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# MITOCHONDRIAL DNA VARIATION IN THE EASTERN POND MUSSEL, *LIGUMIA NASUTA* (BIVALVIA: UNIONOIDA), IN THE GREAT LAKES REGION

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## ABSTRACT

Most freshwater mussel species in the Great Lakes colonized the region from the Mississippi River basin and few appear to have colonized from Atlantic coast rivers. The Eastern Pondmussel, *Ligumia nasuta*, is widespread along the Atlantic coast but occurs elsewhere only in the Great Lakes, suggesting that it is one of the few Great Lakes species of Atlantic origin. Great Lakes populations are now imperiled following invasion of the lakes by dreissenid mussels. We examined patterns of diversity in the mitochondrial CO1 and ND1 genes in *L. nasuta* populations in the Great Lakes and in Atlantic coast rivers. Genetic diversity was low in Great Lakes populations and included only one CO1 and two ND1 haplotypes, all of which were also found in Atlantic coast populations. Genetic diversity was higher in Atlantic coast populations and included four CO1 and six ND1 haplotypes. Pairwise  $\Phi_{ST}$  revealed significant genetic differentiation for both genes between Atlantic coast and Great Lakes populations but not within Great Lakes populations. These results suggest that all populations of *L. nasuta* in the Great Lakes are derived from a single, small founder group that colonized from an Atlantic coast river. As such, Great Lakes populations may be considered a single management unit and conservation efforts based on propagation or translocation should be limited to use of Great Lakes source stock to prevent introduction of non-native haplotypes.

**KEY WORDS** Endangered mussels, genetic variation, Laurentian Great Lakes, phylogeography, glaciation, Atlantic coast

## INTRODUCTION

The diverse mussel fauna of the Laurentian Great Lakes upstream of Niagara Falls (referred to here as the upper Great Lakes) is a result of dispersal into the region following the end of the Wisconsin glaciation about 11,000 years ago. Most species (about 40) colonized the region from the Mississippi River basin (van der Schalie, 1963; Graf, 2002) and genetic evidence suggests that there were multiple colonization routes (Elderkin et al., 2007, 2008). Only two species are thought to have colonized the region from Atlantic coast river systems: the Eastern Pondmussel, *Ligumia nasuta* (Say, 1817) and Eastern Elliptio, *Elliptio complanata* (Lightfoot, 1786). The dearth of Atlantic coast species is a result of the long-standing barrier of Niagara Falls and the limited number of post-glacial colonization routes between Atlantic coast rivers and the upper Great Lakes (Man-

drak & Crossman, 1992; Strayer & Jirka, 1997; Larson & Schaetzl, 2001; Lewis et al., 2012). In contrast, Lake Ontario and the St. Lawrence River system downstream of Niagara Falls have a higher proportion of Atlantic coast mussel species, suggesting that this region has had more exchange with other Atlantic coast rivers (Haag, 2012).

*Ligumia nasuta* is widely distributed in Atlantic coast rivers from South Carolina to Maine (Nedeau et al., 2000; Price, 2005). In the upper Great Lakes, *L. nasuta* was locally common but restricted mainly to the Lake Erie and Lake St. Clair watersheds and a small portion of the Lake Huron and Lake Michigan watersheds, and it was widely distributed downstream of Niagara Falls (COSEWIC, 2007; Watters et al., 2009). The distribution and abundance of *L. nasuta* in the Great Lakes

