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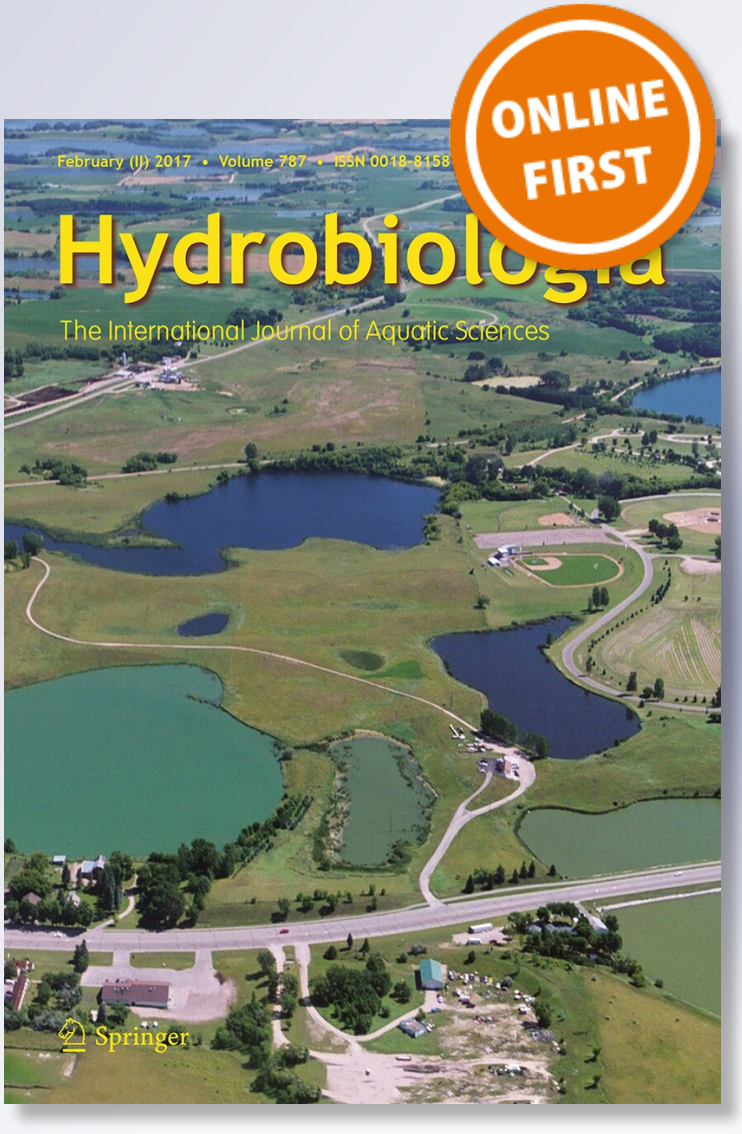

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FRESHWATER BIVALVES

Signature of postglacial colonization on contemporary genetic structure and diversity of *Quadrula quadrula* (Bivalvia: Unionidae)

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Abstract Contemporary species distributions and geographic patterns of genetic structure largely reflect pre-historic events, often with subsequent alterations from human influences. The geographic genetic structure of a relatively common and widespread unionid species, *Quadrula quadrula*, was investigated to reconstruct its postglacial history. Hypotheses regarding colonization routes of *Q. quadrula* into the Great Lakes basin after the most recent glacial retreat were tested. Samples were collected from *Q. quadrula* at sites spanning hypothesized glacial refugia and postglacial expansion routes in the Mississippi River drainage, including the Ohio and Missouri rivers, and the Great Lakes. Broad-scale phylogeography and population structure were assessed by sequencing a fragment of the mitochondrial *CO1* gene and genotyping eight microsatellites. Results of analyses showed marked differences among the Great Lakes, Mississippi River, and Ohio River drainages, and suggested colonization of the Great Lakes basin from a Mississippian source. Populations showed patterns of isolation by distance: geographic and genetic distances were significantly correlated among Great Lakes populations based on colonization through the Chicago–Illinois outlet, but not when following the Wabash–Maumee outlet. All evidence indicates that postglacial colonization of the Great Lakes basin occurred almost exclusively through the Chicago–Illinois outlet, with subsequent expansion into the lower Great Lakes.

Keywords Great Lakes · Freshwater mussels · Phylogeography · Mitochondrial DNA · Microsatellites · Conservation genetics

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Introduction

Contemporary distributions of aquatic species were greatly influenced by Pleistocene glacial events (Bernatchez & Wilson, 1998; Soltis et al., 2006). In North America, freshwater fish and mussel species colonized the Laurentian Great Lakes basin between 14,400 and 9,000 years ago, following the late-Wisconsinan

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retreat of the Laurentide Ice Sheet (Bailey & Smith, 1981; Mandrak & Crossman, 1992). As the glaciers receded and drainages changed, several meltwater outlets in the Great Lakes region potentially allowed fish and mussels to enter the Great Lakes from unglaciated refugia (van der Schalie, 1945; Bailey and Smith, 1981; Mandrak & Crossman, 1992; Larson & Schaetzl, 2001; Graf, 2002). Van der Schalie (1945) and Mandrak & Crossman (1992) identified two major entry points for aquatic organisms into the Great Lakes (Fig. 1). After the lake levels declined and isostatic rebound progressed, some historic glacial outlets no longer flowed (Bailey & Smith, 1981). For example, the current lower Lake Michigan tributaries and Lake Huron tributaries, and the upper Maumee River tributaries and the upper Wabash River tributaries may have been connected hydrologically until isostatic rebound occurred (Mandrak & Crossman, 1992; Herdendorf, 2013).

Unionid mussels (family Unionidae) are considered among the most imperiled faunal groups in North America (Lydeard et al., 2004) with over 70% of unionid species listed as threatened or endangered (Williams et al., 1993; Haag, 2012). Compared to fish species, there is relatively limited knowledge about genetic variation or phylogeographic structure among unionid mussel populations, despite its value for species management and conservation (Lydeard et al., 2004; Bogan and Roe, 2008; Haag, 2012; FMCS, 2016). Emerging knowledge regarding mussel population genetics has informed conservation biologists about populations most in need of management (Jones et al., 2006a, b; Hoftyzer et al., 2008; Galbraith et al., 2015; Paterson et al., 2015). Phylogeographic and population genetics methods have been employed as approaches to gain insight and direction towards conservation efforts for unionid species and populations (Berg et al., 1998; Geist & Kuehn, 2005; Kelly & Rhymer, 2005; Berg et al., 2007; Elderkin et al., 2007; Inoue et al., 2013; Froufe et al., 2014; Galbraith et al., 2015; Hewitt et al., 2016). Phylogeographic studies can be used not only to identify the number and

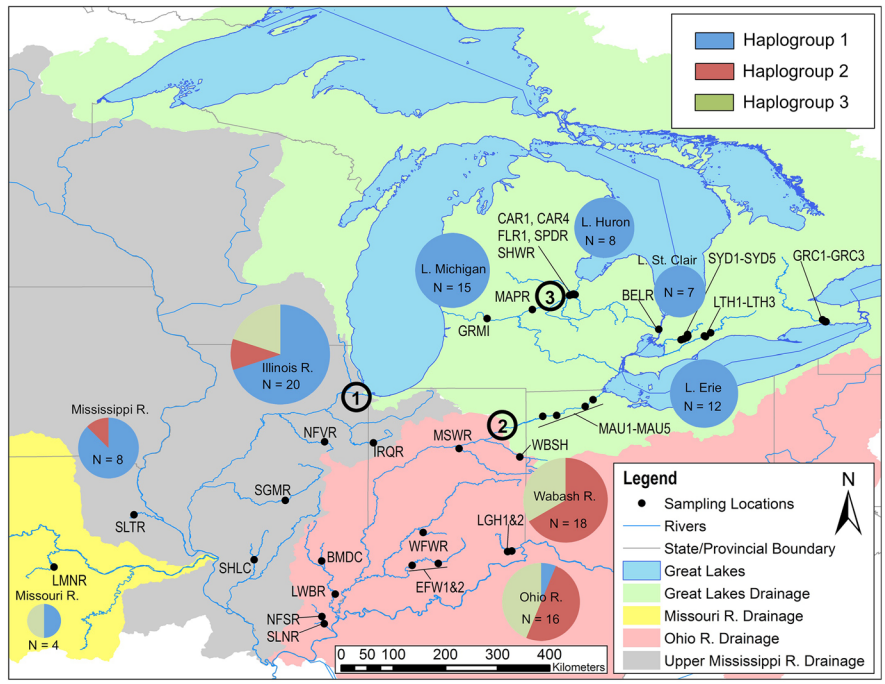


Fig. 1 Collection sites for *Q. quadrula* in the Great Lakes basin and Mississippi River drainage with site codes listed as in Table 1; contemporary drainages are color-coded. *Circled numbers* indicate potential single-entry colonization routes: (*1*) Chicago–Illinois outlet, (*2*) Wabash–Maumee outlet. The third hypothesized route that is a combination of (*1*) and (*2*) is not depicted. A hypothesized postglacial connection between the Grand River (Lake Michigan) and Saginaw River (Lake Huron) drainages is depicted by (*3*). Also shown is the haplogroup composition of *Q. quadrula* from nine drainages in Mississippi River and Great Lakes watersheds. *Pie chart* sizes correlate to sample size from each location. Haplogroup colors correspond with those described in Fig. 2

