Apr 22nd, 10:00 AM - 11:00 AM

Genetic Variation in the Ohio Population of Tonguetied Minnow (Exoglossum laurae)

Emmily Moses
Ohio Northern University

Sarah Armstrong
Ohio Northern University

Madeline Williams
Ohio Northern University

Genetic Variation in the Ohio Population of Tonguetied Minnow (*Exoglossum laurae*)

Emmily Moses, Sarah Armstrong, Madeline Williams, Jamie Siders, and Kenneth Oswald

School of Science, Technology, and Mathematics, Ohio Northern University, Ada, Ohio 45810

Introduction

- **Tonguetied Minnow classified as Endangered in Ohio**
  - **Small population in the Mad River (Figure 1)[1]**
  - **Possible low genetic diversity due to genetic drift[2]**
- **If population has low fitness**
  - **Unable to respond to biotic and abiotic changes**
- **Estimate genetic diversity at multiple loci**

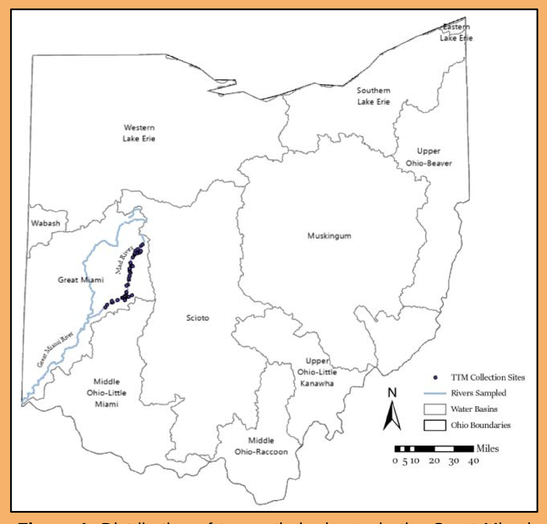

Figure 1. Distribution of tonguetied minnow in the Great Miami River. The population is restricted to the middle and upper Mad River.

Methods

- **Individuals collected via electroshocking or seine net**
- **Samples preserved in 95% ethanol**
- **Genomic DNA extracted via caudal fin clips**
- **Polymerase Chain Reaction (PCR) amplified five genetic loci**
  - **NADH dehydrogenase subunit 2 (*ND2*)**
  - **Mitochondrial Control Region (*D-loop*)**
  - **Myosin Heavy Polypeptide Intron 6 (*Myh6*)**
  - ***β*-actin Intron (*β-act*)**
  - **Major Histocompatibility Complex II (*Mhc-IIβ*; Figure 2)**
- **PCR amplicons sequenced via the Sanger method**
- **DNA sequences edited using Sequencher™**

Results

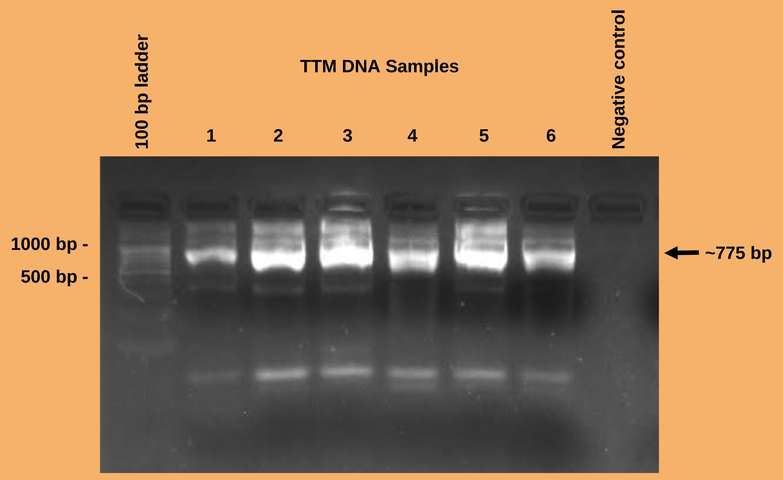

Figure 2. Amplification of tonguetied minnow DNA samples 1-6 using FishC12S and FishC12R primers[3]. Approximated size of the genome *Mhc-IIβ* amplifications was ~750 base pairs (bp). The amplicon was confirmed using Sanger sequencing. PCR amplifications of *Mhc-IIβ* will be cloned into plasmid pUC19 to determine variation in the gene sequence.

