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Developing Methods of Processing and Analyzing Genetic Data to Examine Tiger Salamander Population Structure

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Introduction

Anthropogenic influences in the Prairie Pothole Region of Minnesota have resulted in changing environmental conditions and subsequent declines in amphibian species population sizes (1, 2). Habitat fragmentation disrupts gene flow, causing genetic isolation and low genetic diversity within populations (3, 4, 5, 6, 7). By maintaining genetic diversity and gene flow, dispersal provides opportunities to re-establish populations after local extinction events.

To understand population structure and movement patterns of tiger salamanders in west-central Minnesota, Professor Waye and colleagues conducted a pilot study in 2014 to measure genetic differences between larvae captured in breeding ponds. They expected that ponds closer to each other would have more similar genetic information, and that genetic differences between ponds would increase with geographic distance. However, the initial analysis using standard techniques failed to uncover useful patterns in the data. Reorganization of the data and other quantitative approaches are needed to discover any significant patterns in this sample. In my research, I attempted a different modeling method to determine whether re-manipulating samples will uncover hidden patterns of genetic variation. My results improve understanding of how to use advanced statistical and computational methods tailored to complex problems with real-world data.

Table 1. Summary information for sampling methods in 2014 in the Pepperton WPA, Stevens County, MN.

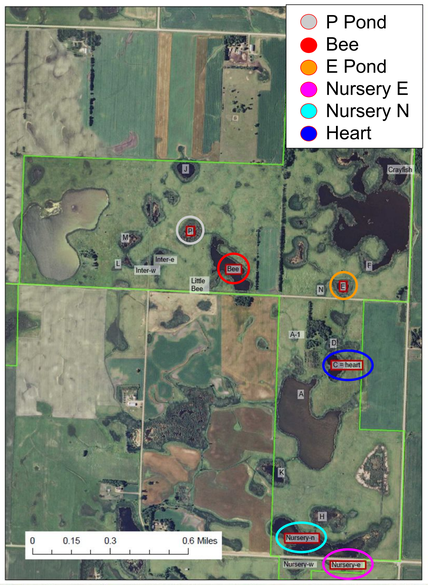
Distance	Name	# Samples
0	P Pond	18
100 m	Bee	18
1000 m	E Pond	12
10 km	Johnson	20
0	Nursery E	21
100m	Nursery N	22
1000 m	Heart	4
10 km	Crystal	16

Methods

- A total of 120 samples were collected from 8 ponds that were separated by 100 m, 1000m, and 10 km in the summer of 2014 (Table 1, Fig. 1)
- We used double-digest Restriction-site Associated DNA sequencing or RADseq process, a technique that interrogates DNA fragments, to generate single nucleotide polymorphism (SNP) data for around 20,000 loci per individual.
- RADseq libraries were run on a software pipeline called Stacks to build loci, fixed positions of genetic markers on chromosomes; this software has the potential to provide the resolution necessary to look for genetic differences between individuals.
- We structured a phylogenetic tree of local tiger salamanders by using a program called Molecular Evolutionary Genetics Analysis (MEGA)

Results

- The P Pond experienced the most genetic divergence from the other groups (Fig. 2)
- The Bee and Johnson ponds experienced the least genetic change compared to the other groups (Fig. 2)
- The genetic data do not match the geographic structure of the ponds (Fig. 1, Fig. 2)



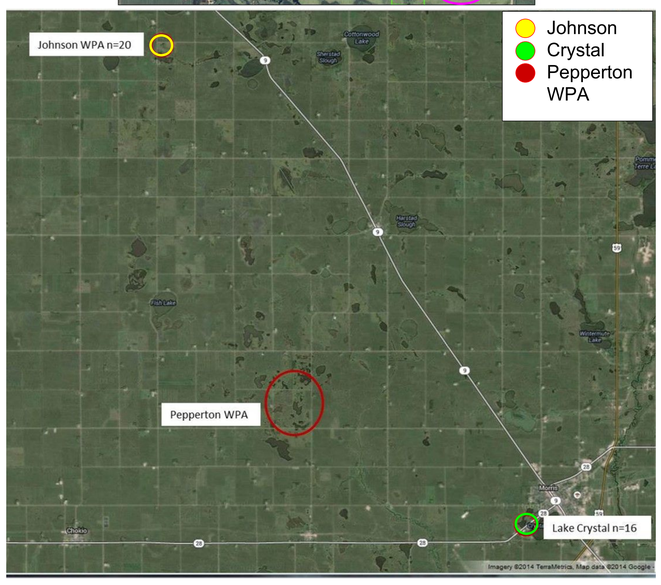


Figure 1. Locations of collected samples in the summer 2014 in the Pepperton Waterfowl Production Area and other locations, Stevens County, Minnesota.

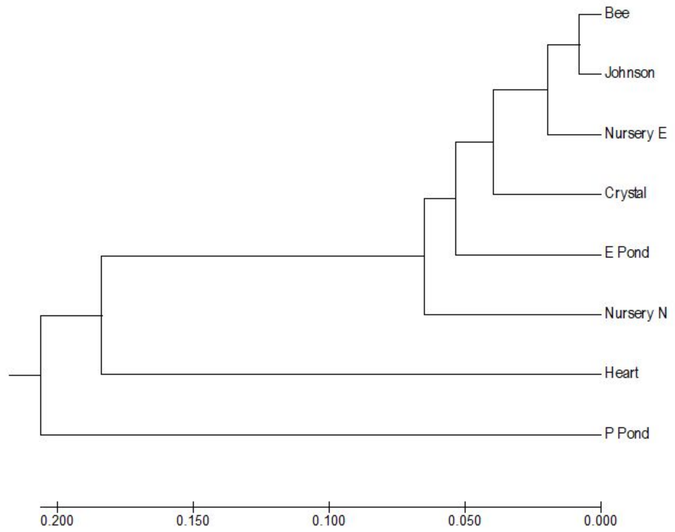


Figure 2. A phylogenetic tree of tiger salamanders from the ponds surveyed in this study. Branch lengths indicate genetic change, with longer branches indicating more genetic divergence.

Discussion

The results of this analysis suggest the genetic data do not fit the geographic structure of the ponds. It appears that there is not a clear pattern of movement of individuals and therefore little genetic differentiation among subpopulations. Another potential factor is that it is highly likely that the study site is occupied by two species of tiger salamanders (Eastern and Western), based on range maps and color pattern differences. This factor needs to be considered for future re-interpretation as there is a possibility that the two tiger salamander species are reproductively segregating within the ponds.

Future work will proceed by re-analyzing the data without taking the pond structure into consideration and finding out if the individuals naturally segregate into groups that are consistent with the pond structure.

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