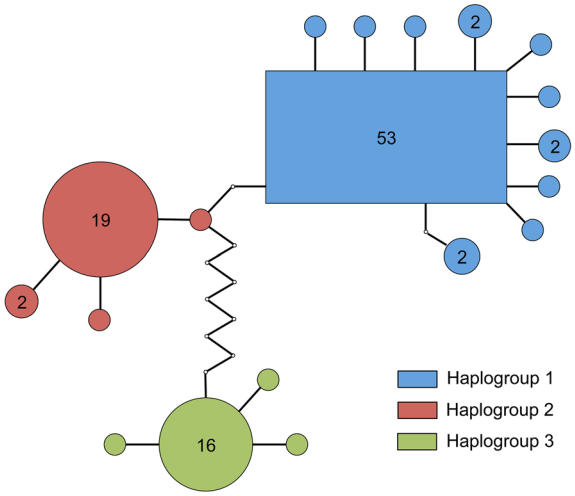


Fig. 2 Haplotype network for 108 *Q. quadrula* CO1 mtDNA sequences from 16 sites. The network was constructed in TCS using 95% connection limit, gaps defined as missing data, and manually removed redundant loops and reticulations following the procedure outlined in Fetzner & Crandall (2003). Haplotypes were categorized into haplogroups based on haplotype network structure. The number of individuals with each haplotype is noted in each *circle*; *unlabeled circles* were represented by only one individual. *Small uncolored circles* represent inferred mutational differences among observed haplotypes

distribution of extant intraspecific lineages, but also to reconstruct their respective postglacial or ancestral colonization routes (Bernatchez & Wilson, 1998; Soltis et al., 2006). Identifying intraspecific genetic lineages can provide valuable information for helping to conserve species and populations (Moritz, 1994); determining patterns of diversity for post-Pleistocene native mussel assemblages and their source populations can therefore provide valuable information for their conservation and management (Elderkin et al., 2007, 2008; Zanatta & Murphy, 2007, 2008; Zanatta and Harris, 2013). As Graf (2002) and Hewitt et al. (2016) suggest that some unionid mussel species that occur in the central Great Lakes (lakes Michigan, Huron, and Erie) used multiple entry points into the Great Lakes following the most recent glacial retreat, the postglacial colonization of the basin has not yet been fully resolved.

The Mapleleaf, *Quadrula quadrula* (Rafinesque, 1820), is a relatively common and widespread unionid mussel found in tributaries of the lower Great Lakes, the St. Lawrence River, the Red River of the North, and Mississippi River drainages in medium to large rivers, shallow lakes, and big river embayments. It is not listed as threatened or endangered anywhere throughout its U.S. range (Williams et al., 1993; Oesch, 1995; Garner & McGregor, 2001; WDNR, 2003; Lydeard et al., 2004; Grabarkiewicz & Crail, 2006; Williams et al., 2008). In Canada, *Q. quadrula* is currently considered threatened in the Great Lakes–St. Lawrence Designatable Unit (DU) and endangered in the Saskatchewan–Nelson River DU (Metcalfe-Smith et al., 2005; COSEWIC, 2006).

Like other unionid mussels, *Q. quadrula* depends on host fish species for their early development and for long-distance dispersal of their larvae, in which larval mussels, or glochidia, attach themselves to a host fish (Williams et al., 2008). These glochidia-bearing fish can travel various distances within rivers and among watersheds. *Q. quadrula* is known to use the flathead catfish (*Pylodictis olivaris*; Rafinesque, 1818) and channel catfish (*Ictalurus punctatus*; Rafinesque, 1818) as glochidial host fishes (Watters et al., 2009). The home range of the flathead catfish can range from several hundred meters to over 1.5 km (Daugherty & Sutton, 2005), with dispersal distances that range from several kilometers to over 50 km (Vokoun & Rabeni, 2005). Channel catfish have similarly large dispersal distances of up to 40 km, and have the potential to travel nearly 350 km (Hubley, 1963). The large distances that these potential host fish can disperse allow for the potential spread of larval mussels over large geographic distances and the potential for connection among several populations. Based on distributional data, host fishes for *Q. quadrula* have been hypothesized to have entered the Great Lakes through the Chicago–Illinois outlet, crossing the Lower Peninsula of Michigan via the Grand–Saginaw outlet to enter the lower Great Lakes (Huron, Erie, and St. Clair), but may have also used the Wabash–Maumee outlet (van der Schalie, 1945; Graf, 2002). Mandrak & Crossman (1992) hypothesized that Ictaluridae most likely entered the Great Lakes via the Chicago–Illinois outlet.

The relative stability of Mapleleaf populations across a moderately broad species range allows *Q. quadrula* to be used as a foundation for comparative studies of other mussel species' geographic genetic structure (Berg et al., 1998), especially those species with similar life history strategies (Haag, 2012). Haag (2012) describes most mussel species belonging to the tribe Quadrulini (which includes *Q. quadrula*) as

having an equilibrium life history strategy: long life span and late maturity, low fecundity (although high in species that use a broadcast host infection method), a short-term brooding strategy for glochidia, and moderate to large body size. By analyzing a relatively intact species such as *Q. quadrula*, a baseline of conservation management options can be designed and then applied as appropriate to threatened and endangered species (Berg et al., 1998). This approach could potentially benefit threatened and endangered mussel species with similar life history strategies without requiring invasive sampling such as tissue collections.

The objective of this project was to assess the phylogeographic origins and history of *Q. quadrula* populations in the Great Lakes basin. As such, the number of genetic groups and their distribution across the species range should reflect the number of glacial lineages, as well as their colonization route(s) following deglaciation. Microsatellite DNA genotyping and mitochondrial DNA sequencing were used to assess genetic structure among contemporary populations and identify the most probable colonization route(s) that *Q. quadrula* followed postglaciation. The three postglacial colonization routes that have been hypothesized to have been used by ictaluridae to enter the Great Lakes are a Chicago–Illinois outlet as suggested by Mandrak & Crossman (1992), a Wabash–Maumee outlet, or a combination of the Chicago-Illinois and the Wabash–Maumee outlets as suggested by van der Schalie (1945) and Graf (2002) (Fig. 1). Accordingly, each of these potential colonization histories were treated as hypotheses with testable predictions of genetic relationships among populations corresponding with shared phylogeographic ancestry: (1) following a connection between the Illinois River (Mississippi River drainage) and Lake Michigan, and (2) following a connection between the Wabash River (Ohio River drainage) and the Maumee River (Lake Erie drainage).

Methods

Study Area, Site Selection, and Sample Collection

Populations of *Q. quadrula* were non-lethally sampled from across much of the species' range, targeting locations in Michigan, Ohio, Indiana, Illinois, Missouri, and Ontario north and south of the Wisconsinan glacial maximum (Fig. 1). Sites were selected based on accessibility from roads and previously known collection sites. In total, *Q. quadrula* were sampled at 40 sites from 26 rivers within both the Great Lakes and Mississippi River drainages from 2008 to 2010 (Fig. 1; Table 1).

