Allozyme analysis of a contact zone between two mtDNA haplotypes in *Desmognathus ocoee* (Amphibia: Plethodontidae)

Noëlle K. J. Bittner and Stephen G. Tilley
Department of Biological Sciences, Smith College, Northampton, MA

Introduction

Recent phylogeographic analyses of mitochondrial DNA (mtDNA) sequences by Yoke et al. have revealed five distinct well supported clades within the salamander species traditionally called *Desmognathus ocoee* Nicholls (Fig. 1 and 5). This information suggests that the organisms currently recognized as *D. ocoee* may belong to more than one species. In the Blue Ridge Mountains of northeastern Georgia, a contact zone between two mtDNA haplotypes has been identified. Populations within this area vary notably in appearance, especially in regard to dorsal pattern and coloration although little has been done to resolve the relationships among these populations. My study sought to augment the mtDNA data with information from nuclear genes by comparing allele frequencies at six allozyme loci among populations of *D. ocoee* at and around the contact zone. Previous studies have found that allozyme frequencies tend to corroborate mitochondrial sequence data, indicating similar patterns of geographic differentiation among populations. The mitochondrial gene sequenced, Cytochrome b (*cyt b*), has proven to be valuable when delineating caudate species in previous publications (ie: Tilley et al., 2008). Independently, allozyme electrophoresis (Fig. 2) has been demonstrated to be an effective tool for elucidating species relationships.

Figure 1: Dorsal (L) and ventral (R) photographs of *D. ocoee*.

Materials and Methods

Individuals were collected from 21 populations across the contact zone of two clades recognized by Yoke et al. in northeastern Georgia. Population sample sizes ranged from ten to 34 specimens; samples of fewer individuals were excluded from analysis. Dorsal and ventral photographs were taken of all specimens pre-sacrifice then individuals were processed and stomach, liver and ventral muscles were extracted for analysis. Tail tips were frozen for future analysis and carcasses were preserved as voucher specimens. Standard methods of horizontal starch gel electrophoresis (Murphy et al., 1990) were utilized to determine allele frequencies at six allozyme systems: aspartate aminotransferase 1 (AAT1), glyceraldehydes-3-phosphase dehydrogenase (G3PDH), isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2, Fig. 2), lactate dehydrogenase 2 (LDH2) and peptidase (PEP). Seventeen additional loci were demonstrated to be essentially monomorphic during preliminary tests and were eliminated from further analysis. As a control, local *Desmognathus fuscus* samples, which have proven to be monomorphic at all loci (excluding G3PDH), were run on each gel. Allele frequencies were computed by Genepop (Raymond and Rousset, 1995).

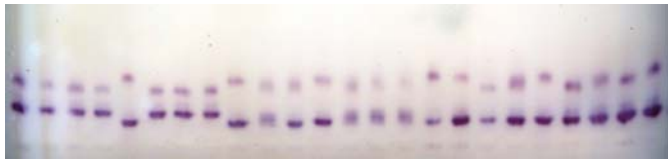

Figure 2: An example of a stained starch electrophoresis gel showing IDH1 (top) and IDH2 (bottom). Different bands within these staining regions represent products (allozymes) of different alleles. Three-banded patterns represent heterozygotes.

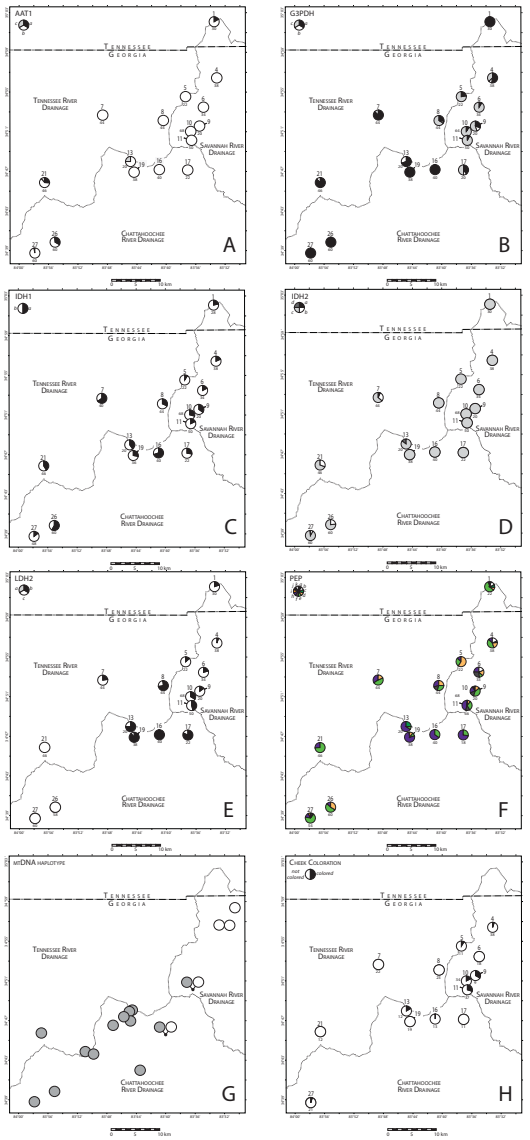

Figure 3A-H: Maps showing the collection sites with allele frequencies (A-F), haplotypes (G), and cheek coloration (H) for each population.

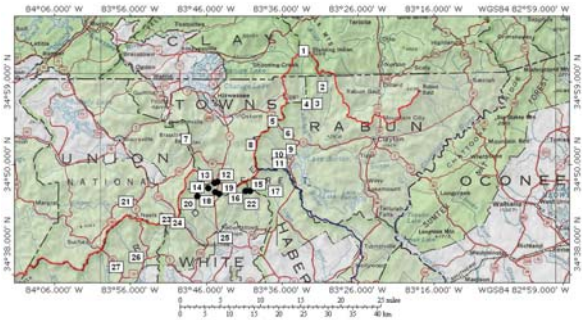

Figure 4: Locations of collection sites in northeastern Georgia, USA.

Results and Discussion

The mtDNA data display a North-South shift between the two haplotypes, with two populations containing both (Fig. 3G). The allozyme patterns do not correlate with this North-South trend (Fig. 3A-F). While varying in frequency among populations, allozyme variants do not show major frequency shifts where the mtDNA haplotypes replace each other. Different species of *Desmognathus* typically do not share alleles at a few to several of the loci surveyed in this study. The allozyme results suggest that the populations belong to a single species.

The origin of the discrepancy between the nuclear allozymes and mitochondrial nucleotide data poses questions of both animal behavior and molecular evolution. The geographic division of mitochondrial haplotypes could be linked to dispersal disparities between the sexes with migrating males and static females within populations. Little is known about the distances desmognathine salamanders disperse from their hatching sites. This study gives evidence for nuclear gene flow among populations greater than 60 km apart suggesting movement throughout the area. The discordance could also suggest very recent evolutionary divergence. Mitochondrial genes evolve at a faster rate than many nuclear genes because they are maternally inherited and thus the effective breeding size is smaller. A combination of behavioral and molecular explanations likely contribute to the discrepancy between the mtDNA and nuclear information.

Figure 5: *D. ocoee* beautifully displaying cheek coloration.
Photo Credit: S. G. Tilley

Sources

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