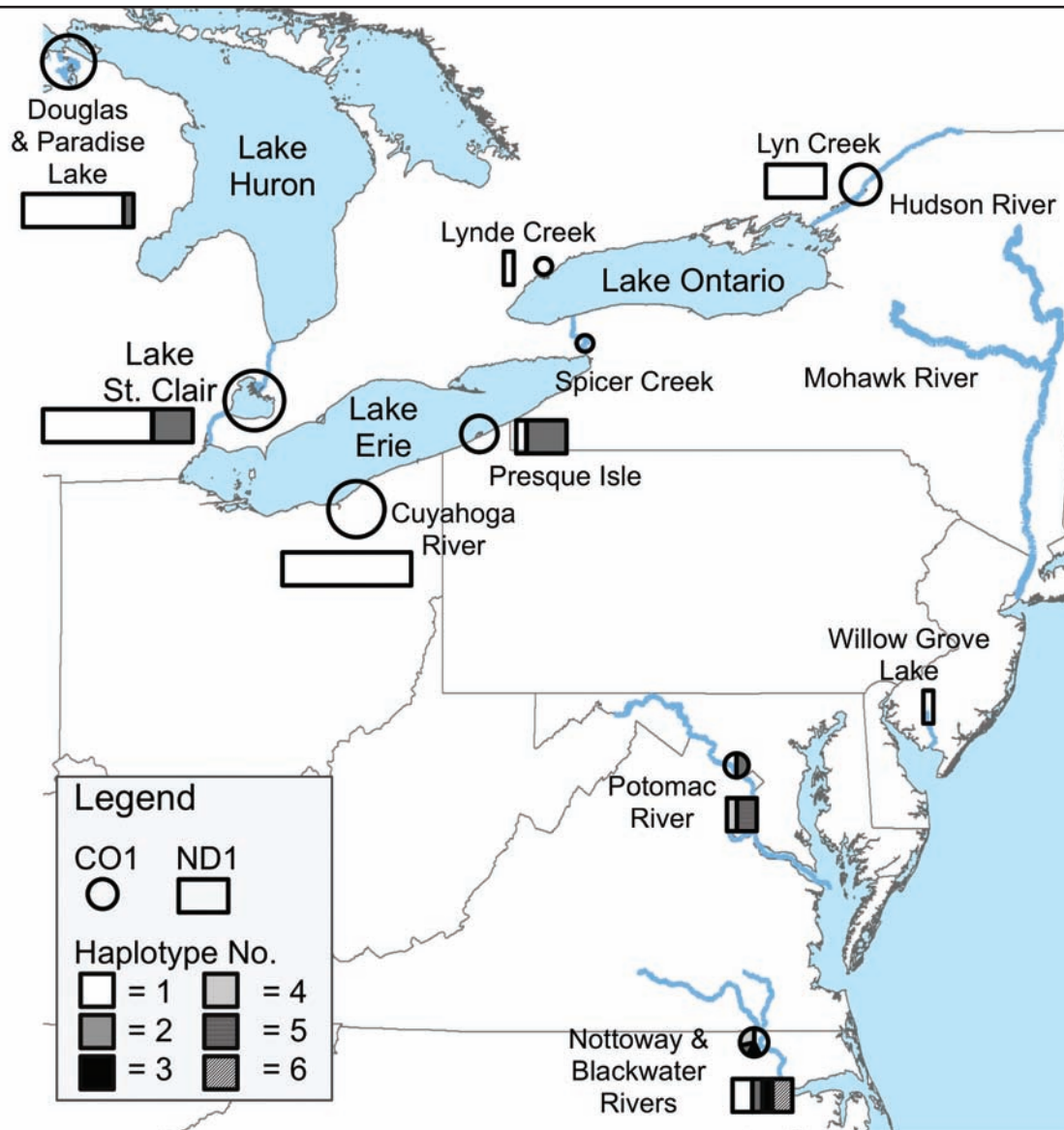
was greatly reduced after introduction of invasive dreissenid mussels (*Dreissena* spp.) (Nalepa et al., 1991; Schloesser et al., 1996; Zanatta et al., in press), and the species is in danger of extirpation from the region. Because it remains widely distributed in Atlantic coast rivers, *L. nasuta* is considered “apparently secure” globally (NatureServe, 2013). However, the genetic relationship of surviving Great Lakes populations to those on the Atlantic coast is unknown.