Individual mussels were collected by hand with the use of underwater viewers, snorkeling, or SCUBA using tactile searches. Small tissue samples for genetic analysis were obtained by taking a non-lethal biopsy from the mantle tissue of each mussel (Berg et al., 1995). Lengths of each mussel were measured using calipers, and voucher photographs were taken for identification purposes. Mussels were opened gently, taking special care to avoid tearing the adductor muscles, and small (<0.25 cm^2) clips of mantle tissue were taken from each mussel. Mantle clips were individually preserved in 95% ethanol in 2-ml cryovials labeled with individual mussel number, sampling site (river and location on the river), and the date collected, and stored at room temperature for later DNA extraction. Sampled mussels were carefully placed back in their river habitat and area of capture in the correct orientation with their apertures able to open into the water column.

Genetic data collection

Approximately half of the tissue clipped from each individual mussel was used for DNA extraction using an isopropanol extraction method modified from Sambrook et al. (1989), with an increased amount of Proteinase K (300 μg per sample) to ensure tissue digestion. Extracted DNA was visualized by UV fluorescence using electrophoresis on a 1.5% agarose gel in the presence of SyBr Green and diluted to a working solution of 10 ng/μL in TE buffer (10 mM Tris pH 8, 1 mM EDTA).

A 651-bp fragment of the female lineage mitochondrial gene cytochrome *c* oxidase subunit I (*CO1*) was amplified for a subset of individuals from geographically representative sites using primers and polymerase chain reaction (PCR) conditions described in Campbell et al. (2005). To confirm amplifications, mitochondrial PCR fragments were stained with SYBR Green and visualized using gel electrophoresis with a 1.5% agarose gel. Excess nucleotides and primers were removed using Exonuclease I

Table 1 Sampling localities for *Q. quadrula* by primary and secondary drainage (Drainage 1 and Drainage 2), showing waterbody names, site codes, and latitude and longitude in NAD 1983 decimal degrees

Drainage 1	Drainage 2	ST/PR	Waterbody	Site code	Latitude	Longitude	n	H_O	H_E	F_{IS}
Great Lakes	Lake Michigan	Michigan	Grand R.	GRMI	42.954459	−85.474561	21	0.471	0.551	0.161
		Michigan	Maple R.	MAPR	43.108500	−84.694440	23	0.509	0.480	−0.018
	Lake Huron	Michigan	Cass R.	CAR1	43.376260	−83.980930	15	0.648	0.543	−0.184
		Michigan	Cass R.	CAR4	43.365050	−83.955460	29	0.641	0.532	−0.131
		Michigan	Flint R.	FLR1	43.353580	−84.051510	30	0.373	0.392	0.289
		Michigan	Spaulding Drain	SPDR	43.367580	−84.001750	12	0.455	0.394	−0.127
		Michigan	Shiawassee R.	SHWR	43.370190	−84.002380	47	0.563	0.525	0.055
	Lake St. Clair	Michigan	Belle R.	BELR	42.768210	−82.511530	22	0.561	0.515	0.024
		Ontario	Sydenham R.	SYD5	42.679674	−82.014891	29	0.499	0.497	0.119
				SYD4	42.650100	−82.008910	38	0.562	0.506	−0.114
				SYD3	42.626688	−82.023251	22	0.542	0.504	−0.002
				SYD2	42.604687	−82.075878	24	0.499	0.466	−0.078
				SYD1	42.589130	−82.125330	23	0.525	0.497	−0.068
		Ontario	Lower Thames R.	LTH3	42.708158	−81.616479	25	0.574	0.508	−0.009
				LTH2	42.654272	−81.724591	25	0.575	0.514	0.005
		Ontario		LTH1	42.642744	−81.704157	25	0.576	0.517	−0.155
	Lake Erie	Ontario	Lower Grand R.	GRC1	42.899211	−79.625234	25	0.582	0.512	−0.177
		Ontario		GRC2	42.901931	−79.640638	25	0.554	0.512	−0.094
		Ontario		GRC3	42.932717	−79.685453	27	0.605	0.523	−0.162
		Ohio	Maumee R.	MAU1	41.562678	−83.644879	30	0.492	0.465	−0.078
		Ohio		MAU2	41.445877	−83.781839	27	0.600	0.530	−0.151
		Ohio		MAU3	41.291420	−84.274880	35	0.612	0.525	−0.182
		Ohio		MAU4	41.275460	−84.514730	30	0.614	0.501	−0.254
Mississippi R.	Missouri R.	Missouri	Lamine R.	LMNR	38.679580	−92.949890	39	0.679	0.642	−0.091
	Mississippi R.	Missouri	Salt R.	SLTR	39.580930	−91.570480	35	0.474	0.598	0.262
	Kaskaskia R.	Illinois	Shoal Creek	SHLC	38.806380	−89.495360	35	0.532	0.597	0.099
	Illinois R.	Illinois	Sangamon R.	SGMR	39.828100	−88.958600	38	0.567	0.564	−0.043
		Illinois	N. Fk. Vermillion R.	NFVR	40.837700	−88.280000	33	0.458	0.521	0.190
		Indiana	Iroquois R.	IRQR	40.820280	−87.437800	30	0.519	0.613	0.245
	Wabash R.	Indiana	Mississinewa R.	MSWR	40.720556	−85.958611	50	0.439	0.563	0.278
		Indiana	E. Fork White R.	EFW1	38.709270	−86.768940	49	0.453	0.554	0.169
		Indiana	E. Fork White R.	EFW2	38.740833	−86.316667	31	0.428	0.566	0.248
		Indiana	W. Fork White R.	WFWR	39.275680	−86.578410	36	0.359	0.456	0.264
		Illinois	Little Wabash R.	LWBR	38.213730	−88.095610	60	0.371	0.475	0.174
		Illinois	Big Muddy Creek	BMDC	38.783300	−88.330600	60	0.541	0.614	0.097
	Ohio R.	Indiana	Wabash R.	WBSH	40.576390	−84.909400	9	0.368	0.391	0.049
		Illinois	Saline R.	SLNR	37.703600	−88.286400	59	0.496	0.507	0.027
		Illinois	N. Fk. Saline R.	NFSR	37.827100	−88.325600	35	0.466	0.475	0.053
		Indiana	Laughery Creek	LGH1	38.956389	−85.046667	13	0.400	0.536	0.293
		Indiana	Laughery Creek	LGH2	38.946389	−85.120000	35	0.483	0.554	0.197

The state or province (ST/PR) in which sampling locations occurred is also listed. Sampling and genetic diversity summary statistics listed are the number of individuals (n) genotyped from each site, as well as observed and expected heterozygosity (H_O and H_E), as well as within-population inbreeding coefficient (F_{IS})

(Amersham Biosciences cat# E70073X, 10 U/ml) and shrimp alkaline phosphatase (Amersham Biosciences cat# E70092X, 1 U/ml), and incubation at 37°C for 40 min and then 80°C for 20 min to denature enzymes. The 5′ end of the amplified *CO1* region was cycle sequenced with the forward *CO1* primer using the BigDye v.3.1 terminator cycle sequencing master mix (Applied Biosystems, Inc.) and visualized on an ABI 3100 automated DNA sequencer.

For microsatellite markers, all sampled individuals were genotyped at eight microsatellite loci. Primers were originally developed for the congeneric species *Q. fragosa* (Conrad, 1835) by Hemmingsen et al. (2009), and were optimized for *Q. quadrula* in this study to compensate for potential genetic differences between the two species. Temperature gradients were performed on an Eppendorf Mastercycler ep gradient thermocycler (Eppendorf, New York), and PCR products were visualized on an Applied Biosystems ABI 3730 automated sequencer. Amplicon quality and yield, as well as genotypes and allele sizes, were scored using GENEMARKER^{TM} (SoftGenetics, LLC) software to determine the optimal annealing temperature for each locus.

