

Testing Hypotheses of Atlantic Coastal Origin for the Endangered Eastern Pondmussel, *Ligumia nasuta*, in the Great Lakes

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Introduction

After the last of the Pleistocene glaciers retreated about 10,000 ybp, most freshwater mussels repopulated the GLR from the Mississippi River system. *Ligumia nasuta* was unique among unionid mussels in that it colonized the GLR from rivers on the Atlantic coast. It is currently unclear what population(s) in the NACR the GLR *L. nasuta* populations have directly descended from. It is hypothesized that they migrated from a region of early Lake Ontario meltwater that connected the GLR to the Mohawk or Hudson Rivers. *Ligumia nasuta* is a highly imperiled species throughout its range (e.g., Endangered in Canada and three U.S. states), largely as a result of competition with invasive Zebra Mussels. Therefore, it is particularly important to understand the genetic lineage of remaining *L. nasuta* populations, so that future recovery efforts are properly managed. Further understanding of *L. nasuta*'s genetic lineage will have large implications on its conservation and management.

Objectives

- (1) What is the genetic diversity of *L. nasuta*, among populations in the Great Lakes region (GLR)?
- (2) Do *L. nasuta* in the Great Lakes region represent a single genetic lineage (indicating a single post-glacial introduction) or multiple lineages (indicating post-glacial introductions from multiple sources)?
- (3) Which Northern Atlantic coastal population or populations belong to a similar or ancestral genetic lineage to the Great Lakes populations?

Methods

- Two mitochondrial DNA loci were used to determine genetic diversity and phylogeographic structure of *L. nasuta* from 63 individuals collected from 26 sampling locations that were pooled into 5 populations (Figure 1).
- Non-destructive foot swabbing or mantle biopsies were used to collect the samples for DNA analysis.
- Two sections of mitochondrial DNA were amplified and sequenced. They were a 453 bp sequence of the CO1 locus and 511 bp of the ND1 locus.
- Sequences were aligned using BioEdit and MacClade software.
- Haplotypes were identified using Collapse v.1.2 software.
- Haplotype networks were constructed using TCS v.1.21 software.
- AMOVA was implemented in ARLEQUIN.

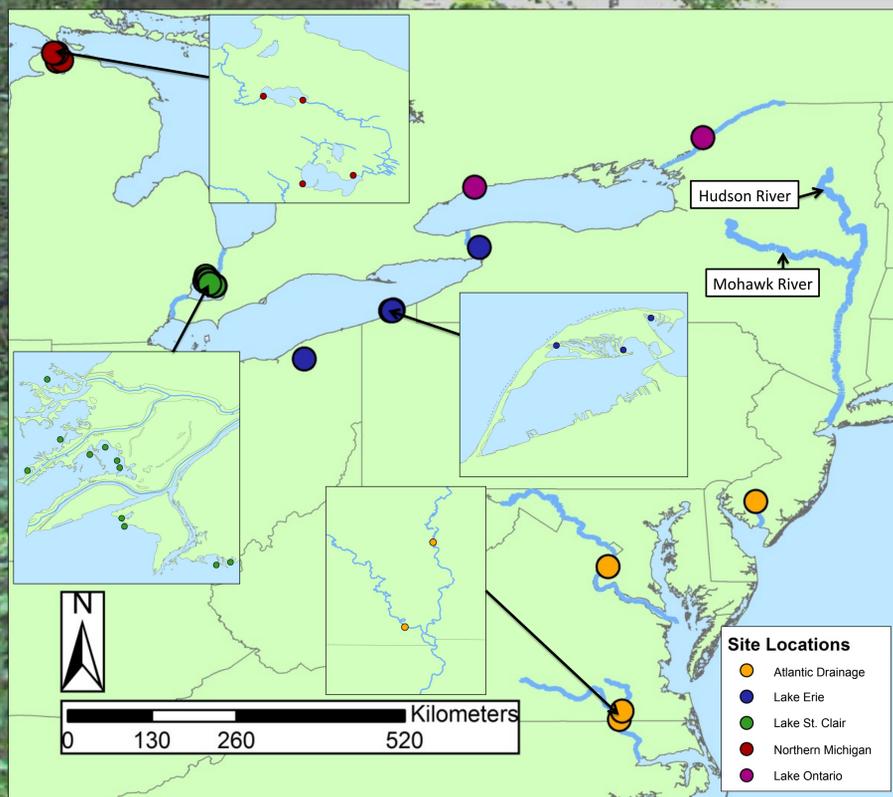


Figure 1. A map of the sampling sites for *L. nasuta*, which have been grouped into five pooled populations. Inset maps display greater detail of the sites in relation to each other. The map also depicts the Hudson and Mohawk Rivers, which are the theorized route that the *L. nasuta* colonized the Great Lakes.



Results

CO1 Results

- Four haplotype sequences were recovered for CO1 (Figure 2).
- The GLR displayed only a single haplotype. This haplotype was found in each of the Atlantic Drainage populations.
- The percentage of variation among populations was 38.34% ($P < 0.0001$) and the percentage of variation within populations was 61.66% (Table 1).
- Pairwise Φ_{ST} comparing the haplotype differences between populations, only showed significant differences between the four GLR populations and the NACR population (Table 2).