We examined DNA sequence variation in the mitochondrial COI and ND1 genes in populations of *L. nasuta* in the Great Lakes and Atlantic coast rivers. We use these

data to 1) examine the colonization history of the species in the Great Lakes, and 2) provide information necessary for management and conservation of the species.

### METHODS

A total of 64 individuals were collected in 2011 and 2012 from 17 sites within five major watersheds or geographical regions: northern Michigan (Lake Michigan and Huron drainages), Lake St. Clair, Lake Erie, Lake Ontario (including the St. Lawrence River system), and Atlantic coast rivers (Fig. 1; Table 1). Mussels were col-



**FIGURE 1**

Sampling sites and haplotype frequencies for *L. nasuta*. The size of circles (CO1) and rectangles (ND1) indicates the relative sample sizes (number of individuals) for each gene. CO1 haplotype circles are centered over the sampling location area they represent. Note that CO1 and ND1 mtDNA sequences were not resolved at some sites and these sites lack the corresponding symbol. Some closely adjacent sample sites are represented by a single symbol representing pooled results for those sites (e.g., Presque Isle); Table 1 provides a complete list of sample sites

**TABLE 1**

Sampling sites for *Ligumia nasuta*. Sites were pooled by region for statistical analysis (see text). Sites were pooled by population for depiction of haplotype frequencies on Fig. 1.

Region	Population	Site
Northern Michigan	Douglas Lake and Paradise Lake	Douglas Lake, Cheboygan Co., Michigan Paradise Lake, Emmet and Cheboygan Co., Michigan
	Lake St. Clair	Big Muscamoot Bay, St. Clair Co., Michigan Goose Bay, St. Clair Co., Michigan Little Muscamoot Bay, St. Clair Co., Michigan Bass Bay, Walpole Island First Nation, Ontario, Canada
Lake Erie	Cuyahoga River	Cuyahoga River, Geauga Co., Portage Co., Ohio
	Presque Isle	Thompson Bay, Erie Co., Pennsylvania Presque Isle Bay, Erie Co., Pennsylvania Duck Pond, Erie Co., Pennsylvania
	Spicer Creek (Niagara River)	Spicer Creek, Grand Island, Erie Co., New York
Lake Ontario	Lynde Creek (Lake Ontario)	Lynde Creek, Durham Region, Ontario, Canada
	Lyn Creek (St. Lawrence River)	Lyn Creek, Leeds and Grenville Co., Ontario, Canada
Atlantic coast	Willow Grove Lake (Maurice River)	Willow Grove Lake, Salem Co., New Jersey
	Potomac River	Potomac River, Montgomery Co., Maryland
	Nottaway and Blackwater rivers	Nottaway River, Southampton Co., Virginia Blackwater River, Franklin, Virginia

lected with clam rakes or by hand with SCUBA and snorkeling. Two methods were used to collect DNA: a swab of mucus from the foot, which was stored in sterile lysis buffer (Henley et al., 2006); or a clip of mantle tissue stored in 95% ethanol (Berg et al., 1995). The collection method depended on permit restrictions for rare species in each state or province. Each mussel was gently opened along the ventral margin <1 cm to obtain the sample, after which the mussel was returned to the substrate. All samples were stored at -20°C in the laboratory. Only female lineage mtDNA was sampled because methods required to obtain gonadal tissue for male lineage mtDNA are typically lethal.

