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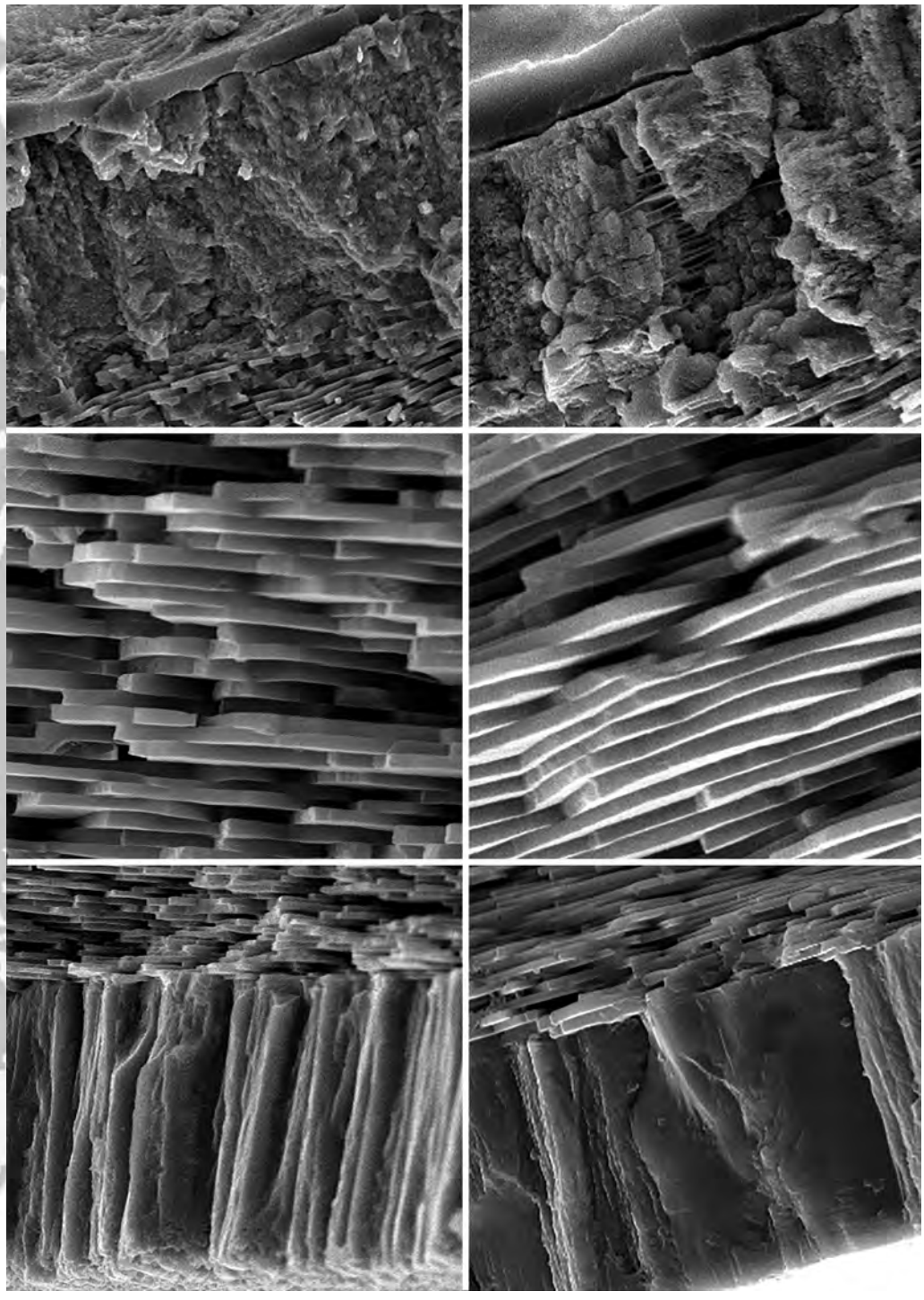

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Mitochondrial DNA structure of *Pyganodon grandis* (Bivalvia: Unionidae) from the Lake Erie watershed and selected locations in its northern distribution