ND1 Results

- Six haplotypes sequences were recovered for ND1 (Figure 3).
- The two most common ND1 haplotypes, Haplotype 1 and 2, were found in all GLR populations. Haplotype 1 was also present in the Nottoway River and Blackwater River (VA), and the Willowgrove Lake Maurice (NJ). Haplotype two was also present in the Nottoway River (VA).
- The percentage of variation among populations was 9.96% ($P = 0.0128$) and the percentage of variation within populations was 90.04% (Table 1).
- Pairwise Φ_{ST} showed significant differences between the four GLR populations and the NACR populations (Table 2).

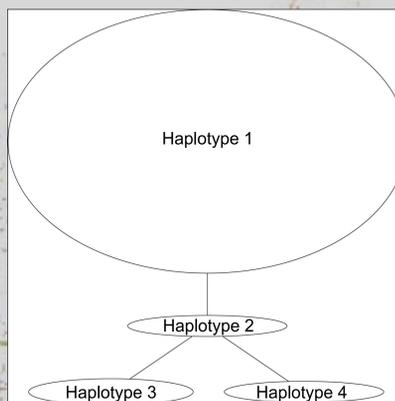


Figure 2. Spanning network of mtDNA haplotypes at the CO1 locus for *L. nasuta*. The connecting lines represent a single base pair difference between adjoining haplotypes. The relative size of the ovals represent the frequency of the haplotypes in the samples sequenced.

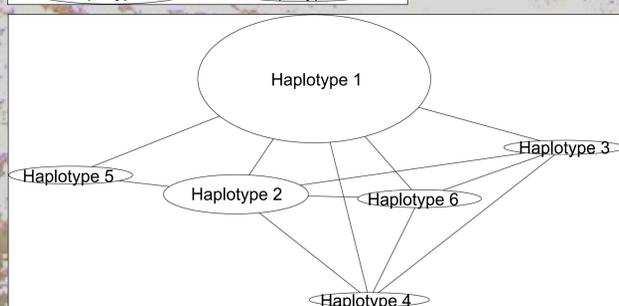


Figure 3. Spanning network of mtDNA haplotypes at the ND1 locus for *L. nasuta*. The connecting lines represent a single base pair difference between adjoining haplotypes. The relative size of the ovals represent the frequency of the haplotypes in the samples sequenced.

Table 1. Number of samples, genetic diversity, nucleotide diversity, and the number of haplotypes found of the five pooled populations. Values are given for the CO1 loci results and then for the ND1 loci results.

Population	CO1			
	N	Gene Diversity	Nucleotide Diversity	No. of Haplotypes
Northern Michigan	12	0.0000	0.0000	1
Lake St. Clair	14	0.0000	0.0000	1
Lake Erie	21	0.0000	0.0000	1
Lake Ontario	7	0.0000	0.0000	1
Atlantic Drainage	9	0.7778	0.0030	4
ND1				
Northern Michigan	12	0.1667	0.0003	2
Lake St. Clair	15	0.4190	0.0008	2
Lake Erie	17	0.3824	0.0007	2
Lake Ontario	7	0.0000	0.0000	1
Atlantic Drainage	10	0.8889	0.0023	6

Table 2. Φ_{ST} values among five pooled populations of *L. nasuta* from ND1 (above diagonal) and CO1 (below diagonal). Values with asterisk are statistically significant ($\alpha = 0.05$).

	Northern Michigan	Lake Saint Clair	Lake Erie	Lake Ontario	Atlantic Drainage
Northern Michigan	-	0.033	0.007	-0.051	0.155*
Lake St. Clair	0.000	-	-0.062	0.121	0.165*
Lake Erie	0.000	0.000	-	0.089	0.172*
Lake Ontario	0.000	0.000	0.000	-	0.114*
Atlantic Drainage	0.386*	0.415*	0.498*	0.290*	-

Discussion

- The genetic diversity within the tested populations was relatively low within the GLR. The NACR genetic diversity studied was slightly higher.
- The similarity of the genetic haplotypes in the study of *L. nasuta* suggests that its post-glacial colonization into the GLR occurred relatively recently from a population with low genetic diversity, not allowing much time for natural mutations of the DNA to accumulate. However, the NACR source population for *L. nasuta* is still unclear.
- This lack of significant genetic differentiation among GLR sampling locations implies that the *L. nasuta* in the GLR can be treated as a single management unit with respect to conservation efforts to repopulate habitats of the endangered species. However the *L. nasuta* in the GLR and NACR cannot be treated as a single management unit.
- A phylogeographic study of a different freshwater mussel with a similar distribution to *L. nasuta*, *Lasmigona subviridis*, found that distinct river systems, flowing into the Atlantic Ocean, had different genetic lineages. This study suggested that the different rivers should be viewed as evolutionally distinct.
- In contrast to the study on *L. subviridis*, the lack of haplotype diversity among *L. nasuta* populations in the GLR and NACR suggest that the species colonized the GLR relatively recently and from a single post-glacial introduction. These data are consistent with the hypothesis that *L. nasuta* entered to the GLR from a region of Lake Ontario meltwater that connected the Great Lakes to the Mohawk or Hudson Rivers. The low genetic diversity observed across the range of *L. nasuta* suggest a possible bottleneck event during the Pleistocene.
- This study was limited by the number of sampled populations and the comparison of only two loci of mitochondrial DNA, thus further analysis, including microsatellite DNA genotyping, is recommended to investigate this phenomenon.

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