Once the annealing temperatures were optimized, the microsatellite loci were amplified using 10-µl PCR reactions containing 1 µl of 10 × PCR buffer (Qiagen Sciences, Maryland), 0.2 µM of $MgCl_2$, 0.2 µl of 10 mM dNTPs, 1 µl of 2 mg/ml BSA, 0.4 µM of forward and reverse primers for QqA112$^{6\text{-FAM}}$, QqA130$^{6\text{-FAM}}$, QqC114^{NED}, QqC4^{5HEX}, QqD102^{5HEX}, or QqN9$^{6\text{-FAM}}$; for QqA103-M13 and QqR9-M13 0.4 µM of M13 fluorescent probe, 0.1 µM forward primer, and 0.4 µM reverse primer, 0.25 units of *Taq* DNA polymerase, approximately 10 ng of DNA (1.0 µl), and sterile deionized water. Primer sequences and annealing temperatures are detailed in Table 2. Amplifications were done in an Eppendorf Mastercycler ep gradient thermocycler (Eppendorf, New York), using cycling conditions of 10 min at 94°C followed by

Table 2 Microsatellite loci from Hemmingsen et al. (2009) used to genotype *Q. quadrula*, listing locus names, GenBank accession numbers, forward (F) and reverse (R) primer sequences, annealing temperature (T_A) for *Q. quadrula*, repeat motif, size range of the alleles in base pairs (bp), number of alleles (*A*), and the fluorescent dye or probe used for visualization

Locus	Genbank accession	Primer sequence (5′–3′)	T_A (°C)	Repeat motif	Size range (bp)	*A*	Dye
QfA103-M13	FJ785629	**F: GCACACCTTATTCATTTGAGA**	48.0	CA	298–302	2	M13-Probe
		R: GGACACCCCAGTGTGTAAGAACA					
QfA112	FJ785630	**F: ACTTGCTCCAAAACTTGTAGAG**	55.0	CA	160–180	18	6-FAM
		R: GGAATGGTTCAGACTATGACC					
QfA130	FJ785631	**F: TGAGAAATCGTGATGACTCAG**	55.0	TG	287–323	29	6-FAM
		R: CCTACCTACCTTCATGTGGTC					
QfC4	FJ785632	**F: TGTCCTTCTCTGTGAATGTTTG**	59.0	TACA	236–380	27	5HEX
		R: GCACTCCATAAATGCAGGTAAT					
QfC114	FJ785634	**F: TCCATGTTTTTCTCCTCCTCTA**	59.0	TACA	246–270	15	NED
		R: CACCCTTGCTTATAGCGTAGTC					
QfD102	FJ785635	**F: TGGACAATTCATCAAGTCAAG**	51.0	ATCT	290–350	5	5HEX
		R: CTTTGTTTTCCAAACCATACAG					
QfN9	FJ785636	**F: TCGTCTACCACCTCTGCAACACATACCG**	68.0	TG	435–473	17	6-FAM
		R: GGCAGAGAGGTCACAACCCCGGA					
QfR9-M13	FJ785639	**F: AGCTTGGGATCGGAGTTGCAGCCAGC**	63.5	CA	212–220	5	M13-Probe
		R: GGACACCCCAGTGTGTAAGAACA					
M13-Probe		**TGTAAAACGACGGCCAGT**					6-FAM

Labeled or M13-tailed primers and probe are shown in bold font

40 cycles of 94°C for 45 s, 45 s at the primer annealing temperature (Table 2), and 1 min at 72°C; with a final extension period of 72°C for 20 min.

After amplification, 0.5 µl of the PCR product was added to 10 µl of a solution containing 1 ml of HiDi formamide and 2 µl of ROX 500 size standard (Applied Biosystems Inc.), and were genotyped on the same ABI 3730 sequencer (Applied Biosystems Inc.). The results were then analyzed using GENEMARKER™ (SoftGenetics LLC) to score allele sizes and genotypes.

Data Analysis

Mitochondrial DNA sequences were proofread using 4PEAKS v.1.7.1 (Griekspoor & Groothuis, 2006) and edited using MESQUITE v.3.0 (Maddison & Maddison, 2008). The program CLUSTAL W v.2.1 (Larkin et al., 2007) was used to align sequences for editing and analysis. Unique haplotypes from sequence reads longer than 400 bp were identified for each species using COLLAPSE v.1.2 (Posada, 2004). Missing nucleotides were defined as missing data. The mean number of polymorphic sites and nucleotide diversity (π) were calculated using ARLEQUIN v. 3.5.2.2 (Exofficer & Lischer, 2010). TCS v.1.21 (Clement et al., 2000) was used to create a haplotype network using a 95% connection limit with gaps defined as missing data. Haplotypes were categorized into haplogroups based on haplotype clusters (Elderkin et al., 2008), and loops and reticulations in the haplotype network were resolved using the method described in Fetzner & Crandall (2003).

For the microsatellite data, only individuals that amplified successfully at five or more of the eight microsatellite loci were used for analysis. GenAlEx 6.5 (Peakall & Smouse, 2006) was used to calculate allele frequencies and estimate observed and expected heterozygosities (H_O and H_E, respectively). GENEPOP 4.2 (Raymond & Rousset, 1995) was used to test for deviations from Hardy–Weinberg equilibrium expectations for each locus and population. MICROCHECKER (van Oosterhout et al., 2004) was used to test for and estimate the frequencies of potential null alleles using the Brookfield 1 method (Brookfield, 1996). The data were tested for pairwise linkage disequilibrium among loci using GENEPOP.

Genetic structuring within and among sample collections was tested using both individual- and population-based analyses. An individual-based Bayesian assignment test was performed using STRUCTURE (Pritchard et al., 2000) to determine the number of hypothesized genetic groups (K) within the dataset as well as individual membership coefficients for each group. Values of K from 1 to 41 (number of sampling locations +1) were run 10 times each to assess their probability and robustness (Pritchard et al., 2000). The STRUCTURE parameters used were 200,000 burn-in iterations, followed by 100,000 sampling iterations, using assumptions of correlated allele frequencies across loci and potential admixture (gene flow) among populations. Separate analyses were run with and without a priori population information. The most probable number of populations (optimal K; Pritchard, 2010) was selected based on the rate of change in the log probability of data between successive K values (Pritchard, 2010) and using the method of Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012).

Genetic distances among populations were estimated in GenAlEx 6.5 (Peakall & Smouse, 2006), using Nei's (1972) genetic distance measure (D). A dendrogram was created in POPTREE2 (Takezaki et al., 2010) from allele frequency data using a neighbor-joining algorithm (Saitou & Nei, 1987) to calculate 1,000 bootstrap replicates. FIGTREE V1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize the topology and display bootstrap nodal support.

Hierarchical genetic divergence (F_{ST} and Jost's D; Jost, 2008) among populations were tested using GenAlEx 6.5 (Peakall & Smouse, 2006). To test for contemporary versus historical groupings, F_{ST} estimates were generated among sampling locations in the upper Mississippi and Missouri River drainages, the Ohio River, and the Great Lakes basin. An analysis of molecular variance (AMOVA; Excoffier et al., 1992) was run using GenAlEx 6.5 (Peakall & Smouse, 2006) to test the statistical significance of genetic divergences within and among these regions.

Population divergences due to isolation by distance (IBD) were assessed using a Mantel test (Mantel, 1967) implemented in GenAlEx 6.5 (Peakall & Smouse, 2006) to test for correlations between genetic and geographic distances among populations. Pairwise genetic differences were calculated as linearized F_{ST} in GenAlEx. Two distance matrices were calculated using river lengths (kilometers) from the U.S.

Geological Survey National Hydrography medium resolution dataset (USGS-NHD; http://nhd.usgs.gov/data.html) flowline shapefiles in ARCMAP 10.1 (ESRI, California). The data were permutated 10,000 times for the Mantel test to determine significance. Several assumptions were made while measuring the hypothetical colonizations through the proposed outlets before isostatic rebound occurred. It was assumed that the Wabash River drainage and Maumee River drainage were historically connected near the Indiana–Ohio border through a 7-km gap (calculated using the shortest straight-line distance between the USGS-NHD flowline shapefiles) between the Little River (Wabash River watershed) and the Saint Mary's River (Maumee River watershed). The Chicago–Illinois outlet was similarly assumed to have drained through the Calumet River to the Illinois River near Gary, Indiana as suggested by Larson & Schaetzl (2001). The connection between the Grand River drainage and Saginaw River was assumed to be through a 3.5-km gap between the Maple River (Lake Michigan drainage) and the South Fork Bad River (Lake Huron drainage) near Maple Rapids, Michigan using the shortest straight-line distance between the USGS-NHD flowline shapefiles (Fig. 1). The distances through and across the Great Lakes were assumed to be the shortest (straight-line) distance across the lakes. Assumed distances between sampling locations were measured using the 'measure' tool in ArcMap 10 (ESRI); river distances were summed from the River_km column of the flowline shapefile after being selected in ArcMap 10 (ESRI).