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Abstract: The distribution of *Pyganodon grandis* (Say, 1829) and the entire community of unionid freshwater mussels within the Laurentian Great Lakes became greatly reduced following invasion of dreissenid mussels. While some populations remain, how much gene flow still occurs is not known, nor has the level of population structure been examined within a large lake subsequent to dreissenid infestation. *Pyganodon grandis* is a common and relatively abundant lacustrine species that utilizes diverse host fish, and, therefore, it may disperse as much as or more than any other unionid species in the region. To test for population structure, we examined a fragment of the maternal mtDNA COI gene from 300 individuals encompassing shallow areas from Lake Erie's western and central basins, Sandusky Bay, Lake St. Clair, and the upper Niagara River. Another 94 individuals from the upper Great Lakes and upper Mississippi River watersheds were added to the analysis. A total of 34 different haplotypes were found for *P. grandis* in the Lake Erie watershed, but just one was common and composed > 80% of all individuals. No other haplotype exceeded a frequency of 2% and most were found only once. Just thirteen haplotypes were found west of Lake Erie, and only the common haplotype and one other were shared with the Lake Erie watershed. However, structure in haplotype frequencies and the presence of one very different clade were limited to samples abutting the Red River of the North. Thus recent population declines in Lake Erie appear not to have significantly impacted levels of genetic variation.

Key words: conservation, driftless area, freshwater mussels, gene flow, glaciation, streams

The diverse fauna of native freshwater mussels (Bivalvia: Unionidae) in Lake Erie (Graf 2002) has experienced habitat degradation, species invasions, and eutrophication (Hartman 1973), but a precipitous decline began after the invasion of zebra and quagga mussels from the Black and Caspian Seas (Schloesser and Nalepa 1994, Schloesser *et al.* 1996), beginning with *Dreissena polymorpha* (Pallas, 1771) about 1986 followed by *Dreissena rostriformis bugensis* (Andrusov, 1897) in the early 1990s (Hebert *et al.* 1989, Mills *et al.* 1993). Species that once would have littered beaches with their shells (Goodrich and Vander Schalie 1932) had largely disappeared by the 1990's, yet in the last few years, surveys conducted along shorelines, in river mouths, marshes and shallow coastal areas suggest some recovery along the lake (Crail *et al.* 2011).

The state of recovery of mussels is difficult to assess because populations are generally small and scattered (Sherman *et al.* 2013). *Pyganodon grandis* (Say, 1829) is a fast growing lacustrine species (Haag 2012) that is still considered widespread and common in North America (www.natureserve.org). The species possesses hooked glochidia that are typical of unionids described as generalists in host use (Hoggarth 1999). In *P. grandis*, the glochidia are held in a marsupium along the outer gills and released between October and February (Watters and O'Dee 1998) when they attach to the scales or gills of fish. Watters *et al.* (2009) list over 40 confirmed and potential host fish species. Therefore, *P. grandis* may be better able than most native freshwater mussels to recolonize habitat and to maintain gene flow across great distances (Haag 2012).

To assess populations affected by dreissenid mussels, extensive surveys focused on identifying potential refuges along Lake Erie's American coast (Prescott 2014, Zanatta *et al.* pers. comm.), during which mantle tissue clips were collected. Targeted habitat were those areas likely to possess soft or fine sand sediments that allow unionids to burrow (Nichols and Wilcox 1997), areas of high productivity to reduce food competition with dreissenids (Higgins and Vander Zanden 2010), and regions where water level fluctuations, wave action, offshore currents or other features periodically produce shallow water depths (Schloesser *et al.* 1997, McGoldrick *et al.* 2009, Sherman *et al.* 2013). Known refuges have included Crane Creek (Bowers and de Szalay 2004, 2005), Metzger Marsh (Nichols and Wilcox 1997, Nichols and Amberg 1999) and Presque Isle Bay (Schloesser and Masteller 1999) of Lake Erie, and the delta region of Lake St. Clair (Zanatta *et al.* 2002, Sherman *et al.* 2013, Lucy *et al.* 2014). We collected across all of these areas as well as shallow zones of Sandusky Bay and river mouths along the American side of Lake Erie.

We report genetic variation in *Pyganodon grandis* across a large lake system, as this species may be more resilient with

respect to maintaining genetic variation than species suffering greater population losses or those utilizing fewer and less vagile host fish (Krebs *et al.* 2010), and, thus, prove suitable for investigating lower limits of consequences from habitat change and population decline in the presence of dreissenid mussels. Thus, our goals were to first, assess the genetic variation in the maternal COI mtDNA gene for this widespread species of freshwater mussel, second, estimate genetic connectivity among these freshwater mussel populations across a large lake, and finally, contrast variation in Lake Erie to published sequences of *P. grandis* from outside the Lake Erie watershed (Doucet-Beaupré *et al.* 2012).