DNA was extracted from samples using an overnight digestion with proteinase K. The alcohol extraction method of Sambrook et al. (1989) was used for mucus samples and Qiagen DNeasy extraction kits were used for mantle clips. Genomic DNA was stained with SYBR Green (or Ethidium Bromide) and electrophoresed in a 1.5% agarose gel to confirm presence. Two mtDNA regions were amplified, the mitochondrial cytochrome c oxidase subunit 1 (CO1) and the NADH dehydrogenase subunit 1 (ND1) using primers described in Campbell et al. (2005). Samples from the Cuyahoga River were run at Cleveland State University in 25 µL volumes consisting of 10 µL of deionized water, 5.5 µL of a 5X buffer, 2.75 µL of 2.5 mM dNTPs, 2.75 µL of each primer at 2.5 mM, 2.75 µL of 0.25 mM MgCl<sub>2</sub>, and 0.15 µL Taq polymerase. All other samples were run at Central Michigan University in 10 µL volumes, made of the mixture of 1 µL 10X Buffer, 1 µL bovine serum albumin, 0.3 µL of forward primer, 0.3 of reverse primer, 0.2 µL of dNTP, 5.15 µL of deionized water, and 0.05 µL Taq polymerase per sample. To each assay 1 µL extracted DNA was added. If the initial PCR reaction did not work, an additional 0.2 µL of MgCl<sub>2</sub> replaced an equal amount of water. The thermocycler amplification conditions for both mtDNA regions were as follows: denaturation at 92-94°C for 2 minutes; five cycles of 92-94°C for 40 seconds; 40°C for 40 seconds; 72°C for 90 seconds; 25 cycles of 92°C for 40 seconds; 50°C for 40 seconds (or 49°C for all cycles), and 72°C for 90 seconds. Completed reactions were held at 4°C and then placed in the freezer. Primers were removed from amplified samples using a QIAquick® PCR Purification Kit or an Exonuclease I (Amersham Biosciences cat# E70073X, 10 U.ml) and shrimp alkaline phosphatase (SAP) (Amersham Biosciences cat# E70092X 1U.ml) (78 ml ddH<sub>2</sub>O, 2 ml ExoI, 20 ml SAP) reaction to denature enzymes, and incubation at 37°C for 40 min followed by 80°C for 20 min. Amplified samples were sequenced on an ABI 3730 (Applied Biosystems).

The sequences of the two mtDNA regions were aligned and edited using BIOEDIT (Hall, 1999) and MAC-

CLADE (Maddison and Maddison, 1997) software. Haplotypes were identified using COLLAPSE v.1.2 software (Posada 2011). A haplotype network for both mtDNA regions was constructed using TCS v.1.21 software (Clement et al., 2000). Due to limited sample sizes at many sites, we pooled sites within the five watersheds or geographical regions described previously (see Table 1) to examine large-scale patterns of genetic diversity. Differences among these regions in haplotype differentiation ( $\Phi_{ST}$ ), gene diversity, nucleotide diversity, and the number of haplotypes per group were examined using analysis of molecular variance (AMOVA) implemented in ARLEQUIN (Schneider et al., 2000). CO1 and ND1 mtDNA sections were analyzed separately because sequencing was not successful for both genes in all individuals.

## RESULTS

The CO1 sequencing provided a 453 bp fragment from 64 individuals and the ND1 sequencing gave a 511 bp fragment from 61 individuals (Genbank Accession numbers KM656075-KM656083). Both mtDNA gene segments exhibited little variation within the Great Lakes including Lake Ontario and the St. Lawrence River. Only one CO1 haplotype and two ND1 haplotypes were found in these populations (Fig. 1; Table 2). In contrast, four CO1 and six ND1 haplotypes were recovered in Atlantic coast populations. All Great Lakes haplotypes were present in and among the most common haplotypes in Atlantic coast populations even though sample numbers were generally low across all of the Atlantic coast populations sampled. All haplotypes in all regions differed by just one or two point mutations from the most common type (Fig. 2). Gene diversity and nucleotide diversity for both genes also were low in Great Lakes populations; CO1 was invariant and ND1 showed very low diversity except in Lake Ontario where it was invariant (Table 2). Gene diversity and nucleotide diversity for both genes were substantially higher in Atlantic coast populations than in the Great Lakes (Table 2).