Results

Mitochondrial phylogeography

Mitochondrial *CO1* sequences greater than 400 bp were acquired from 108 *Q. quadrula* from nine drainage basins and 16 sampling locations across central North America (Appendix 1—Supplementary materials). Nineteen unique haplotypes were identified from these sequences (Genbank Accession #KX853887–KX853982). The number of individuals sequenced per population (range 4–20), the number of haplotypes per population (range 2–7), the number of haplotypes unique to each population (range 0–4), mean pairwise differences (range 0.167–7.000), and mean nucleotide diversity (π) are displayed in Appendix 1—Supplementary materials.

Three distinct haplogroups were identified based on haplotype network structure (Fig. 2). Only haplogroup 1 was detected within the Great Lakes basin (Fig. 1). Haplogroups 2 and 3 were present at sites associated with Ohio and Mississippi river drainages, but were particularly dominant in the Ohio, Wabash, and Missouri rivers, and not found in the Great Lakes (Fig. 1). Haplogroup 1 was not found in the Wabash River, and was rare in the Ohio River. Two Upper Mississippi River sites, Iroquois River and Sangamon River, which are close to the hypothesized Chicago–Illinois outlet, shared haplotypes with Great Lakes sites, with 70% of haplotypes belonging to haplogroup 1 (Fig. 1). By contrast, sites in the Wabash River drainage had no haplotypes in common with any Great Lakes sites (Fig. 1).

Microsatellite Diversity

A total of 1,284 individuals from the Great Lakes ($n = 694$) and Mississippi/Ohio River drainages ($n = 590$) were successfully amplified at a minimum of five of the eight microsatellite loci. Microsatellite diversity varied considerably among the genotyped loci: only two alleles were observed at locus QqA103, whereas locus QqA130 showed the most variation with 29 alleles (Table 2). A substantial proportion of locus-by-population Hardy–Weinberg tests appeared to deviate from equilibrium expectations, with 52 of 320 tests showing significant deviations after table-wide Bonferroni correction ($\alpha = 0.0001873$; many more were significant without a Bonferroni correction) that excluded locus-collection site combinations with only one amplified allele or that had failed amplifications (Appendix 2—Supplementary materials). Of these, 35 showed a significant heterozygote deficit, and 17 showed greater heterozygosity than expected. The highest and lowest mean levels of heterozygosity ($H_O = 0.679$ and 0.359) were found in the Lamine River (LMNR) and West Fork White River (WFWR) collection sites, respectively (Table 1). Some departures from Hardy–Weinberg expectations appeared to be due to the presence of null alleles (Appendix 3—Supplementary materials). The frequency of null alleles was highest within locus QqC4, which ranged from 0.16 to 0.32 for the 12 populations with a null allele apparent (frequency >0.05). Null alleles were

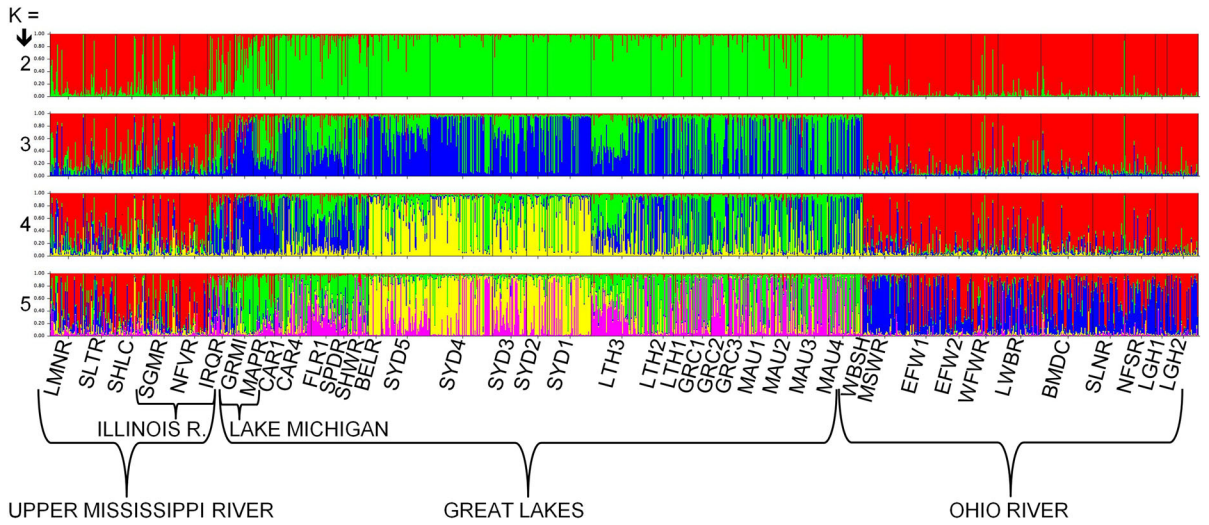


Fig. 3 STRUCTURE bar plot output for 40 *Q. quadrula* collection sites without a priori populations assigned (admixture and correlated alleles were assumed) for multiple genetic groupings (K), ranging from $K = 2$ to $K = 5$. Sampling localities are labeled with site codes and are divided by their Drainage 1 and/or Drainage 2 listed in Table 1

also present in four populations at locus QqA103 with a range of 0.21–0.32, and a single population each at loci QqA112 (0.16) and QqD102 (0.28). Most locus–population combinations with null alleles present were found in the unglaciated (southern) portions of this study. The Grand River in Michigan (site GRMI), which grouped with the Iroquois River (IRQR, see below), had two loci with null alleles, the most of any Great Lakes populations. Locus QqC4 exhibited null alleles only in the unglaciated sampling locations and the site from the Grand River, Michigan (GRMI). No significant linkage disequilibrium was observed among the microsatellite loci or sampling locations.

Genetic Structure

Individual-based analysis of multilocus genotypes showed clear evidence of spatial structure among geographic sample collections. The most probable number of genetic clusters for *Q. quadrula* detected with STRUCTURE without using a priori sampling location information was two groups using the ΔK method (Evanno et al., 2005), but greater resolution and higher log-likelihood probability [$\ln P(K)$] suggested five genetic groups ($K = 5$) as the most informative solution (without a priori population assignments $\Delta K = 1{,}027.92$ for $K = 2$ and mean $\ln P\ (K) = -24{,}241.32$ for $K = 5$; Fig. 3, Appendix 5—Supplementary materials). Excluding the two loci with common null alleles or sampling locations out of HWE (QqA103 and QqC4) did not change the clusters identified or individual assignments to genetic groups (data not shown). STRUCTURE outcomes for $K = 2$ showed a strong distinction between sites sampling locations in the Great Lakes basin and those in the Mississippi, Missouri, and Ohio River drainages (Fig. 3). Two exceptions to this were individuals from the site nearest the Chicago–Illinois outlet in the Iroquois River (upper Illinois River watershed) and upper Wabash River (Ohio River drainage), which both showed affinities to the Great Lakes dataset (Fig. 3). At higher values of *K in the STRUCTURE analysis*, sites in the Mississippi and Ohio river drainages retained genetic affinities with geographically proximal sites in the Great Lakes drainages; Iroquois River with the Grand River and Wabash River with individuals in the Maumee River (MAU1-4) (Fig. 3). As more genetic clusters were considered ($K > 3$), sampling locations from the Mississippi and Ohio River drainages were more clearly distinct, but showed little evidence of substructure within either river basin (Fig. 3). By contrast, genetic substructure was evident among Great Lakes sites as values of K increased, with two Lake St. Clair tributaries (Sydenham and Belle rivers) showing clear differences from other sampling locations. Differences among other Great Lakes tributaries were most apparent at $K = 5$, with Thames River, Ontario