MATERIALS AND METHODS

As restricted by permit rules for small populations, mantle clips were made for *Pyganodon grandis* collected in shallow coastal areas and stream mouths across the Lake Erie watershed in the summers of 2010 and 2011 (Fig. 1): Lake St. Clair delta region (N =13), the western basin of Lake Erie (N = 103), Sandusky Bay area (N = 54), the central basin of Lake Erie (N = 75), and the Niagara River (N = 55). These basins differ in depth, with a maximum in Lake St. Clair of 6 m, a maximum of 10 m in the western basin of Lake Erie, and of 25 m in the central basin, an area also impacted by hypoxia (Zhou *et al.* 2013). Sandusky Bay is a shallow zone at only 1–3 m depth draining at the western edge of the central basin. A ridge and islands separate these basins, and another deeper ridge separates the central and eastern basins, the latter of which drops below 60 m. The lake drains east through the Niagara River. These differences in depth impact the structure of rivers, which once extended farther before Lake Erie rose to its present level at about 174 m elevation (Bolsenga and Herdendorf 1993). Streams in the western basin and Sandusky Bay are lake-influenced with low flow (sometimes called freshwater estuaries), supporting mussels (Prescott 2014). Many eastern rivers drop off quickly and with high flow rates limiting sampling.

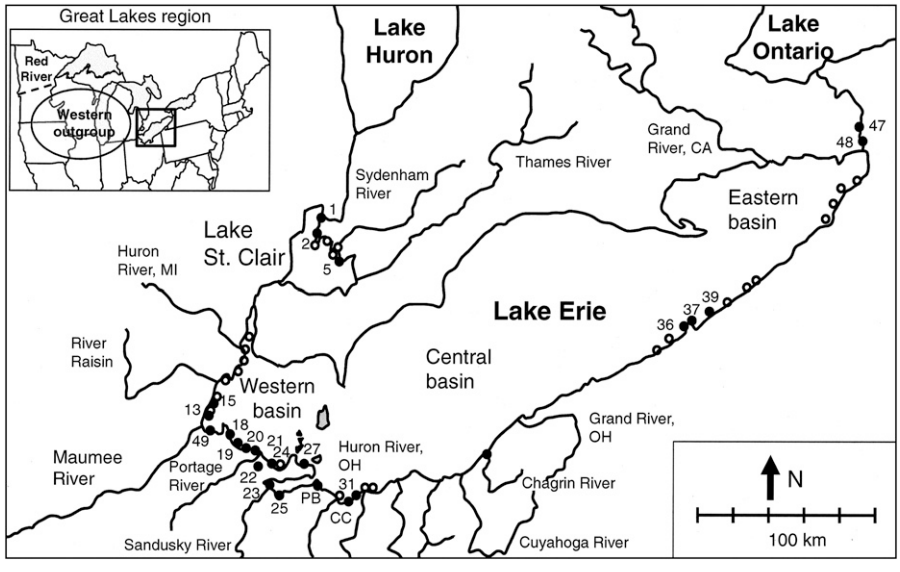

Figure 1. Filled circles represent sampling sites of *Pyganodon grandis* within the major bathymetric basins of the Lake Erie watershed within the Laurentian Great Lakes region of North America: Lake St. Clair delta region in (1) Pollet Bay, (2) Goose Bay, and (5) Little Muscamoot Bay; in the western basin of Lake Erie, (13) Maumee Bay-Bayshore, (49) North Maumee Bay, (15) Monroe Power Plant Discharge, (18) Cedar Creek Marina, (19) Crane Creek Marsh, (20) Turtle Creek Marsh, (21) Toussaint Creek mouth, (22) Portage River, and (23) Young Marsh; in Sandusky Bay, (24) Muddy Creek Bay, (25) Sandusky Bay and diverse small tributaries (Yellow Swale, South Creek, Raccoon Creek); in the central basin of Lake Erie, (PB) Plum Brook, (31) Old Woman Creek, (CC) Cranberry Creek, (36) Presque Isle Bay, (37) Thompson Bay, and the Duck Pond at Presque Isle; and in the Niagara River, (47) Strawberry Island and (48) Spicer Creek near Grand Isle. Collection areas from Doucet-Beaupré *et al.* (2012) and 7 additional specimens from the upper Mississippi River and the Red River of the North are indicated on the inset map. Open circles indicate areas surveyed where no *P. grandis* were obtained.

Additional sequences were derived from Doucet-Beaupré *et al.* (2012). This material was broken into two groups, samples in the upper Mississippi River and upper Great Lakes watersheds, which included Michigan, Indiana, Wisconsin, and southern Minnesota as one clade (their Fig. 5), and samples from above Minneapolis as a second clade: Rice River (46.533°N, -93.320°W), Pfeiffer Lake (47.752°N, -92.477°W), Prairie River (47.239°N, -93.482°W), Deer Lake (47.825°N, -93.375°W) and the Minnesota River and several small lakes above Montevideo, MN (44.996°N, -95.691°W).

