

Population status and genetic variability of two remaining populations of U.S. Endangered Dusky Gopher Frogs, *Rana sevosa*



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Abstract

As humans continue to encroach on the natural landscape, habitat is lost, altered, and fragmented. As a result, non-human populations are often reduced and connections among them are lost. The Dusky Gopher Frog, *Rana sevosa*, is an endangered species with only two populations remaining. Our primary objective for this study was to determine the genetic variation within each population and to quantify population differentiation between the two populations.

We genotyped individuals of each population for six microsatellite DNA loci and performed genetic analyses of variability within and between each population. We found that one of the ponds had lower genetic variation as was predicted based on population size estimates. We also found that neither population had a signature of recent reduction in population size. The two populations showed significant population genetic differentiation. The results of this research will help conservation efforts by aiding decision making about potentially mixing these two populations in a translocation of eggs to historic breeding sites.

Introduction

Reduction and fragmentation of natural habitat leads to an overall decline of populations of non-human organisms. The most severe consequence of this is complete geographic isolation of populations. Isolation leads to genetically reduced populations with no ability to exchange genes or to be recolonized following local extinctions.

Dusky gopher frogs (*R. sevosa*) are an example of such a species. Only two isolated populations are known to exist from a historic three-state distribution (Fig. 1). The population in Harrison County, Mississippi (Glen's Pond) has had demographic and genetic data collected over the last two decades (e.g., Richter et al. 2003) and is located on protected land in De Soto National Forest.

Conversely, the status of the population in Jackson County, Mississippi (Mike's Pond) is unknown because of its recent discovery (March 2004). It is located on unprotected land.

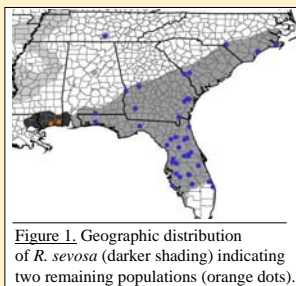


Figure 1. Geographic distribution of *R. sevosa* (darker shading) indicating two remaining populations (orange dots).

Our objectives were 1) to assess the status of the Jackson County population by examining genetic variability and testing for historic population declines and 2) to determine genetic differences between the two populations.

Methods

- Five eggs were collected from every egg mass deposited in 2005 at both study sites (Fig. 2) (n = 20 for Glen's pond; n = 5 for Mike's Pond).

- DNA was extracted from all eggs (n = 125) using Qiagen® DNEasy tissue kits.

- Six fluorescently labeled microsatellite primers were used in PCRs of all eggs according to protocol developed by Richter and Broughton (2005).

- Amplified microsatellite DNA was multiplexed, and genotypic data were collected on an ABI Prism® 310 Genetic Analyzer.

- We tested for differences in population genetic variation (observed heterozygosity; expected heterozygosity, and number of alleles) and for population differentiation using Arlequin® software. We used Bottleneck® to examine the data for signatures of large, historic reductions in population size.



Figure 2. Image of study ponds.

Results

- Genetic variation was low in both populations, although it was higher in the Glen's Pond population (Table 1).

- No significant signature of bottleneck was detected in either population.

- Significant population differentiation was found, and amount of differentiation was within the range of population-level expectations ($F_{ST} = 0.11$).

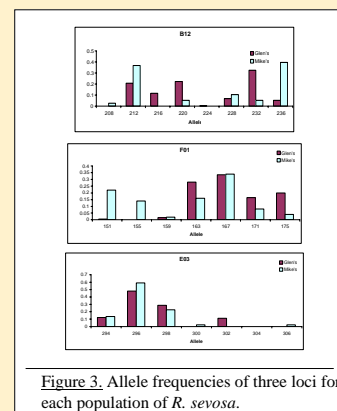


Figure 3. Allele frequencies of three loci for each population of *R. sevosa*.

Population	H _O	H _E	Mean # of alleles
Mike's Pond	0.446	0.522	4.2
Glen's Pond	0.626	0.612	5.3

Table 1. Genetic variation of each population.

Results are somewhat preliminary due to small sample sizes, which resulted from low reproductive efforts in 2005.

Discussion

Complete geographic isolation is detrimental to the survival of endangered species. Small population size results in low genetic variation and leads to genetic differentiation of populations. (Knaepkens et al. 2004). Although both populations of *R. sevosa* appear stable (i.e., no historical signature of bottleneck), the lack of gene flow constitutes a problem for their prolonged existence. Without gene flow into populations, inbreeding occurs which can lead to inbreeding depression. Also, no recolonization can occur in cases of local extinction. Because only two populations exist, local extinctions would lead to the extinction of the species.

Implementation of conservation efforts are vital for the long-term survival of this endangered species. One potential conservation strategy is to transfer eggs from the two populations to historic breeding sites. One application of our genetic data is to aid in determining whether we should mix individuals from these populations.

Literature Cited

- Knaepkens G., et al. 2004. *Biological Conservation* 115:403–410.
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Acknowledgments

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