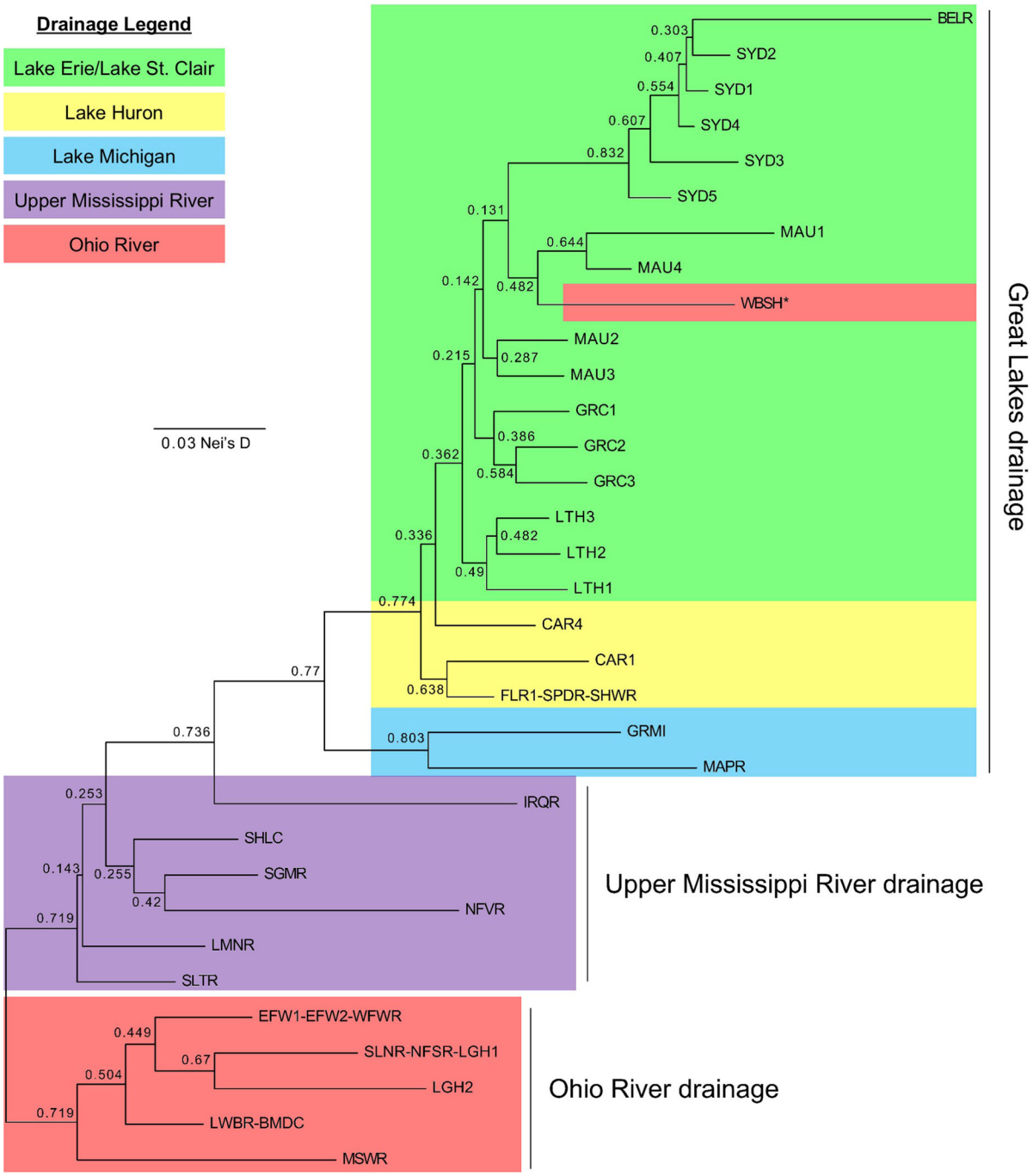


Fig. 4 Neighbor-joining dendrogram of genetic divergences among populations based on Nei's (1972) genetic distance (*D*), using sample location codes in Table 1. The *scale bar* represents genetic distance and the *numbers* at each node indicate nodal support based on 9,999 iterations

genotypes sharing affinities with Lake Huron tributaries (Fig. 3). Lake Erie tributaries and the Wabash River (WBSH) site were also grouped together and showed similarities to the Thames River and Lake Huron sites. We note that there was distinct separation between individuals collected within the Great Lakes

basin and the Wabash River drainage collection sites at all values of K, despite the latter's proximity to Maumee River collection sites (Fig. 1). Similarly, all Ohio River sites other than Wabash River remained distinct from Great Lakes sites at all scenarios of K.

Population-based analyses showed similar patterns of hierarchical genetic structure. The neighbor-joining dendrogram showed evidence of genetic differences with strong nodal support between the upper Mississippi and Ohio Rivers, with Great Lakes populations grouping within the Mississippi cluster (Fig. 4). For the most part, genetic distances among population pairs were greater in the Ohio and Mississippi River basins than in the Great Lakes. The Great Lakes sampling locations formed a subset of the larger Upper Mississippi, Missouri River, and Illinois River group, with the Iroquois River site appearing to be intermediate between upper Mississippi River populations and those in the Great Lakes. The collection site in the upper Wabash River grouped with the Maumee River sites (MAU1, MAU2, MAU3, and MAU4), while the nearby and hydrologically connected Mississinewa River (MSWR) site was strongly differentiated from the upper Wabash River site and grouped with sites in the Ohio River drainage. The Ohio River populations, with the exception of the upper Wabash River, were clearly divergent from all sampling locations in the upper Mississippi River and Great Lakes. Major branches (Mississippi versus Ohio drainages, and the distinctiveness of the Great Lakes cluster within the Mississippi group) showed strong nodal support, as did the separation from Sydenham River, Ontario sites from other Great Lakes locations (Fig. 4).

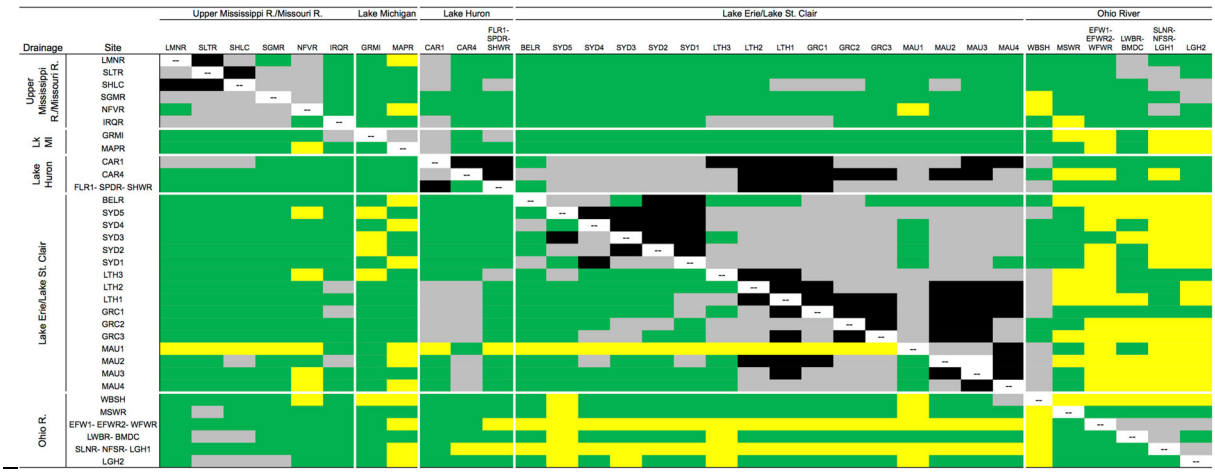
Genetic divergence estimates among sites using AMOVA and pairwise F_{ST} and Jost's D_{est} yielded findings similar to the genetic distance analysis (Tables 3, 4, Supplemental Information). Mean estimates of pairwise population divergence showed significant differences between Great Lakes populations and those in the Mississippi and Ohio River drainages (mean $F_{ST} = 0.067$, $P < 0.05$). Higher-order F_{ST} comparisons also were considered among the upper Mississippi River drainage (UMS), Ohio River drainage (OH), and Great Lakes drainage (GL), resulting in mean F_{ST} values of 0.028 ($P < 0.05$) for UMS–OH, 0.050 ($P < 0.05$) for UMS–GL, and 0.095 ($P < 0.05$) for OH–GL. The highest level of genetic differentiation was between the upper Wabash River and West Fork White River, Indiana ($F_{ST} = 0.356$) collection sites, despite both occurring in the Ohio River watershed (Fig. 1). By contrast, the majority of Great Lakes tributaries showed little genetic differentiation ($F_{ST} < 0.05$). Within-river divergence estimates among sites within Great Lakes tributaries (Sydenham, Thames, and Maumee rivers) were low or not significant (Table 4). Sites close to both hypothesized colonization routes (North Fork Vermillion River and Iroquois River for the Chicago–Illinois outlet, and upper Wabash River for the Wabash–Maumee outlet) showed little to moderate divergence from Great Lakes populations (Table 4). By contrast, most of the Ohio River populations, with the exception of the upper Wabash River (WBSH) site, were much more divergent from Great Lakes populations than from populations in upper Mississippi River drainage showing moderate to high levels of divergence (Table 4). The AMOVA showed evidence of significant genetic structuring at each level of geographic partitioning (Table 3), although the amount of variation explained by differences among hydrogeographic regions (Mississippi and Missouri rivers, Ohio River, and the Great Lakes basin) was small (6%), but significant ($P < 0.001$) based on 9,999 resampling iterations. Genetic structuring among sampling