Direct sampling in the lake is generally difficult because of low unionid densities and high water depth, although 30 of the samples were juveniles collected in Maumee Bay of the Western Basin following a seiche in December, 2011 (Bryan *et al.* 2013). Mussel rakes were used to collect most samples, and brailing with hands and feet was applied in shallow river mouths and marshes adjacent to these basins, with sampling as close to the lake as possible. All tissue clips were preserved in 95% ethanol.

Total DNA was extracted using a Qiagen DNeasy extraction kit, except that only 1– 2 mg of mussel tissue was

used in each extraction (Krebs *et al.* 2013). Any more tissue can cause extraction problems, as the exudate becomes viscous (Liu and Mitton 1996). A segment of the female mitochondrial DNA cytochrome oxidase subunit 1 (COI) was amplified using the primer pair LCOI490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G; HCO2198 5′-TTA ACT TCA GGG TGA CCA AAA AAT CA (Folmer *et al.* 1994) in 25 µL volumes consisting of 10 µL of deionized water, 5.5 µL of GoTAQ buffer (Promega), 2.75 µL of 2.5 mM dNTPs, 2.75 µL of each primer at 2.5 mM, and 2.75 µL of 0.25 mM $MgCl_2$. To this reaction mix, 0.15 µL per reaction GoTAQ polymerase was added, from which 24 µL were added to 1 µL DNA template. The initial denaturation phase was 2 min at 94 °C, followed by 35 cycles of DNA denaturation for 30 sec, primer annealing at 49 °C for 30 sec, and polymerization extension at 72 °C for 45 sec. After amplification, unused primers were degraded using 3 µl of a mixture of Exonuclease I (Amersham Biosciences cat# E70073X, 10 U/ml) and Shrimp Alkaline Phosphatase (SAP) (Amersham Biosciences cat# E70092X 1 U/ml) combined as 78 µl ddH2O, 2 µl ExoI, and 20 µl SAP. Reactions were incubated at 37 °C for 40 min followed by 80 °C for 20 min to denature enzymes. For sequencing at the Cleveland Clinic sequencing facility, 5 µl of each amplified product was transferred to a 96-well plate with 3 µl of primer LCOI490 to provide 630 bp of readable sequence, as new technology has greatly reduced signal-to-noise ratios. Samples with ambiguities were sequenced in the reverse direction, except in a few samples where the first few base pairs were not readable, but they otherwise matched an identified haplotype.

Sequences of individuals collected across the Lake Erie watershed were entered into DnaSP V 5.1 to define haplotypes from which phylogenetic relationships and haplotype networks were constructed in Network V 4.6.1 (Röhl 2004), applying the median-joining algorithm (Bandelt *et al.* 1999) followed by the post-processing Steiner algorithm (Polzin and Daneschmand 2003) to remove unnecessary median vectors and non-parsimonious links. A weighted genetic distance, ε, was zero, the default value (Röhl 2004). Polymorphic sites, transitions, and transversions were weighted equally. Allele frequencies by regions were compared with rarefacted frequencies based on one less than the smallest sample size, or an *N* of 12, in PAST 3.0 (Hammer *et al.* 2001). Analysis of Molecular Variance (AMOVA), pairwise F_{ST} analyses and a test of neutrality, *i.e.*, Tajima's *D* (Tajima 1989), were run in ARLEQUIN version 3.5.1.2, using 30,000 permutations for tests of significance (Excoffier and Lischer 2010). Tajima's *D* contrasts Θ_s, the estimation of 4Nµ based on the number of segregating sites, and Θ_π, the estimation of 4Nµ based on nucleotide differences, which are predicted to be the same under neutral evolution and a stable population size. Mismatch analysis was run to compare observed pairwise differences and the frequency of segregating sites to that predicted with population expansion in ARLEQUIN and DnaSP 5.10 (Librado and Rozas 2009).

RESULTS

Haplotype frequencies were separated by area of origin within the network (Fig. 2) to show spatial dispersion and to identify differences among 34 haplotypes across Lake Erie (Table 1, accession numbers (KM262507- KM262540). The most common haplotype (H1) was observed for 250 of 300 (83%) *Pyganodon grandis* sampled in the Lake Erie watershed, which included coastal refuges and stream mouths near Lake Erie, Lake St. Clair, and within the Niagara River above