The percentage of variation explained by partitioning among the five regions was 38% ( $P < 0.0001$ ) for CO1 and 10% for ND1 ( $P = 0.0128$ ), and more variation was present within Atlantic coast populations than within all of the Great Lakes samples combined (Table 2). Pairwise  $\Phi_{ST}$  revealed significant genetic differentiation for both genes only between the Atlantic coast populations and each of the four Great Lakes regions and there were no differences within the Great Lakes (Table 3).

## DISCUSSION

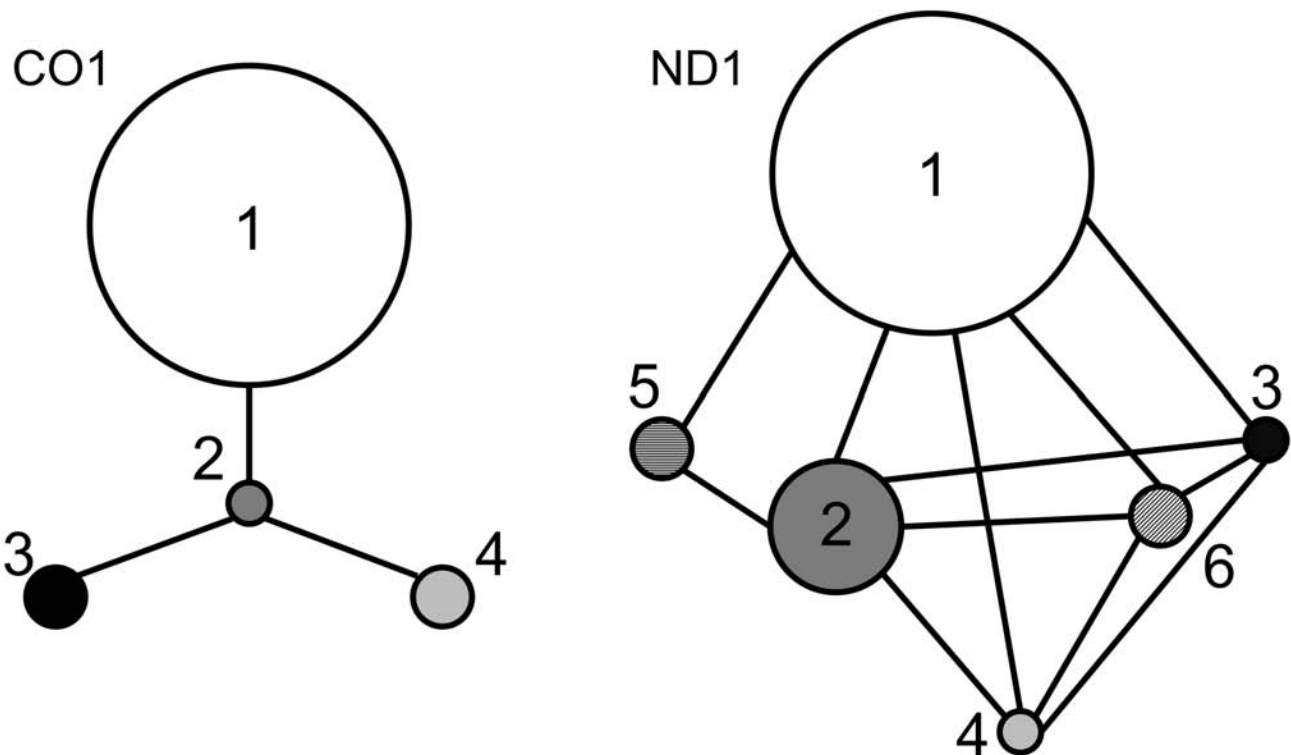
Genetic variation in *Ligumia nasuta* was low in all Great Lakes populations compared to Atlantic coast



**TABLE 2**

Variation in the mitochondrial CO1 and ND1 genes of *Ligumia nasuta* among five regions. N is the number of individuals sampled.

