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An Evaluation of the Genetic Structure of Mapleleaf Mussels (Quadrula quadrula) in the Lake Erie Watershed

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Introduction

Freshwater pearly mussels in the family Unionidae are large bivalves that live in the sediments of rivers, streams, and lakes worldwide, with over 300 species in North America (Graf and Cummings, 2007) and over 40 species native to the Great Lakes watershed (Graf, 2002; Metcalfe-Smith et al., 1998; Strayer and Jirka, 1997). These long-lived molluscs often survive for decades (Haag, 2012) and have a unique life cycle. While most bivalves have free-living larvae, unionid larvae are obligate parasites on fish. This adaptation, which enables mussels to move upstream over long distances, may also help maintain genetic diversity within spatially isolated populations.

Unionidae have experienced drastic declines in diversity and abundance throughout North America (Lydeard et al., 2004; Haag, 2012). In 1986, *Dreissena polymorpha* (Pallas, 1771), a Ponto-Caspian native, was introduced into the Great Lakes region from ballast water of trans-oceanic ships (Hebert et al., 1989; Carlton, 2008). Shortly after occurred the introduction of *Dreissena rostriformis bugensis* (Andrusov, 1897), another Ponto-Caspian region native (Mills et al., 1993). Both dreissenid species are highly competitive epifaunal filter feeders that attach to hard surfaces, including the hard shells of unionids, using byssal threads (Mackie, 1991). Live unionids may even be a preferred substrate (Burlakova et al., 2000; Mackie, 1991; Ricciardi et al., 1995), and fouling by dreissenids can impede filter feeding, reduce motility, and fitness and may be fatal when the number attached is high (Bowers and de Szalay, 2007; Haag et al., 1993; Mackie, 1991; Ricciardi et al., 1995).

The decline in unionid abundance and local extirpations (Schloesser et al., 2006; Schloesser and Nalepa, 1994) is likely to reduce gene flow among species composing the remaining assemblage. In the western basin of Lake Erie, Herdendorf (1987) reported 35 unionid species, including *Quadrula quadrula* (Rafinesque, 1820), although this species was apparently rare in the open waters prior to 1990 (Brown et al., 1938; Nalepa et al., 1991). More *Q. quadrula* could have existed prior to the present study, but most coastal unionid survey studies were recent (Crail et al., 2011) and suggest increases in this habitat generalist species that prefer muddy, mud mixed with sand, and cobble substrates (Metcalfe-Smith et al., 2000, 1998; Schloesser and Masteller, 1999; Bowers and de Szalay, 2004; Griffith, 2013; Prescott, 2014).

As one of the more common species remaining in western Lake Erie, *Q. quadrula* could be used for making generalized assessments of genetic variation in rare, threatened, and endangered species remaining or returning to the lake. Understanding populations of common species is one way to develop a recovery plan for communities, as studies of population genetics help predict which populations are thriving and which populations are potentially experiencing inbreeding depression (Freeland, 2005), especially for species of similar life history strategies (Elderkin et al., 2007; Haag, 2012). Understanding dispersal routes is also critical, as historical patterns could indicate where modern barriers inhibit recolonization. For instance the Maumee River drainage has long been considered one of the likely post-glacial colonization routes for many unionid mussels into Lake Erie (Graf, 2002; Haag, 2012; Watters, 1995); however, there may be alternative origins (Krebs et al., 2013; Scott et al., 2014), suggesting a need to compare extant lake populations to those in the mouths of tributary streams.

Underlying dispersal patterns in *Q. quadrula* may match host fish movement. Possible host fish are thought to be flathead catfish (*Pylodictis olivaris*, Rafinesque, 1818) and channel catfish (*Ictalurus punctatus*, Rafinesque, 1818) (COSEWIC, 2006; Watters et al., 2009). The latter species is common in western Lake Erie (Bowers and de Szalay, 2007). Adequate gene flow could create an almost panmictic population even in a large lake (Krebs et al., 2015), while at the other extreme, low levels of genetic exchange or rare expansion events during colonization could create a genetically structured population. Analysis of microsatellite DNA loci can identify source or sink populations and relationships between geographic and genetic distance within the range of the species (Freeland, 2005). The major objectives of this study were to determine levels of genetic diversity and structure of *Q. quadrula* within and among Lake Erie (open embayments and drowned rivermouths) and its tributaries; and to identify whether divergence exists; and if so, whether patterns of variation better correspond to an island model (Wright, 1940) of variation or a stepping-stone model of differentiation by distance (Kimura and Weiss, 1964).

Methods and materials

The American (U.S.A.) shore of Lake Erie was exhaustively surveyed in 2011 and 2012 (total of 132 sites from the Detroit River mouth to the Niagara River) for mussels, where a minimum of two person hours of search time (e.g., 4 searchers × 30 min) was used at each site using several methods (visual, tactile, SCUBA, snorkel, raking; Zanatta et al., 2015). *Q. quadrula* were located in large numbers only within the western basin (Fig. 1); this species has not been found alive in Canadian waters of Lake Erie in recent survey efforts (COSEWIC, 2006). From Lake Erie tributaries, sufficient samples of *Q. quadrula* for genetic analyses were obtained from three sites in the Maumee River, Ohio, from two sites pooled as a single location in the lower Grand River, Canada (upstream of Dunnville dam; Mathias and Zanatta, unpublished data; Galbraith et al., 2015), and from one site in the lower Huron River, Ohio (Table 1). Position and geographic distances among sites were calculated using the ARCMAP™ measuring tool in ARCGIS® software.