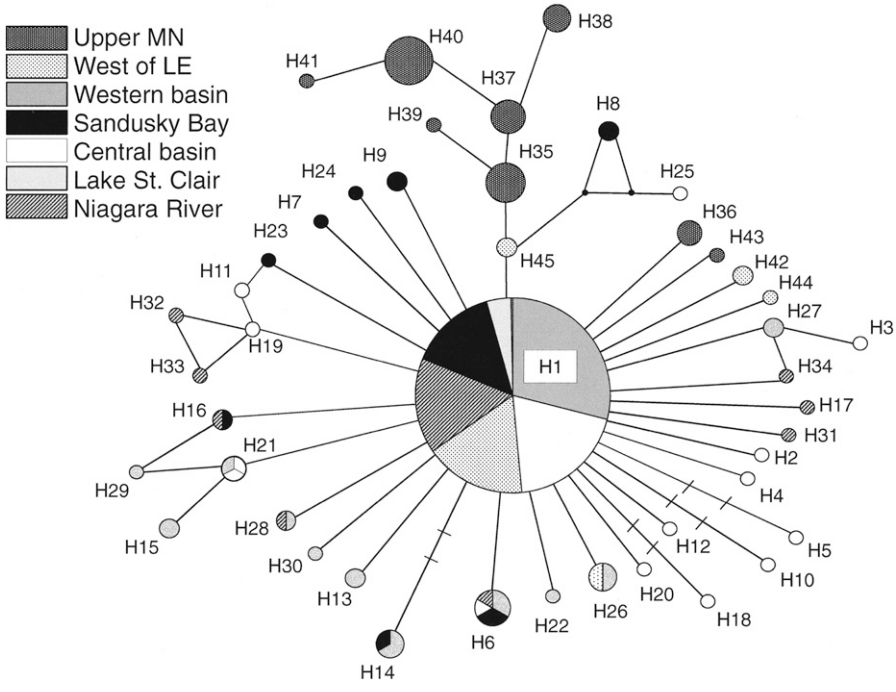

Figure 2. Haplotype network for a fragment of the COI gene amplified for *Pyganodon grandis* collected across the Lake Erie watershed: Lake St. Clair, Western basin, Sandusky Bay, Central Basin and from the Niagara River off the Eastern Basin, as well as areas west of Lake Erie and Upper MN, including the upper Minnesota River and the Red River of the North.

Table 1. Genetic diversity in *Pyganodon grandis* across five bathymetrically separated regions in Lake Erie and samples from two outgroup regions, one west of Lake Erie and a second group from above Minneapolis, Minnesota. Reported are sample size (N), allele number, rarefacted allele number on the minimum sample size minus one (or 12), polymorphic sites, haplotype diversity (H) or the likelihood that two sequences drawn at random will differ, π, the mean sequence variation among all haplotypes within a population, Θ_s, the estimation of 4Nμ based on the number of segregating sites, Θ_π, the estimation of 4Nμ based on nucleotide differences, and Tajima's D, a test of the difference between Θ_s and Θ_π which is predicted to be equivalent under neutral evolution.

Region	N	Allele Number	Rarefacted allele freq. (if $N = 12$)	Polymorphic sites	Diversity H	π x 100	Θ_s	Θ_π	Tajima's D
St. Clair	13	2	1.9 ± 0.3	1	0.15 ± 0.12	0.024	0.32	0.154	-1.15
Western	103	12	2.9 ± 1.1	12	0.30 ± 0.06	0.067	2.30	0.429	-2.16***
Sandusky	54	9	3.3 ± 1.1	11	0.37 ± 0.08	0.093	2.41	0.586	-2.18***
Central	75	12	3.1 ± 1.2	20	0.32 ± 0.07	0.089	4.09	0.559	-2.60***
Niagara	55	9	2.7 ± 1.1	8	0.27 ± 0.08	0.057	1.74	0.362	-2.13**
West of Lake Erie[1]	57	5	2.3 ± 0.9	4	0.23 ± 0.07	0.038	0.87	0.242	-1.59*
Upper Minnesota[1]	37	9	5.7 ± 1.0	10	0.82 ± 0.04	0.318	2.40	2.000	-0.50

[1]all samples outside the Lake Erie watershed except $N = 7$ from Doucet-Beaupré *et al.* (2012)
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Niagara Falls. The second most common haplotype from the Lake Erie region (H6, $N = 6$, or 2% of all individuals) occurred in 4 of 5 regions; H6 was not found in Lake St. Clair, although only 13 *P. grandis* were obtained for this lake. No other haplotype was found more than 3 times (*i.e.*, above a frequency of 1%).Where three copies were observed (H14 and H21), each derived from more than one basin; even with haplotypes found twice, 2 of 8 came from different basins. The consistency of haplotype sharing suggested little genetic differentiation across this large watershed, a result confirmed by AMOVA, in which the among-basin component of variation was almost non-existent ($F_{ST} = 0.00$), and neither were any pairwise F_{ST} values within the Lake Erie watershed significantly different from zero (Table 2). The only pattern to genetic variation within the lake was less haplotype diversity at the edges of the survey range, Lake St. Clair (0.15) and the Niagara River (0.27) compared to that observed (0.30–0.37) in the western and central basins and Sandusky Bay, which was a pattern that held for nucleotide diversity (π) and rarefied allele numbers as well (Table 1).

