

Conservation Genetics of Green Salamanders (*Aneides aeneus*)

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Introduction

The Green Salamander is a species of threatened lungless salamander that occurs in the Appalachian Highlands of the eastern US. In Tennessee and Alabama, most known populations occur along the Cumberland Plateau. Little is known about the genetic relationships among populations, and it is possible that several distinct genetic clusters of populations exist within this species, which may require their own conservation and management priorities. This project examines the genetic diversity present in populations within the Cumberland Plateau and also looks at how connected are the populations within the Cumberland Plateau.

Methods

- Tissue samples (tips of tails) were collected from 15 sites and preserved in 100% EtOH.
- DNA was extracted from preserved tissue samples using the Qiagen DNeasy kit.
- DNA for mtDNA *cytb* and *nd4* genes was amplified using PCR. Gel electrophoresis was used to confirm DNA amplification.
- DNA was then sent for Sanger sequencing.
- Resulting sequences were aligned and trimmed in MEGA.
- Phylogenetic relationships among populations were assessed using maximum likelihood in RaXML with support assessed via bootstrapping.



Figure 1. Rock outcrops in forested areas at 25 sites along the Cumberland Plateau in Alabama and Tennessee were searched (left) for Green Salamander (right).

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Results and Discussion



Figure 2. Maximum likelihood genealogy based on mitochondrial *cytb* and *nd4* loci of 79 samples from 23 sites in AL, NC, TN, and WV.

- Three genetic lineages were discovered based on mtDNA
 - Southern Clade: southern Cumberland Plateau in northeast AL, south-central TN
 - Northern Clade: northern Cumberland Plateau, Central Appalachians, and Ridge and Valley in TN and WV
 - Blue Ridge Clade: Blue Ridge Mountains of NC
- Uncorrected sequence divergence between clades ranged 7.2–9.0%
- In the southern Cumberland Plateau, no genetic structuring was observed indicating substantial population connectivity
- Likewise, little genetic structuring was observed between populations sampled in WV and northern TN

Future Directions

- Additional populations will be sampled in TN, AL, and KY to address sampling gaps
- Genetic dataset will be expanded using a population genomic approach developed in collaboration with Tangled Bank Conservation