Genetic data collection

Mantle tissue was non-lethally excised from live mussels varying in size from 53 mm to 108 mm using biopsy techniques outlined in Berg et al. (1995). Each individual clip was stored in a 1.5 ml microtube filled with 95% ethanol, which was placed in a −20 °C freezer within four days of collection. A fraction of the entire tissue sample (1–2 mg) was removed from each clip, and DNA was extracted and precipitated using an alcohol solution following the procedures of Sambrook et al. (1989).

Six microsatellite loci developed by Hemmingsen et al. (2009) for *Quadrula fragosa* (Conrad, 1835) were amplified using polymerase chain reaction (PCR) at conditions optimized for use with *Q. quadrula* (Table 2). Each reaction consisted of 1.0 μl of working DNA (1:10 dilution from extracted DNA) mixed in 9.0 μl of PCR cocktail [10× *Taq* buffer (Qiagen™), bovine serum albumin (BSA), deoxyribonucleotide triphosphate (dNTP), forward and reverse primers, $MgCl_2$, and Taq (Qiagen™)]. An Eppendorf Mastercycler® was used for DNA amplification with locus-specific settings. PCR products [including a positive (previously genotyped sample of known size) and negative control] were visualized electrophoretically under UV light on agarose gels to verify amplification.

Genotyping was conducted on an ABI 3730 at the Natural Resources DNA Profiling & Forensic Centre, Trent University (Peterborough, ON, Canada). Alleles based on fragment size classes were assigned using GENEMARKER™ (SoftGenetics LLC®). The fragment sizes assigned by GENEMARKER were proofread and independently verified by two of the co-authors (WLP and TAG). The frequency of null alleles was estimated in MICROCHECKER v. 2.2.3 (van Oosterhout et al., 2004) using the Brookfield 1 method to incorporate excess in homozygosity and any individuals for which amplification failed (Brookfield, 1996). In

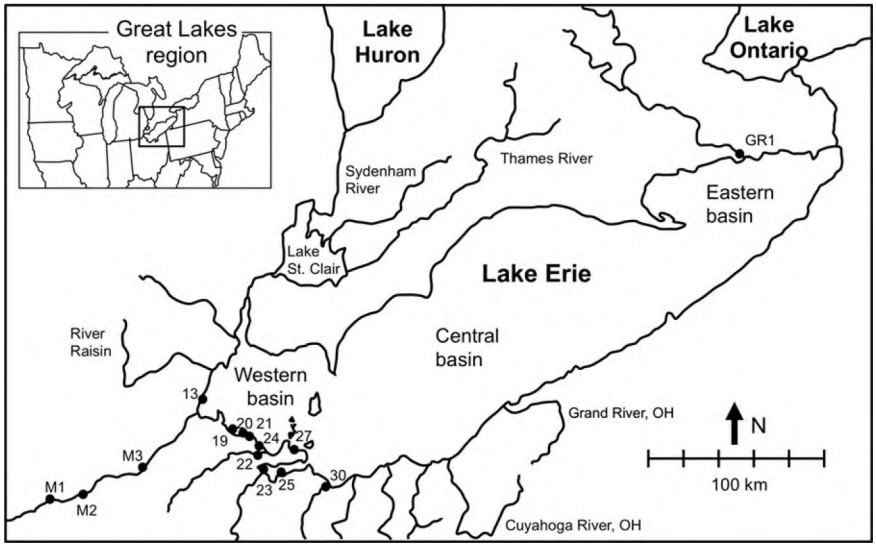

Fig. 1. Lake Erie watershed and sampling locations where tissue clips were collected from *Quadrula quadrula* in 2011–2012: three locations in the Maumee River (M1–M3), in Lake Erie's Western Basin, North Maumee Bay (13), Crane Creek Marsh (19), Turtle Creek (20), Toussaint Creek (21), Portage River (22), Muddy Creek Bay (23) and Young Marsh (24), Sandusky Bay (25), East Harbor (27), Huron River, Ohio (30), and one location in the lower Grand River (GR1), Canada.

Table 1
Sampling locations for *Quadrula quadrula* in 2011 and 2012 including location abbreviations (code), number of sites at each location, number of *Q. quadrula* found at each location, and the number of tissue clips obtained.

Sampling location name (state or province)	Location code no.	Number of sites	Number of *Quadrula quadrula* within sites	Number of tissue samples
Maumee River[1]	M1–3	3	122	98
North Maumee Bay	13	3	42	30
Crane Creek Marsh	19	5	197	30
Turtle Creek	20	4	84	30
Toussaint Creek	21	4	158	30
Young Marsh	24	2	23	30*
Portage River	22	4	150	30
East Harbor	27	2	2	2
Sandusky Bay	25	14	34	14
Muddy Creek Bay	23	17	105	30
Huron River	30	3	8	12*
Grand River, Ontario[1]	GR1	4	45	45
All location totals	14	73	974	386

[1] Tissue samples from Mathias and Zanatta, unpublished data.
* Additional specimens found outside of 0.5 ha sites.

addition to calculating the frequency of null alleles MICROCHECKER also tested for errors in scoring as a result of peak stuttering or large-allele dropout (van Oosterhout et al., 2004).