Table 1. Genetic parameters for PCR-amplified loci used in this study based on DNA sequence results. N_1 = number of individuals processed, N_2 = number of haplotypes/alleles discovered, N_3 = number of segregating sites, N_4 = length of DNA sequence. Population genetic summary statistics include gene diversity (*h*) and nucleotide diversity (π)[4]. *indicates approximate value.

Locus	N_1	N_2	N_3	N_4*	*h*	π
ND2	35	3	2	890	0.22	0.0005
D-loop	35	1	0	350	0	0
Myh6	32	1	0	440	0	0
β-act	35	1	0	550	0	0
Mhc-IIβ	35	?	?	775	?	?

Discussion

- **Overall (Table 1):**
  - **No diversity**
    - ***D-loop***
    - ***Myh6***
    - ***β-act***
  - **Low diversity**
    - ***ND2***
  - **Unknown diversity**
    - ***Mhc-IIβ***

Future Directions

- **PCR amplifications of *Mhc-IIβ* will be ligated into plasmid pUC19 and cloned into *E. coli* (Figure 3)**

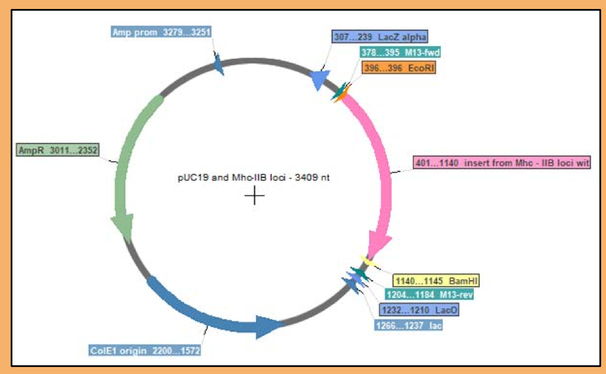

Figure 3. The pUC19 vector and *Mhc-IIβ* ligation using restriction enzyme sites of *EcoRI* and *BamHI*. This plasmid will be cloned into competent *E. coli* cells to identify alleles at the *Mhc-IIβ* locus.

- **Clonal sequences will determine genetic variation of *Mhc-IIβ***
  - **Effects of genetic drift *vs*. natural selection in the population will be estimated**
  - **Assist in conservation and management**

References

[1]Trautman MB. 1981. *The fishes of Ohio, 2nd Edition*. Ohio State University Press. Columbus, Ohio.

[2]Oswald KJ, and 15 others. 2020. Drainage history, evolution, and conservation of tonguetied minnow (*Exoglossum laurae*), a rare and imperiled Teays River endemic. *Copeia* 108:381-391.

[3]Ottová E, A Šimkova, J-F Martin, JG de Bellocq, M Gelnar, J-F Allienne, and S Morand. 2004. Evolution and trans-species polymorphism of MHC class IIβ genes in cyprinid fish. *Fish and Shellfish Immunology* 18:199-222.

[4]Hahn M. 2018. *Molecular population genetics*. Oxford University Press. Oxford, England.

Acknowledgements

We thank previous members of the Oswald Laboratory for their contributions to this project. Several organizations and agencies have generously supported this research, including the Ohio Environmental Protection Agency, the Columbus Zoo and Aquarium, the Ohio State University Museum of Biological Diversity, the North American Native Fishes Association, Miami University's Center for Bioinformatics and Functional Genomics, and Cleveland Metroparks Zoo.