Table 3 Analysis of molecular variance (AMOVA) results for *Q. quadrula*

Source of variation	Degrees of freedom	Sum of squares	Percent variation (%)	Parameter value	*P*
Among regions (Ohio R. drainage, Great Lakes drainage, Mississippi/Missouri R. drainage)	2	287.2	6	$F_{RT} = 0.063$	<0.001
Among sampling locations within regions	37	613.2	9	$F_{SR} = 0.093$	<0.001
Within sampling locations	2528	2486.0	85	$F_{ST} = 0.149$	<0.001

All variance components were significantly different from zero

Table 4 Pairwise population differentiation (F_{ST} below diagonal and D_{est} above diagonal) with each site represented by a rectangle separated by drainage



Black is insignificant, zero, or very low genetic differentiation (F_{ST} or $D_{est} < 0.01$); gray is low genetic differentiation (F_{ST} or $D_{est} \geq 0.01$–0.05); green is moderate genetic differentiation (F_{ST} or $D_{est} \geq 0.05$–0.15); yellow is high genetic differentiation (F_{ST} or $D_{est} \geq 0.15$–0.25). The Lake Michigan drainage is abbreviated as Lk MI. Complete values are available as Supplementary Information (Appendix 4—Supplementary materials)

locations within each region was similarly significant (Table 3).

The two Mantel tests of isolation by distance showed contrasting results for the two main hypothesized colonization routes. There was a significant positive relationship between geographic river distance (km) and genetic distance (linearized F_{ST}) when testing postglacial colonization through the hypothesized Chicago–Illinois outlet ($r^2 = 0.157$; $P < 0.001$; Fig. 5a). When testing the geographic–genetic distance relationship for postglacial colonization of the Great Lakes through the hypothesized Wabash–Maumee outlet, no significant relationship was detected ($r^2 = 0.020$; $P = 0.054$; Fig. 5b).

Discussion

Mitochondrial and microsatellite DNA analyses revealed substantial intraspecific variation and structure among sampled populations of *Q. quadrula*, reflecting both phylogeographic history and contemporary population genetic processes among major drainage basins. The mitochondrial data showed a pattern of diversity similar to those observed in fishes and other species (Bernatchez & Wilson, 1998; Soltis et al., 2006), with populations in unglaciated areas (southern rivers) having greater haplotype diversity than populations in the recently glaciated (Great Lakes) part of the species' range. By contrast, populations in and outside of the Great Lakes basin showed comparable levels of diversity at microsatellite loci, reflecting these markers' faster mutation rate and higher effective population size (Galbraith et al., 2015). Data from both marker systems supported the hypothesis that *Q. quadrula* entered the Great Lakes at or shortly after the end of the last glaciation via the Chicago–Illinois glacial outlet, and refuted the hypothesis of colonization solely through a Wabash–Maumee entry point.

Genetic Diversity

The substantial genetic diversity observed within and among populations of *Q. quadrula* reflects the species' biology, and was consistent with findings from other studies (Berg et al., 1998; Elderkin et al., 2007; Galbraith et al., 2015). Long-lived unionid mussels such as *Q. quadrula*, with a maximum age over 60 years (Watters et al., 2009), may have multiple

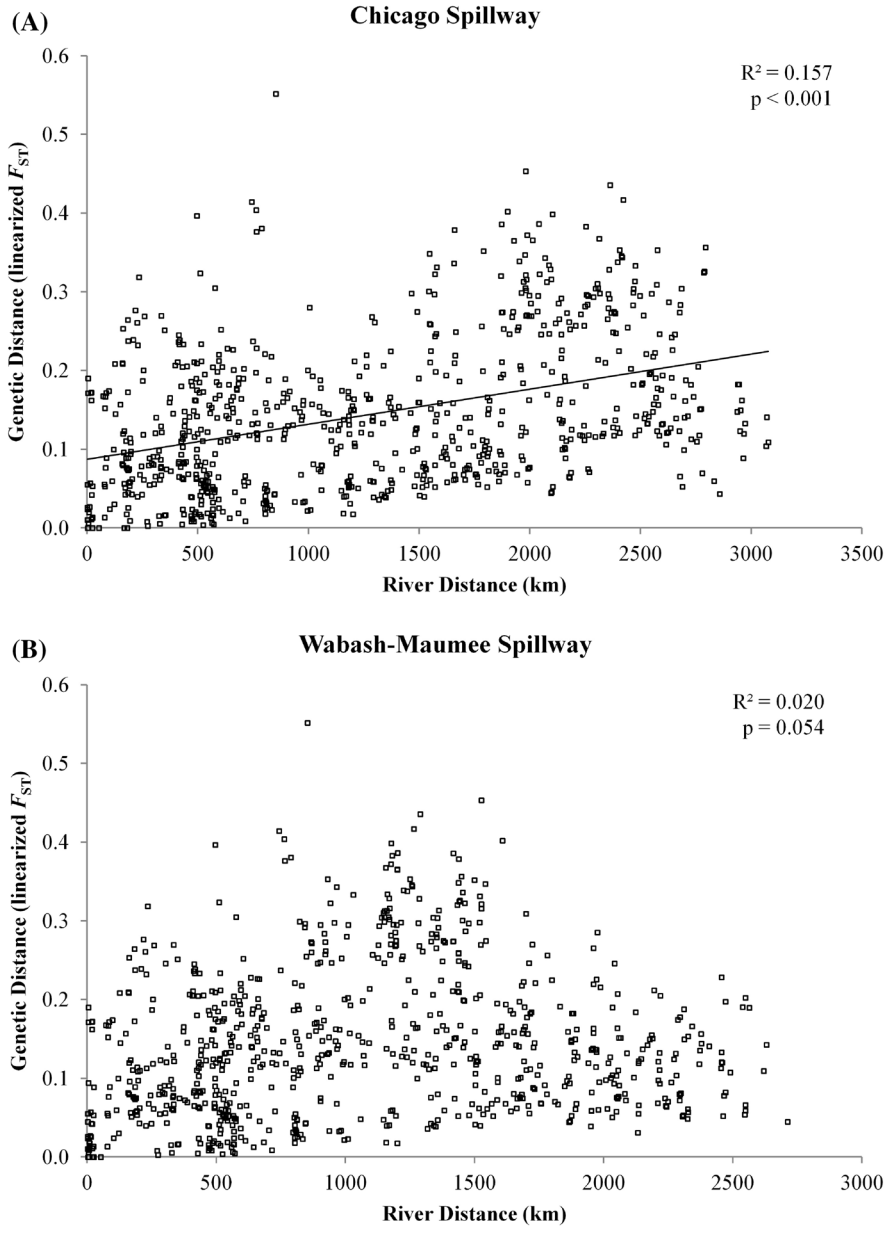


Fig. 5 Mantel tests of isolation by distance testing the postglacial colonization routes of the Great Lakes by *Q. quadrula* using either the Chicago–Illinois outlet (**A**) or Wabash–Maumee outlet (**B**). River distances were calculated between each site using the proposed outlet

generations reproducing at one time, with this multi-generational diversity providing a buffer against loss of genetic variation through poor recruitment years. Unionids and other bivalve molluscs commonly deviate from Hardy–Weinberg model assumptions, with overlapping generations, host fish dispersal, null alleles, and inbreeding potentially contributing to heterozygote excess or deficiency (Nagel et al., 1996; Bierne et al., 1998; Astanei et al., 2005; Jones et al., 2006b; Chapuis & Estoup, 2007). The evidence of null alleles at two of the eight loci used in this study likely contributed to apparent heterozygosity deficiencies in some populations; using primers developed for the congeneric *Q. fragosa* (Hemmingsen et al., 2009) may have contributed to null alleles at some loci. Null alleles at microsatellite loci are known to be common in other molluscs (Pemberton et al., 1995; Brookfield, 1996; McGoldrick et al., 2000), but their presence typically does not affect assignment tests or population differentiation estimates (Chapuis & Estoup,

2007; Ferguson et al., 2013). A typical (spatial) Wahlund effect, or inadvertent pooling of discrete gene pools (Hedrick, 2005), is unlikely within the dataset because individual-based assignments within sampling localities, as well as among multiple sampling locations within the same rivers, did not exhibit genetic substructuring. This homogeneity within rivers may reflect the long-distance dispersal abilities of *Q. quadrula*'s host fish (ictaluridae) (Hubley, 1963; Scott & Crossman, 1973; Shrader et al., 2003). With the large dispersal potential of glochidia, it is possible and even probable that younger mussels within a sampling location will not be the offspring of co-occurring adult mussels. With *Q. quadrula*'s longevity, this could create a temporal Wahlund effect from the spatial co-occurrence of overlapping but unrelated generations.