Only locations with ten or more individuals with successful amplifications from minimally four of the six loci were used in non-individual based analyses. The probability of linkage disequilibrium was determined in GENEPOP 4.2 (Raymond and Rousset, 1995). Calculations of heterozygosity and tests for Hardy–Weinberg equilibrium were performed in GENALEX v. 6.5 (Peakall and Smouse, 2006), but all loci were used as common practice suggests the use of all microsatellite locus whether in or out of equilibrium (Selkoe and Toonen, 2006). Rarefacted allelic richness was calculated using FSTAT v 2.9.3 (Goudet, 1995) to correct for variation in sample size (Manier and Arnold, 2005). Heterozygosity and rarefacted allelic richness were compared among sampling locations using Kruskal–Wallis tests (Kruskal and Wallis, 1952) in MINITAB 16 Statistical Software (2010).

Bayesian analyses of genetic structure were conducted in STRUCTURE v 2.3.4 (Pritchard et al., 2000) to compare the probability of a genotype for each individual being derived from a hypothesized set of genetic populations (K), which can then be mapped geographically. Each analysis set parameters at 100,000 iterations after a burn-in period of 200,000 iterations; admixture and correlation among allele frequencies was assumed. Ten iterations of each K were run for a maximum number of 26 populations (25 total number of sites sampled plus one) in STRUCTURE v 2.3.4 with and without the a priori geographic groupings indicated to determine if this had any effect on interpretation of population structure. Mean log likelihood was calculated using STRUCTURE HARVESTER v 0.6.93 (Earl and von Holdt, 2012) to determine which K best fit (Evanno et al., 2005) the STRUCTURE results.

Genetic differentiation was analyzed by two methods: Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992; Huff et al., 1993; Peakall et al., 1995; Michalakis and Excoffier, 1996) and pairwise F_{ST}, developed by Wright (1965). AMOVA was implemented in GENALEX to compare variation among the 12 sampling locations and the three geographic regions (Maumee River, Lake Erie, and Grand River). To determine if there was both genetic divergence among populations (e.g., F_{ST}) and isolation by distance, as would be observed in stepping-stone pattern for dispersal, we used a permutation algorithm (pairwise F_{ST} implemented in GENALEX) to examine site-specific differences both within and among regions (Allendorf and Luikart, 2007) and examined correlation between geographic and genetic distance in a Mantel test (Mantel, 1967). The Mantel test (Mantel, 1967) was implemented in GENEPOP comparing genetic distance in the form of linearized F_{ST} ($[F_{ST}/(1 - F_{ST})]$; calculated in GENALEX) to geographic distance. Geographic distances were calculated as the average shortest linear distance over water between sampling sites within each region. Linearized F_{ST} was also used to create an unrooted neighbor-joining tree in TREEFIT (Kalinowski, 2009); explanatory power was based on the R^2 value, or the proportion of variation explained.

Evidence of recent bottlenecks was tested as observed excesses in heterozygosity within populations (Freeland, 2005) in BOTTLENECK v 1.2.02 (Cornuet and Luikart, 1996). These analyses were run twice, once for ten populations (eight western basin sampling locations, Maumee River, and Grand River) and once with three populations after pooling all individuals in each sampling region: Lake Erie, Maumee River, and Grand River. As recommended by Piry et al. (1999), each run was conducted using 10,000 iterations with the variance set at twelve and the probability set at 95%. A signed-rank Wilcoxon test compared expected to observed heterozygosity among three models of evolution: Infinite Allele Model (I.A.M.), a Two Phase Model (T.P.M.), and a Stepwise Mutation Model (S.M.M.). Mode-Shift in allele frequencies was also examined to determine whether frequencies had the normal L-shaped distribution expected for stable populations, one with high frequencies of only a few alleles and many rare alleles, or after a genetic bottleneck, which can produce evenness of alleles following loss and/or increase in rare alleles (Piry et al., 1999).

Results

A total of 324 individuals from Lake Erie (211 individuals), the Maumee River (79 individuals), and the Grand River, Canada (34 individuals) were successfully amplified at a minimum four of the six loci. Over 90% of individuals used in the analyses successfully amplified at all six of the loci. No microsatellite loci were genetically linked.

Table 2
Amplification conditions for microsatellite loci optimized for *Quadrula quadrula* from *Quadrula fragosa* (Hemmingsen et al., 2009).

