

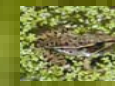
Preliminary Genetic Analysis of the Distribution of *Rana pipiens* and *Rana sphenoccephala* Across a Zone of Sympatry

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Rana pipiens



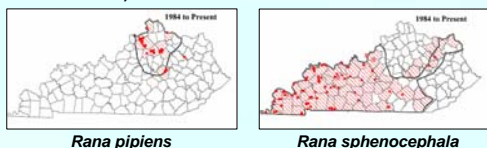
Rana sphenoccephala



Introduction

Hybridization can occur when two closely related species are sympatric. Based on our current understanding in Kentucky, the ranges of the northern (*Rana pipiens*) and southern (*R. sphenoccephala*) leopard frog overlap (Fig. 1).

Figure 1. Distribution of *Rana pipiens* and *Rana sphenoccephala* in Kentucky (Kentucky Department of Fish and Wildlife Resources 2005).



Hybridization can affect a species positively or negatively. Understanding the role that hybridization plays can greatly affect management strategies and conservation status.

R. pipiens and *R. sphenoccephala* are very similar morphologically. In Kentucky, distribution maps have been made based on morphology and mating calls. However, this is not always reliable, especially when hybridization may be occurring (Fig. 2). Therefore we combined morphological and vocalization data with genetic analysis to accurately identify these two species in Kentucky.

Figure 2. A white dot on the tympanum is used to identify *R. sphenoccephala*.



Objectives

- The objectives of this preliminary study were to:
- (1) collect samples from areas of range overlap for initial, coarse-scale analysis
 - (2) determine if the two species are interbreeding
 - (3) determine areas to target for data collection during the breeding season of 2008

Methods

Field

- Assisted John MacGregor (Kentucky Department of Fish and Wildlife Resources) and Martina Hines (Kentucky State Nature Preserves Commission) in surveying 62 ponds and wetland sites across Kentucky
- Collected adults, tadpoles, and eggs: clipped one toe from each adult, clipped 3mm from tail of each tadpole, and removed 5 embryos from each egg mass
- Samples were stored in 95% ethanol
- Leopard frogs samples were collected from 15 ponds in 10 counties in Kentucky (shaded blue in Fig. 3)

Figure 3. Counties in Kentucky where leopard frog samples have been collected.



Laboratory

- Chose sites from 5 counties for preliminary analysis (numbered 1-5 in Fig. 3)
- Extracted DNA from tissue samples using Qiagen DNeasy Blood and Tissue Kits
- Amplified a portion of mitochondrial gene ND2 for each
- Sequenced the genes using a 310 ABI Genetic Analyzer
- Aligned and compared nucleotide sequences of each individual and a reference *R. pipiens* from GenBank (Accession #: XXXXXXX) using Sequencher 4.7

Results

- When comparing sequences (513 bases) from sites 1 and 3 to the ND2 sequence of the reference *R. pipiens* and the *R. sphenoccephala* from site 5, 95 positions were variable. Of these, 15 were consistent with *R. pipiens* and 70 were consistent with *R. sphenoccephala*.
- When comparing sequences (551 bases) from sites 2 and 4 to *R. pipiens* and *R. sphenoccephala*, 114 were variable. Of these, 60 were consistent with *R. pipiens* and 22 were consistent with *R. sphenoccephala*.

Conclusions and Future Direction

We found discrepancies between morphological traits and mitochondrial genotypes, which suggests that *R. pipiens* and *R. sphenoccephala* are hybridizing in Kentucky (Table 1). The individuals from Scott Co. and Lewis Co. have genotypes consistent with *R. pipiens*, however, the morphology of the individual from Lewis Co. is consistent with *R. sphenoccephala*. The individuals from Madison Co. and Rowan Co. have genotypes consistent with *R. sphenoccephala*, however, the morphology of the individual from Madison Co. matches *R. pipiens*. Additionally, frogs from Lewis Co. (*R. sphenoccephala* morphology; *R. pipiens* genetically) have a vocalization that is *R. pipiens* like (J. MacGregor, pers. comm.).

Future work will focus on collecting more individuals to define the the hybrid zone, recording vocalizations and collecting detailed morphological analyses of individuals across the zone, incorporating a nuclear DNA marker (e.g., ITS-1), and performing fitness trials of hybrid offspring versus pure species offspring.

Table 1. Morphological appearance of leopard frogs from our study sites compared to their genotype.

Site	County	Morphological Appearance	Genotype
1	Scott	<i>R. pipiens</i>	<i>R. pipiens</i>
2	Madison	<i>R. pipiens</i>	<i>R. sphenoccephala</i>
3	Lewis	<i>R. sphenoccephala</i>	<i>R. pipiens</i>
4	Rowan	<i>R. sphenoccephala</i>	<i>R. sphenoccephala</i>
5	McCracken	<i>R. sphenoccephala</i>	<i>R. sphenoccephala</i>

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