Within regions, the pattern of haplotype variation did not match that predicted under a mutation-drift or population-size equilibrium. Tajima's D differed significantly ($\Theta_s >> \Theta_\pi$) except in Lake St. Clair where sample numbers were small. A complementary test, mismatch analysis (in ARLEQUIN and DnaSP), indicated no significant difference from predictions under population expansion ($P > 0.05$) either per site or when pooling data for all of Lake Erie; pairwise differences produced a unimodal plot and a plot of variation in segregating sites was overrepresented by small differences among individuals (results not shown).

Variation outside of Lake Erie comprised 13 haplotypes of which only two (H1 and H26) overlapped with haplotypes found in Lake Erie. Concordant with levels of variation across all of Lake Erie, 50 of 57, or 87% of individuals collected west of the Lake Erie watershed, but excluding those individuals from areas upstream of Minneapolis, MN (about 45° lat.) possessed the H1 haplotype. Pairwise F_{ST} for this group compared with the five Lake Erie regions suggested no significant differences at an experiment-wise level, and only a possible difference with the farthest population, that from the Niagara River (Table 2).

However, haplotypes of *Pyganodon grandis* from above Minneapolis differed from those of all other areas. Only one of 37 individuals possessed the H1 haplotype, 4 others possessed related but private haplotypes, and those collected farthest north composed a separate lineage in the network (Fig. 2) that came out as basal in a phylogenetic tree (Fig. 3). Inclusion of these data produced significant population structure (F_{ST} of 0.416, $P < 0.001$) with pairwise F_{ST} values ranging between 0.56 and 0.71 (Table 2, all $P < 0.001$). Furthermore, haplotype frequencies followed those expected

Table 2. Population pairwise-F_{ST} values among individuals collected from five bathymetrically separate regions of the Lake Erie watershed, as well as areas west of Lake Erie, and Upper Minnesota, including the upper Minnesota River and the Red River of the North[1].

Region	Lake St. Clair	Western Basin	Sandusky Bay	Central Basin	Niagara River	West of Lake Erie
Western Basin	-0.033					
Sandusky Bay	-0.019	0.007				
Central Basin	-0.030	0.002	-0.000			
Niagara River	-0.016	0.004	0.005	-0.004		
West of Lake Erie[1]	-0.003	0.006	0.007	0.001	0.010*	
Upper Minnesota[1]	0.559***	0.707***	0.627***	0.658***	0.664***	0.677***

[1] all samples outside the Lake Erie watershed except $N = 7$ from Doucet-Beaupré *et al.* (2012)
* $P < 0.05$, *** $P < 0.001$

under neutrality, with Tajima's *D* not significantly different from zero (Table 1), and P values in mismatch analysis closer to significance than for any other population. Restricting mismatch analysis to the 32 most northern individuals (those at the very top of the Mississippi watershed or within the Red River watershed), suggested rejection of a hypothesis of demographic ($P = 0.054$) and spatial ($P = 0.023$) expansion.

All sequences in Fig. 2 are monophyletic and closely related members of *Pyganodon grandis* (Fig. 3) within clade B for this species (Doucet-Beaupré *et al.* 2012). The one anomalous haplotype (identified as H lac in Fig. 3) from an individual reported morphologically to be a *P. grandis* from the Lake St. Clair region differed from all *P. grandis* haplotypes and was just 87.9% similar to the common haplotype, H1 (differing for 76 of 630 bases). A blast search in GenBank identified the mtDNA haplotype as 94% identical to a *P. lacustris* (Lea, 1852) sequence (GenBank accession # HM849110.1) and 96% identical to accession # EF418018.1, which Cyr *et al.* (2007) called *Pyganodon* species A, and which Doucet-Beaupré *et al.* (2012) placed within a *P. lacustris* haplotype lineage.

DISCUSSION

The distribution of female COI mtDNA haplotype variation in the Lake Erie watershed provided no evidence of population structure; genetically, *Pyganodon grandis* in Lake Erie appears to be one panmictic population. However, the observed distribution was significantly different from that expected under a neutral equilibrium model despite the often weak power of Tajima's *D* (Zhai *et al.* 2009). Basically, while a neutral, infinite-allele model is appropriate for assessing variation in this species, drift-mutation equilibrium was not indicated. For one, that process is predicted to generate a Poisson frequency distribution of haplotypes and not a star pattern of one common haplotype and many rare ones (Braverman *et al.* 1995).