	QfC4	QfC114	QfD102	QfR9	QfA112	QfA130
GenBank accession no.	FJ785632	FJ785634	FJ785635	FJ785639	FJ785630	FJ785631
Annealing temp. (C°)	59	59	51	63.5	59	55
Repeat motif	TACA	TACA	ATCT	CA	CA	TG
Size range (bp)	199–263	204–232	196–224	207–231	135–161	176–280
Number of alleles	12	8	6	17	19	25
ddH_2O	5.7 μl	5.6 μl	5.7 μl	5.6 μl	5.9 μl	5.7 μl
10× PCR buffer (contains 1.5 mM $MgCl_2$)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
$MgCl_2$	0.5 mM	0.5 mM	0.5 mM	0.5 mM	0.5 mM	0.5 mM
BSA	0.2 mg/ml	0.2 mg/ml	0.2 mg/ml	0.2 mg/ml	0.2 mg/ml	0.2 mg/ml
dNTPs	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM
M13 universal primer	–	0.4 mM	–	0.4 mM	–	–
Forward primer	0.4 mM	0.1 mM*	0.4 mM	0.1 mM*	0.4 mM	0.4 mM
Reverse primer	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM
Taq	0.5 U	0.5 U	0.5 U	0.5 U	0.5 U	0.5 U
DNA (~5 ng)	1 μl	1 μl	1 μl	1 μl	1 μl	1 μl

* With M13 tag on 5′ end.

MICROCHECKER calculated that null alleles were present at four of the six loci with probabilities of null alleles ranging from 0 to 7% at the seven loci used. No errors in scoring due to stutter or large allele dropout were found. Simulations suggest that null alleles minimally bias results when their frequencies are below 20% (Dakin and Avise, 2004; Carlsson, 2008), which applied to all loci in this study. Significant deviations from Hardy–Weinberg (H–W) equilibrium occurred at 16 out of 72 locus-population combinations after Bonferroni's correction ($p = 0.0042$, Table 3). Seven of the 12 sampling locations were out of equilibrium at locus R9 where all but the Huron River samples expressed lower than expected heterozygosity, suggesting null alleles at this one locus. The other nine differences were scattered throughout the results with four excesses in heterozygosity and five deficiencies, and therefore no overall difference was found when pooling across loci (Table 3). Likewise, sampling locations did not vary in mean rarefacted allelic richness (Kruskal–Wallis: $p = 0.905$ and $p = 0.998$ respectively, Table 3), and no recent bottleneck effects were indicated in any sensitivity model whether results were separated for each site or analyzed after grouping sites within the three regions (only Turtle Creek was significant ($p = 0.016$) after adjusting for multiple comparisons).

Among sampling locations, genetic variation in *Q. quadrula* was highly structured (Fig. 2). In the absence of assigning individuals to sampling locations, the entire data set had the highest likelihood of being divided into $K = 4$–6 genetic clusters, all with similar mean log likelihoods (Ln[Pr(X|K)] = −3927.4 for K = 4, Ln[Pr(X|K)] = −3926.9 3926.9 for K = 5, Ln[Pr(X|K)] = −3926.4 for K = 6). However, delineations of different groups were not visually distinct, as can be seen Fig. 2A where many of the individual bars are made of equal parts two or three clusters. When geographic distribution of individuals was added as a priori information, separation of genetic populations was pronounced with K = 3 (Ln[Pr(X|K)] = −3800.0, Fig. 2B). The three clusters were primarily comprised of individuals from the Grand River, Canada, another cluster of individuals of Maumee River origin, and a third as those individuals from Lake Erie.

The Lake Erie cluster showed some heterogeneity that corresponded to the edge localities. Individual samples from North Maumee Bay had almost the same probability of being assigned to the Maumee cluster (at 44%) as they did for Lake Erie (56%), suggesting intermediate multilocus genotypes (Fig. 2B). To the east, individuals from the Huron River, Ohio, possessed similarity with the Grand River cohort (68% Lake Erie and 32% Grand River). A related pattern, but to a lesser degree, was observed in the Sandusky Bay samples, which came from a location just west of the Huron River, also showed similarity with the Grand River Custer (Fig. 2). Other samples from within the western basin, most notably those from Toussaint Creek, Young Marsh, and the Portage River, possessed genotypes partially intermediate among all three clusters (Fig. 2B).

AMOVA results were congruent to those obtained from STRUCTURE analyses. When individuals were separated into twelve distinct sampling locations (Table 4) the global F_{ST} was 0.118 ($p = 0.0001$), a similar value was obtained when defining just three regions, Maumee River, Lake Erie, and Grand River ($F_{ST} = 0.102$, $p = 0.0001$). Moderate levels

Table 3
Across 12 locations, the number of *Quadrula quadrula* collected, mean rarefacted allelic richness, total number of rarefacted alleles, number genotyped, observed heterozygosity (*Ho*), and expected heterozygosity (*He*) Also, the number of rarefacted alleles, number genotyped, *Ho*, and *He* by locus and sampling location for *Q. quadrula*.