Region	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 coast	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 coast	10	0.8889	0.0023	6

**FIGURE 2**

Spanning network of mtDNA haplotypes at the CO1 and ND1 loci for *L. nasuta*. The connecting lines represent a single base pair difference between adjoining haplotypes. The relative size of the circles represents the frequency of the haplotypes in all samples. Haplotype numbers are referenced on Fig. 1.

**TABLE 3**

Pairwise  $\Phi_{ST}$  values for *Ligumia nasuta* mitochondrial ND1 (above diagonal) and CO1 (below diagonal) genes among five regions. Values with asterisk are statistically significant ( $\alpha = 0.05$ ).

	Northern Michigan	Lake St. Clair	Lake Erie	Lake Ontario	Atlantic coast
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 coast	0.386*	0.415*	0.498*	0.290*	-

populations where limited sampling revealed numerous haplotypes and much higher overall genetic diversity. Together with the common occurrence of all Great Lakes haplotypes in Atlantic coast populations, these results suggest that Great Lakes populations were established by a single, small founder group from an Atlantic coast river system or a larger group from a single source population with low genetic variation. Either scenario is consistent with the hypotheses that 1) *L. nasuta* is one of the few upper Great Lakes species to have colonized the region from Atlantic coast rivers, and 2) there were few opportunities for such exchanges. An unexpected result was the low genetic diversity of Great Lake populations downstream of Niagara Falls. Our sample sizes were lowest in this region, but these results suggest that there also have been few opportunities for faunal exchange between the St. Lawrence River system and other Atlantic coast river systems.

The low genetic diversity of Great Lakes populations of *L. nasuta* is in contrast to other species that colonized the region from the Mississippi River basin. In the Lake Erie watershed alone, *Amblema plicata* (Say, 1817) had at least six CO1 haplotypes out of 36 known haplotypes across its range (Elderkin et al., 2007), and *Pyganodon grandis* (Say, 1829) had 34 CO1 haplotypes out of 45 haplotypes across the northern portion of its range (Krebs et al., in press). Across the Great Lakes region, *Elliptio dilatata* (Rafinesque, 1820) had four to seven haplotypes per site out of 38 haplotypes across its range, and *Actinonaias ligamentina* (Lamarck, 1819) had six to eleven haplotypes per site out of 73 haplotypes across its range (Elderkin et al., 2008). These results are consistent with the idea that some Mississippi River basin species reached the Great Lakes via multiple routes.

Other Mississippian species in the Great Lakes have lower genetic diversity comparable to that seen

in *L. nasuta*. *Fusconaia flava* (Rafinesque, 1820), had only three CO1 haplotypes in the Lake Erie watershed compared to 13 found across its range (Burdick & White, 2007), and *Epioblasma torulosa rangiana* (Lea, 1839) in the Sydenham River (Lake St. Clair watershed) had two CO1 haplotypes out of 10 haplotypes found across its range (Zanatta & Murphy, 2007). *Venustaconcha ellipsiformis* (Conrad, 1836) in the Lake Huron and Lake Michigan watersheds had three CO1 haplotypes and one ND1 haplotype out of 13 haplotypes found in the Mississippi River basin (Zanatta & Harris, 2013). These mixed results highlight the diverse and complex history of post-glacial dispersal into the Great Lakes from the Mississippi River basin (see Graf, 2002) as opposed to the apparently more limited dispersal from Atlantic coast rivers.

The genetic similarity among *L. nasuta* populations throughout the Great Lakes suggests that they can be treated as a single management unit. However, the Great Lakes management unit clearly is genetically distinctive from the Atlantic coast populations we sampled. Until more information becomes available, recovery efforts in the Great Lakes based on captive propagation or translocation should be limited to use of Great Lakes source stock to avoid introduction of non-native haplotypes. Sampling from populations in additional Atlantic coast rivers, particularly those in previously glaciated regions (e.g., Hudson and Mohawk rivers), may reveal other suitable source populations for conservation efforts and may refine our understanding of the evolutionary history of Great Lakes populations of *L. nasuta*.

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