Instead, two alternative processes are likely occurring, which can generate the high number of rare haplotypes observed. First, an excess of low frequency variant haplotypes is consistent with strong purifying selection, especially for a large population within a continuous environment (Hedrick 2011, p. 327, Bickel *et al.* 2013, Krebs *et al.* 2013); no observed variants changed the amino acid coded suggesting that a large proportion of the gene cannot be changed. Second, a sweep of a common haplotype, even independently of selection, may characterize a population that expands rapidly (Avise 2000, Śmietanka *et al.* 2009). Population expansion, a process supported by mismatch analysis as well as Tajima's *D*, has been an explanation applied to star phylogenies in groups as diverse as alpine shrubs (Li *et al.* 2010), wild boar (Djan *et al.* 2013) and domestic sheep (Arora *et al.* 2013). The starburst pattern appears in several widely dispersed freshwater mussel species: *Fusconaia flava* (Rafinesque, 1820) in the Mississippi watershed (Burdick and White 2007), *Amblema plicata* (Say, 1817) (Elderkin *et al.* 2007), *Elliptio dilatata* (Rafinesque, 1820), and *Actinonaias ligamentina* (Lamarck, 1819) (Elderkin *et al.* 2008), and likewise for the less common *Venustaconcha ellipsiformis* (Conrad, 1836) across the upper Midwest (Zanatta and Harris 2013). Presumably, new variants arise but lack time to increase in frequency. As populations of *P. grandis* are likely recent (< 12,000 ybp) in this young lake (Lewis *et al.* 2012), and because glochidia of *P. grandis* can attach to scales of diverse fishes (Watters *et al.* 2009), this species is predicted to disperse easily, enabling high rates of gene flow and a likely original rapid population growth after entering the Great Lakes (Doucet-Beaupré *et al.* 2012).

The 57 sequences from Michigan and the upper Mississippi watershed mirrored Lake Erie in diversity, expressing the same common haplotype and a few rare and related sequences. Variation appeared similar across most of the distribution except for the lower levels present in Lake St. Clair where the fewest individuals were collected, based on both actual allele number and rarefaction analysis. The only

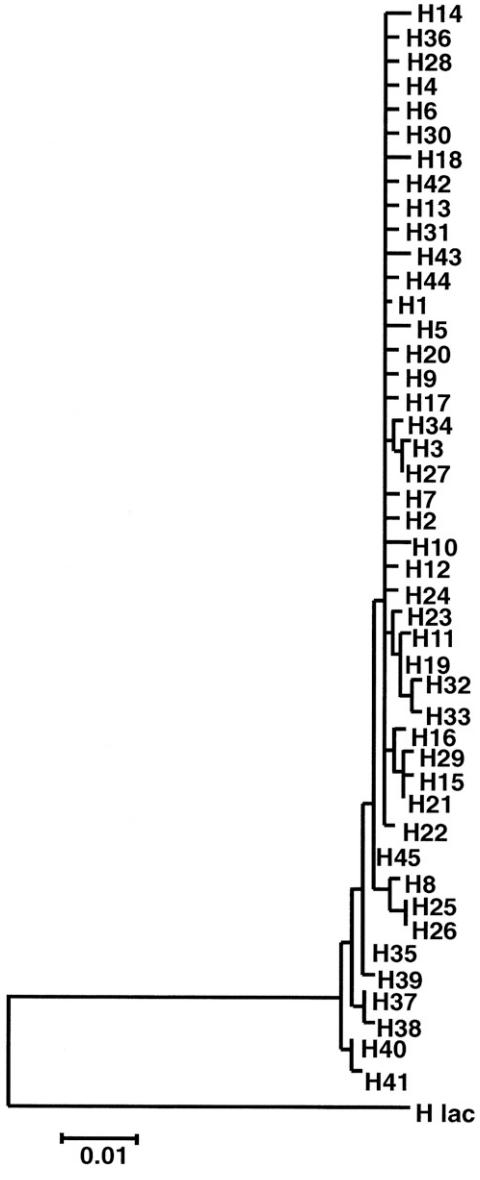

Figure 3. A Neighbor Joining tree for the 45 haplotypes of a fragment of the COI gene presented in the network in Fig. 2, and one anomalous haplotype identified as "H lac" at the base of the tree, which was an individual from the Lake St. Clair region reported morphologically to be a *Pyganodon grandis*. This one sequence was just 87.9% similar to the common haplotype, H1 (differing for 76 of 630 bases).

structure among the *Pyganodon grandis* populations analyzed relates to the very top of the Mississippi River watershed where the Mississippi watershed abuts the tributaries of the Red River of the North. Although all of these sequences fall within the *P. grandis* clade B, and they differ sequentially by just one or two base changes (Fig. 2), they compose the base of clade B (Fig. 5 in Doucet-Beaupré *et al.* 2012). Allelic richness was largest and those haplotypes were not found in individuals collected farther south in the Mississippi or in the Great Lakes watershed. The genetic connection to this lineage was H45, found in northern Wisconsin (Annabelle Lake and the St. Germaine River). That related sequences of this lineage occurred in Lake Erie (H8, $N = 2$, Sandusky Bay and H25, Central Basin), while H1 occurred high the Mississippi River watershed, suggests some historical or recent exchange across this region, but also significant isolation.