	M1	M2	M3	13	19	20	21	24	22	23	30	GR1
# Collected	36	35	27	30	30	30	30	30	30	30	12	65
Mean allelic richness	5.667	5.500	5.833	5.833	5.167	5.167	6.667	7.167	6.000	4.667	3.333	5.000
Global												
Ave # rarefacted alleles	1.576	**	**	1.611	1.531	1.547	1.550	1.634	1.565	1.528	1.503	1.497
# Genotyped	27	28	24	26	27	28	27	28	27	29	10	34
Ho	0.548	0.584	0.626	0.470	0.586	0.557	0.480	0.419	0.464	0.554	0.569	0.437
He	0.539	0.563	0.575	0.594	0.518	0.533	0.538	0.619	0.550	0.516	0.456	0.482
C4												
# Rarefacted alleles	1.723	**	**	1.758	1.537	1.716	1.784	1.723	1.743	1.596	1.894	1.648
# Genotyped	25	27	23	19	17	16	27	24	23	21	6	22
Ho	0.640	0.778	0.783	0.737	0.588	0.813	0.630	0.667	0.652*	0.524	1.000	0.727
He	0.578	0.706	0.810	0.738	0.521	0.693	0.770	0.707	0.727	0.582	0.819	0.633
C114												
# Rarefacted alleles	1.638	**	**	1.577	1.573	1.467	1.592	1.590	1.577	1.592	1.556	1.789
# Genotyped	15	20	18	23	23	24	20	18	24	23	5	10
Ho	0.733	0.850	0.889	0.522*	0.652	0.375	0.650*	0.500	0.417	0.609	1.000	0.400
He	0.584	0.671	0.588	0.564	0.560	0.457	0.578	0.574	0.565	0.579	0.500	0.750
D102												
# Rarefacted alleles	1.000	**	**	1.367	1.040	1.000	1.050	1.444	1.190	1.034	1.000	1.086
# Genotyped	1	0	0	18	25	24	20	19	20	29	10	34
Ho	0.000	0.000	0.000	0.111	0.040	0.000	0.050	0.000*	0.100*	0.034	0.000	0.088
He	0.000	0.000	0.000	0.356	0.039	0.000	0.049	0.432	0.185	0.034	0.000	0.084
R9												
# Rarefacted alleles	1.631	**	**	1.406	1.494	1.527	1.284	1.345	1.297	1.507	1.395	1.058
# Genotyped	24	24	23	17	24	23	19	21	21	28	10	34
Ho	0.458	0.333	0.304	0.000*	0.750	0.870*	0.105*	0.095*	0.190*	0.821*	0.500	0.000*
He	0.576	0.622	0.629	0.394	0.484	0.515	0.277	0.337	0.290	0.498	0.375	0.057
A112												
# Rarefacted alleles	1.725	**	**	1.744	1.719	1.708	1.753	1.777	1.774	1.747	1.857	1.698
# Genotyped	21	20	20	20	23	24	20	25	22	23	4	18
Ho	0.857*	0.750	0.850	0.700	0.696	0.708	0.750	0.680*	0.591	0.783	0.750	0.778*
He	0.766	0.660	0.709	0.725	0.703	0.694	0.734	0.762	0.756	0.731	0.750	0.679
A130												
# Rarefacted alleles	1.739	**	**	1.813	1.824	1.863	1.836	1.922	1.808	1.690	1.318	1.702
# Genotyped	20	24	14	16	19	19	26	21	12	18	6	27
Ho	0.600	0.792	0.929*	0.750	0.789	0.579	0.692	0.571	0.833	0.556	0.167	0.630
He	0.728	0.720	0.712	0.787	0.802	0.841	0.820	0.900	0.774	0.671	0.292	0.689

* Observed heterozygosity out of Hardy–Weinberg equilibrium after Bonferroni's correction ($\alpha = 0.0042$).

** The rarefacted allelic richness listed is that of all Maumee River individuals. This is because rarefacted allelic richness for each Maumee River site (M1, M2, and M3) could not be calculated as a result of limitation in amplifying locus D102 beyond site M1.

