results provide an initial characterization

of MHC variation in the Eastern Massasauga and contribute to our understanding of MHC evolution in vertebrates.

Next, I used 454 deep amplicon sequencing to obtain MHC genotypes for Eastern Massasaugas in wild populations and in captivity. I examined MHC and microsatellite variation to assess the relative roles of natural selection and random genetic drift in shaping genetic variation within and among three Eastern Massasauga populations representing a gradient of demographic histories. Within Illinois, Eastern Massasaugas were sampled from (1) a large, stable population in Clinton County, (2) a small, declining population in Piatt County, and (3) a very small population on the verge of extirpation in Cook County. I identified 24 putatively functional alleles and one putative pseudogene based on 149 Eastern Massasaugas, with individuals possessing 1–12 alleles. As expected, levels of microsatellite diversity varied among the populations in a pattern consistent with demographic histories. While the number of MHC alleles per individual showed a parallel pattern to microsatellite variation across populations, MHC nucleotide diversity was highest in the population experiencing the most severe declines. These results suggest that genetic variation in the natural populations of the Eastern Massasauga is shaped by a combination of natural selection and genetic drift.

Finally, I examined MHC and microsatellite variation within a captive Eastern Massasauga population maintained as part of a captive breeding program, the Eastern Massasauga Species Survival Plan (SSP)®. These analyses revealed relatively high levels of microsatellite and MHC diversity within (i) the captive breeding population as a whole and (ii) each of three distinct subunits previously identified based on mtDNA variation. Thus, the captive population shows no evidence of inbreeding and appears to possess the functional variation necessary to adapt to future selective pressures, if these animals are reintroduced to the wild.