Prehistorically, the south end of Lake Traverse, MN (45.70°N, -96.75°W) was the southern outlet of glacial Lake Agassiz across the Traverse Gap, which drained south as Glacial River Warren up to 9400 ybp and carved out the valley now occupied by the present-day Minnesota River (Ojakangas and Matsch 1982, pp. 109–110, Fisher 2003). Because present dikes exceed the height of the continental divide (299.9 m), floods potentially may allow water to flow from Traverse Lake into the Little Minnesota River, and the Little Minnesota River has flooded high enough to cross the Traverse Gap (Minnesota Department of Natural Resources 2007), a result that may occasionally connect the fauna of these two drainages (Graf 1997). Because *Fusconaia flava* (Rafinesque, 1820) from the Red River area possessed little genetic variation, Burdick and White (2007) argued for recent migration by mussels northward. The opposite pattern was found in *Pyganodon grandis*, as more genetic variation and many private alleles characterized the northern population. This result raises the intriguing possibility that *P. grandis* survived in a northern glacial refuge that remained largely separate from the Mississippi River watershed. The "Driftless Area" of the upper Midwest (Rowe *et al.* 2004) is a possibility, with populations of this lacustrine species possibly expanding northward into Glacial Lake Agassiz.

With respect to Lake Erie, the present results for genetic variation in *Pyganodon grandis* paint a simple and perhaps predictable pattern within the region, one of panmixis, or a population sufficiently large that genetic drift following population declines even after invasion of dreissenid mussels (Schloesser *et al.* 1997) had little effect on patterns of variation. Corroborating these results with nuclear markers and male mtDNA is of interest, but collection of whole animals for gonadal tissue while assessing population presence in refuges was not appropriate. In the future, primers may be derived from those reported to work in *Anodonta cygnea* (Linnaeus, 1758) (Chong *et al.* 2009) and *Lasmigona costata* (Rafinesque, 1820) (Galbraith *et al.* 2011). Relevant to mitochondrial results, population structure is significantly greater for maternal than paternal mtDNA haplotypes in *Lampsilis siliquoidea* (Barnes, 1823), and variation in allozymes for *L. siliquoidea* (Berg *et al.* 2007) was similar to patterns seen in male haplotypes (Krebs *et al.* 2013); few differences occurred among streams. Multiple studies show longer branch lengths

within trees using male versus female mtDNA sequence data (Breton *et al*. 2007). However, while within-species divergence in paternal mtDNA sequence exceeds that of maternal mtDNA in *P. grandis* (Liu, Mitton and Wu 1996, Krebs 2004), differences between mitotypes in *P. grandis* from two Colorado watersheds also could not be explained by gene flow, and both genetic forms suggested similar levels of isolation (Liu, Mitton and Herrmann 1996).

Sample sizes in the present study were comparable to other recent haplotype studies of unionids (Burdick and White 2007, Elderkin *et al*. 2007, 2008), providing the power to detect differences in haplotype variation among basins. Population size is thought to have remained greater in the shallow western basin (Herdendorf 1987, Metcalfe-Smith *et al.* 1998), which often correlates with higher levels of genetic variation. No differences were suggested, however, even between samples from the Niagara River and those from the Lake St. Clair region, which are separated by ~500 km of water. The one caveat was that the most eastern site (Niagara River) differed most from samples west of the Lake Erie watershed. The only sample set differing from all of Lake Erie was for individuals 1000 km farther west, reaching an area with an abrupt haplotype transition.

Regionally, our results may provide a null model for other species within the Laurentian Great Lakes and the Upper Mississippi watershed. Most species in Lake Erie were historically numerous even if now they are reduced in abundance (Crail *et al*. 2011). More work will be needed to identify whether extant populations have become genetically structured. Understanding what regulates their distribution is critical, as perhaps 70% of freshwater mussels in North America are listed as endangered, threatened, or as species of concern (Williams *et al*. 1993, Master *et al*. 2000). Here we show that even as the distribution of *Pyganodon grandis* has been reduced, bathymetric features may not appear to restrict gene flow in this lacustrine freshwater mussel, although the short number of generations since the decline has limited the time when alleles would be lost through lineage sorting.

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