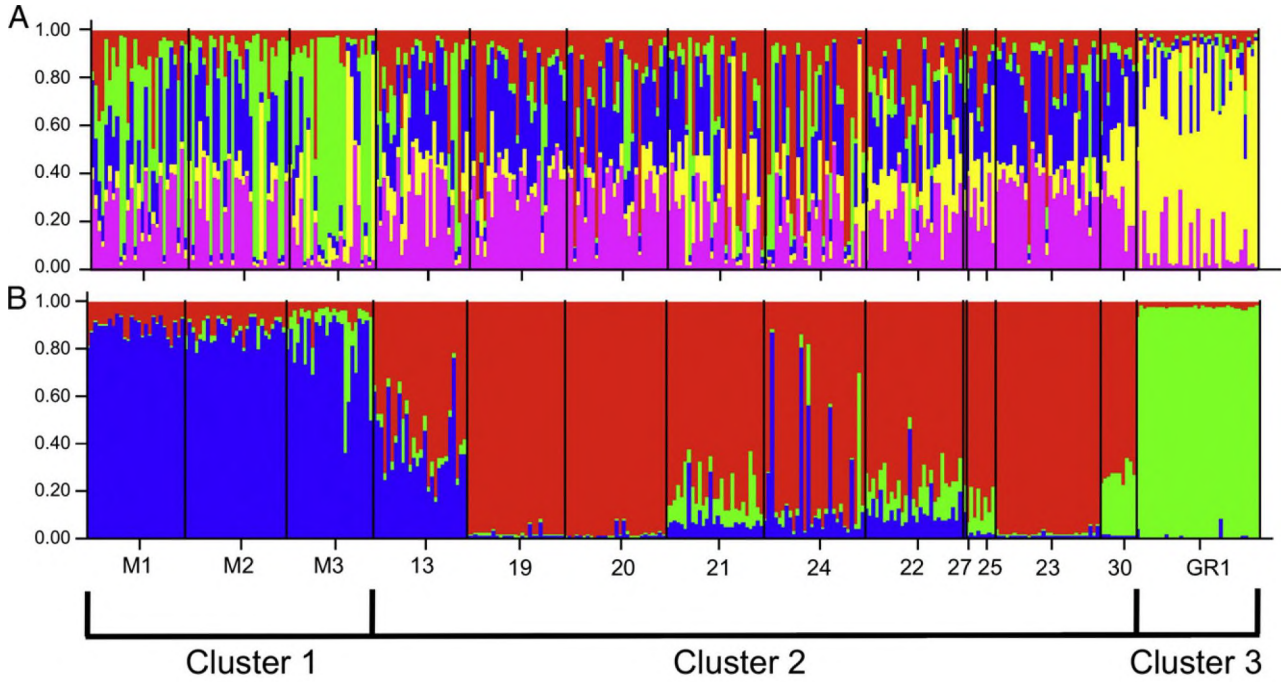

Fig. 2. Summary of STRUCTURE (Pritchard et al., 2000) analyses for *Quadrula quadrula* from Lake Erie and major tributaries. Each color represents a cluster and each bar represents an individual with the probability of membership to a cluster. Black lines separate sampling locations aligned west to east on the x-axis (Fig. 1): (A) results for all genotyped individuals without assigning locality a priori (K = 5), and (B) results with prior geographic information included in the analysis (K = 3).

of genetic differentiation were also suggested by pairwise F_{ST} values (Maumee River & Lake Erie, $F_{ST} = 0.080$; Lake Erie and Grand River, $F_{ST} = 0.111$; and Maumee & Grand rivers, $F_{ST} = 0.204$).

The neighbor-joining topology constructed using TREEFIT (not shown) indicated a good fit with genetic distance ($R^2 = 0.930$) and had three clusters of branches: Maumee River, Lake Erie, and Grand River, supporting the other methods used to determine genetic structure. The greatest branch length, which represents genetic distance, was between the Maumee and Grand River locations, Lake Erie was intermediate, and the Huron River lay between those of the Grand River and the Western Basin localities. Thus, using a Mantel test, a regression of geographic distance on genetic distance was significant ($p < 0.0001$) (Fig. 3).

Discussion

Population structure

The recent invasion of dreissenid mussels caused near extirpation of unionids but may not have had a currently observable genetic impact on the *Q. quadrula* populations within Lake Erie. A progressive change in multilocus genotypes extends between the Maumee River in the west, across Lake Erie, and to the Grand River, Canada, in the east, with differentiation among groups discrete, but not absolute. The Maumee River, which empties into western Lake Erie at Toledo, Ohio, is a known habitat of *Q. quadrula* (Clark and Wilson, 1912; Grabarkiewicz and Crail, 2006) and is considered a possible post-Pleistocene source of *Q. quadrula* for Lake Erie (Graf, 2002). Similar to the Maumee, the Grand River is a major tributary that possessed a diverse mussel community prior to the industrial age, and the persistence of *Q. quadrula* there suggests pollution tolerance (Metcalfe-Smith et al., 2000).

Evidence of admixture within Lake Erie populations indicates that both rivers may continue to function as sources of recolonization for *Q. quadrula* and points to a stepping-stone model of genetic structure for *Q. quadrula* in Lake Erie watershed (Wright, 1940; Kimura and Weiss, 1964). Clear regional genetic differentiation exists in the Lake Erie watershed, nonetheless a high degree of gene flow among neighboring locations is demonstrated in each of the analyses utilized (STRUCTURE outputs, AMOVA, pairwise tests of genetic differentiation, tests for isolation by distance, and the neighbor-joining analysis). The

Table 4
Pairwise F_{ST} (below diagonal) and associated *p*-values (above diagonal) for *Q. quadrula* among Maumee River (M1, M2, and M3), Lake Erie, and Grand River (GR1) sampling locations. All results are from an AMOVA run with 9999 permutations.

	M1	M2	M3	13	19	20	21	24	22	23	30	GR1
M1	–	0.4457	0.0068	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
M2	0.0000*	–	0.1300	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
M3	0.0208*	0.0069*	–	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
13	0.1028	0.1101	0.1079	–	0.0007	0.0026	0.0012	0.0718	0.4295	0.0001	0.0001	0.0001
19	0.1969	0.2072	0.2079	0.0326*	–	0.4300	0.0001	0.0001	0.0026	0.4367	0.0006	0.0001
20	0.1935	0.1996	0.1931	0.0264*	0.0000*	–	0.0001	0.0001	0.0003	0.1607	0.0001	0.0001
21	0.1220	0.1210	0.1400	0.0303*	0.0391	0.0498	–	0.1078	0.0003	0.0001	0.0001	0.0001
24	0.0834	0.0982	0.0957	0.0094*	0.0450	0.0486	0.0077*	–	0.0201	0.0001	0.0001	0.0001
22	0.1349	0.1514	0.1361	0.0000*	0.0288*	0.0362	0.0378	0.0164*	–	0.0003	0.0001	0.0001
23	0.2210	0.2344	0.2344	0.0526	0.0000*	0.0065*	0.0505	0.0642	0.0341	–	0.0060	0.0001
30	0.2451	0.2645	0.2699	0.0848	0.0627*	0.0759	0.0828	0.0941	0.0832	0.0467*	–	0.0075
GR1	0.2951	0.3162	0.3249	0.1801	0.1624	0.1815	0.1457	0.1508	0.1786	0.1551	0.0376*	–

* Indicates F_{ST} values are not significantly different from zero after Bonferroni's correction for multiple comparison ($\alpha = 0.0005$).

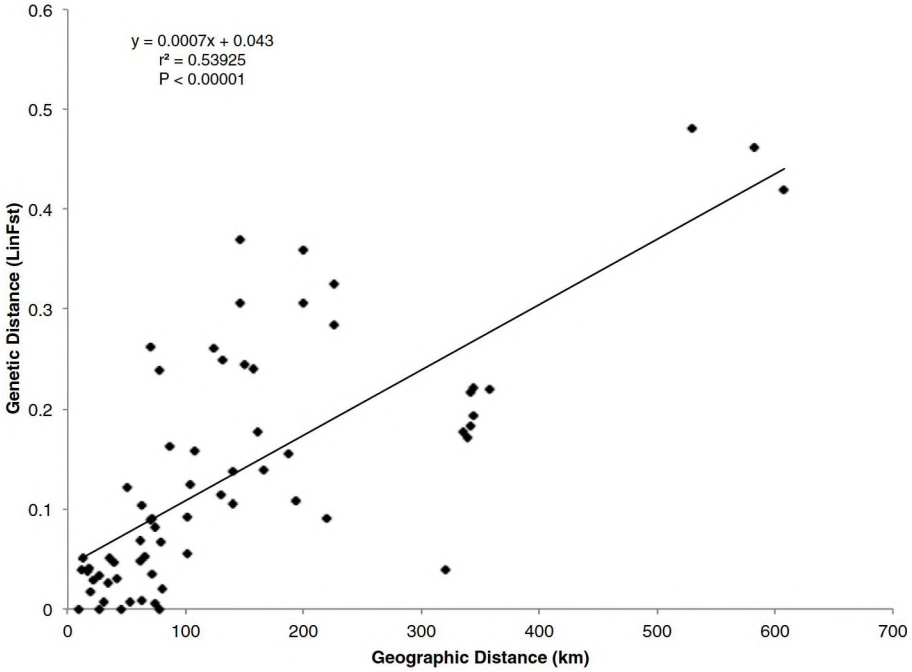

Fig. 3. Linear regression of geographic distance over water between sampling locations and genetic distance (linearized F_{ST}). *p*-Value calculated using a Mantel test (Mantel, 1967).

western basin sites clustered together geographically and did not show obvious genetic structure while pairwise F_{ST} values were above 0.25 among Maumee River sites and the Grand River, indicating increasing differentiation among populations that are most geographically distant, as predicted by a stepping-stone model in contrast to islands models of isolation (Wright, 1940; Kimura and Weiss, 1964).

Unfortunately, the rarity of *Q. quadrula* in other Lake Erie tributaries limits testing further for similarities between *Q. quadrula* in the western portion of the watershed to Canada's Grand River. For example, *Q. quadrula* is likely extirpated from the Rocky River (Krebs and Rundo, 2005); it is rare in the Cuyahoga River (Smith et al., 2002); and Huehner et al. (2005) report its presence only in deep water just above the shipping channel in Ohio's Grand River. An explanation for the observed genetic structure may be found in the movement patterns in channel catfish (*I. punctatus*), the probable host for *Q. quadrula* in Lake Erie. Channel catfish can move sufficient distances to connect all three regions, but not to span the length of the lake (Hubley, 1963; Shrader et al., 2003). Zanatta and Wilson (2011) found evidence of a combined influence of both geographic distribution and host distribution on populations of *Epioblasma triquetra* (Rafinesque, 1820). No genetic information on *I. punctatus* is presently available even though they are a popular sport fish in Lake Erie. Channel catfish rank as the fifth most harvested species in the lake, but greater than 85% of the catch is caught in the western basin (ODW, 2012).

At local scales, dams located in both the lower Grand River at Dunnville, Ontario since 1829 (Bunt et al., 2000) and the in the Maumee River at Defiance and Grand Rapids, Ohio (Independence and Providence Dams), each may impede upstream fish movement (http://www.dnr.state.oh.us/indpndam/tabid/747/Default.aspx). Note that STRUCTURE plots (Fig. 2) provided evidence of downstream admixture into the western basin and from the Grand River, but much less evidence of gene flow upstream in either river even though a fishway was constructed in 1994 in the Grand River to facilitate upstream fish dispersal (and thus mussel glochidia; Metcalfe-Smith et al., 2000). By contrasting population structure in the doubly-uniparental inheritance systems of mussel mitochondria, Krebs et al. (2013) found that dams and waterfalls consistently separated haplotype variants of female-inherited forms, limited to the movement of glochidia, but not male inherited mtDNA haplotypes, which could also move downstream as spermatozeugmata (sperm balls).

Genetic diversity

While dreissenid mussels have been identified as a serious threat to unionid assemblages in the Great Lakes (Zanatta et al., 2002; Schloesser et al., 2006), their effects on the genetic diversity of *Q. quadrula* in Lake Erie, as well as on *Pyganodon grandis* (Say, 1829) and *Lampsilis siliquoidea* (Barnes, 1823) appear as yet small (Krebs et al., 2015; Rowe and Zanatta, 2015). However, we stress that all of these results may represent an overly optimistic situation for other unionids in Lake Erie. Only species with large populations have been tested, and most populations appear to have many alleles for which their frequencies remain in Hardy–Weinberg equilibrium (77.8% locus-population pairs of *Q. quadrula* in this study), and allelic diversity of microsatellite loci was high.

Although there are no studies on genetic diversity of *Q. quadrula* in Lake Erie, Berg et al. (1998) assessed *Q. quadrula* using allozymes and concluded that they vary little across widely separated river systems. Later, Elderkin et al. (2008) examined variation in *Elliptio dilatata* (Rafinesque, 1820) and *Actinonaias ligamentina* (Lamarck, 1819) across even larger distances and similarly found clear effects of genetic isolation in mtDNA haplotypes. Separately, Elderkin et al. (2007) used mtDNA and allozymes to examine population structure of *Amblema plicata* (Say, 1817), and found decreasing genetic diversity in a northerly direction from the Mississippi drainage across to the Lake Erie drainage. *A. plicata*, like *Q. quadrula*, is considered to have an equilibrium life history strategy that could persist during disturbances and thrive once the disturbance subsides (Haag et al., 1993; Haag, 2012). Therefore, the Lake Erie drainage may be sufficiently small of scale not to expect large differences in genetic diversity based on microsatellites, yet differentiation was clear in *Q. quadrula* among sampling locations across the Lake Erie watershed.

Management implications

Rare, threatened, or endangered species have a greater risk of low genetic diversity and in turn are influenced more by differentiation,

isolation, and genetic drift (Freeland, 2005). The low to moderate levels of differentiation of the relatively common *Q. quadrula* found in the western basin of Lake Erie indicates that uncommon unionid species in the same area may be in jeopardy of inbreeding depression. Likewise the more rare and imperiled species may show a disrupted stepping-stone pattern in the Lake Erie watershed with lower connectivity across the lake and its tributaries, thus resulting in lower genetic diversity and greater population instability.

Haag et al. (1993) suggested that species belonging to the unionid tribes Amblemini and Quadrulini are less harshly affected by dreissenids in the western basin of Lake Erie and have generally higher survival rates than members of other unionid groups. Life histories often differ among these species, as equilibrium species like *Q. quadrula* have relatively long lifespans, late maturity, variable and delayed fecundity, and moderate to large body size (Haag, 2012). Several other equilibrium species still occur in the coastal areas, but at lower abundance: *A. plicata*, *Fusconaia flava* (Rafinesque, 1820), *Pleurobema sintoxia* (Rafinesque, 1820), and *Quadrula pustulosa* (Lea, 1831) (Sherman et al., 2013; Crail et al., 2011). Recent findings using *L. siliquoidea* [a member of the tribe Lampsilini with a periodic life history (Haag, 2012)] in Lake St. Clair showed similar results to those found in this study with little evidence of impact of the dreissenid invasion on genetic diversity, despite major demographic effects (Rowe and Zanatta, 2015). The two other more common species remaining in Lake Erie are *P. grandis* (Say, 1829) and *Leptodea fragilis* (Rafinesque, 1820) (Crail et al., 2011; Bryan et al., 2013), both of which are short-lived and highly fecund opportunistic species (Haag, 2012) that may quickly recover naturally from river mouths (Prescott, 2014). The life histories of various mussels in the Lake Erie watershed are likely to result in differing genetic structure post the initial extirpation by dreissenid mussels. Although equilibrium species, such as *Q. quadrula*, may be able to retain populations longer, they cannot repopulate quickly and are at greater risk than opportunistic species should additional stressors occur.

Without further degradation of the current populations and based on *Q. quadrula* as a representative species, a diverse gene pool may remain should unionids repopulate vacated habitat in Lake Erie. As Kimura and Weiss (1964) expected in the stepping stone model, population structure is most evident when genetic distance is examined in relation to geographic distance; and if *Q. quadrula* of the Lake Erie watershed are indeed in a stepping-stone pattern of dispersal, then geographically distant populations remain connected to each other via intermediate locations in the western basin. Unfortunately, dreissenids are not likely to decline soon to densities permissive for large scale, lake-wide recovery of the unionid community in the open waters of Lake Erie.

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