Genetic Structure

Geographic genetic structuring was apparent among populations of *Q. quadrula* with both mitochondrial and microsatellite datasets. The distribution of the *CO1* haplogroups showed substantial differences between the Ohio and Mississippi River drainage basins, albeit with some degree of mitochondrial exchange. The detection of only one haplogroup within Great Lakes tributary populations suggests an ancestral founding event from a Mississippian source and subsequent dispersal from glacial refugia in this region.

The microsatellite data provided finer-scale resolution to population relationships and geographic patterns, with evidence of hierarchical genetic structure within and between the Mississippi and Ohio River basins, as well as among Great Lakes tributaries. Although Berg et al. (1998) reported only low levels of genetic variation across large river distances based on allozyme data, the scale of this study was considerably greater for both geographic coverage and sample sizes, as well as using more variable markers. The observed genetic structuring within the river basins is consistent with historical connectivity and potential for host fish movements (van der Schalie, 1945; Mandrak & Crossman, 1992; Graf, 2002), and may also reflect historical population sizes of both *Q. quadrula* and their host fishes. As such, the observed levels of genetic divergence within the major river basins likely reflect historical as well as contemporary dispersal by host fishes and availability of local habitat patches suitable for *Q. quadrula*. The lower levels of genetic differentiation among Great Lakes tributaries reflect their recent shared ancestry, as well as the absence of pre-historic barriers to local migration. Before the construction of low-head barrier dams for lamprey control, it is probable that host fish were able to move freely among low-gradient rivers within each of the Great Lakes basins, possibly with occasional long-distance dispersal between lake basins. Patterns observed within lower Great Lakes tributaries were congruent with those reported for *Q. quadrula* by Galbraith et al. (2015), with the current study providing greater spatial resolution. The observed patterns were also similar to those reported for other unionid species in the upper Mississippi River, Ohio River, and Great Lakes basins (e.g., Elderkin et al., 2007, 2008; Inoue et al., 2013; Hewitt et al., 2016).

The evidence of shared ancestry between sites in the Illinois River drainage with those in the Grand River (Michigan) may also reflect recent historical as well as postglacial gene flow. Man-made canals have connected the Great Lakes and Mississippi River drainages since 1848 to remove waste and allow ship travel between the Mississippi River and the Great Lakes (Willson, 1966). It is therefore possible that our data may partly reflect recent historical travel of fish hosts carrying *Q. quadrula* larvae between the two systems, prior to the construction of the electrical barrier in the Chicago Sanitary and Ship Canal. Similarly, although the genetic similarity between the upper Maumee River locations and upper Wabash River may reflect stream capture via isostatic rebound (Graf, 2002), it may also have resulted from fish movements following the construction of the Wabash and Erie Canal that connected the Wabash and Maumee Rivers between 1843 and 1887 (Willson, 1966). The subsequent separation of these rivers would not necessarily have resulted in substantial genetic divergence, as dams constructed in southern Ontario rivers in the twentieth century have not yet had detectable genetic effects on the within-river homogeneity of resident unionid species (Galbraith et al., 2015). To discriminate between competing hypotheses of postglacial, recent historical, or contemporary host movements as the primary drivers of observed genetic patterns would require strategic sampling with high spatial resolution and coverage within and among major drainages and associated canal corridors.

Postglacial colonization

Phylogeographic inference based on both mitochondrial and microsatellite DNA showed clear evidence of colonization of the Great Lakes and tributaries by *Q. quadrula* through a Chicago-Illinois River connection. Of the three competing hypotheses for colonization of the Great Lakes by *Q. quadrula* (Chicago–Illinois outlet only; Wabash–Maumee outlet only, or both), the microsatellite and mitochondrial data best support a colonization route into the Great Lakes basin through the Chicago–Illinois outlet. Because of the slow evolutionary rate of mtDNA genes with respect to glacial cycles and postglacial colonization (Bernatchez & Wilson, 1998), the timing of *Q. quadrula*'s arrival in the Great Lakes basin cannot be determined from the *CO1* sequence data. Although modern-day Lake Michigan was connected to the Mississippi River basin several times during the late Pleistocene and early Holocene (Dyke, 2004), the readvance of the Michigan Lobe of the Laurentide Ice Sheet between 13,000 and 11,800 years ago (Larson & Schaetzl, 2001; Dyke, 2004) would have displaced any early colonists in southern Lake Michigan.

The observed genetic substructuring of the microsatellite data among the Great Lakes and their tributaries is consistent with 'stepping-stone' dispersal and gene flow (Hedrick, 2005). As unionids and host fishes share a parasite–host relationship (Watters, 1992), it can be inferred that they would have colonized the Great Lakes postglaciation following a similar colonization route (Graf, 2002). Patterns of genetic relationships as indicated by the genetic distance dendrogram and isolation-by-distance plots both support initial arrival in the Great Lakes basin via the Chicago–Illinois spillway, and expanding into the Lake Huron–Lake Erie corridor. Following colonization of southern glacial Lake Chicago (now Lake Michigan) via the Chicago–Illinois spillway, glochidial *Q. quadrula* likely were carried by host fish around or across the lower peninsula of Michigan into Lake Huron with subsequent expansion into Lake St. Clair and Lake Erie, eventually colonizing the upper Wabash River (site WBSH) from Lake Erie. The results from the Mantel test for the Chicago–Illinois outlet hypothesis were congruent with those from Berg et al. (1998), Elderkin et al. (2007), and Zanatta & Harris (2013) as a route for postglacial colonization into the Great Lakes. Elderkin et al. (2007) and Hewitt et al. (2016) also suggest multiple glacial refugia for Great Lakes populations during the most recent glaciations. Although Hewitt et al. (2016) did not find strong evidence in support of the Chicago–Illinois outlet as a colonization route, this may be due to differences in host use and dispersal by *Lasmigona costata* (Rafinesque, 1820), which is a generalist on a variety of host fishes, and *Q. quadrula* and to different distributions at the end of the Wisconsinan glaciation.

All lines of evidence refuted the hypothesis of colonization of the Great Lakes through the Wabash–Maumee connection, despite the genetic similarity between the Wabash and Maumee sites suggesting a shared postglacial history. The clear segregation of mitochondrial haplotypes between the Ohio River basin and Great Lakes populations strongly refuted the Wabash–Maumee colonization hypothesis. As Graf (2002) suggested for central Great Lakes unionid fauna (including *Q. quadrula*) based on biogeographic records, glacial Lake Maumee was not supported as the only colonization route for many unionid species. Once the glaciers fully receded and the meltwater outlets subsided, hydrologic connections were severed through isostatic rebound (Larson & Schaetzl, 2001; Graf, 2002). Geologic evidence shows that the upper Maumee River drainage historically drained to the southwest through the Wabash River, and later was captured by the Maumee and began flowing northeast into Lake Erie (Mandrak & Crossman, 1992; Herdendorf 2013). This flow reversal would have captured the organisms within the streams and could therefore explain the dispersal of the source population of unionid mussels into the Great Lakes from their preglaciation populations. Based on distributional data and timing among proglacial lake connections, Mandrak & Crossman (1992) hypothesized that *I. punctatus*, one of the host species for *Q. quadrula*, colonized the Great Lakes basin via the Chicago–Illinois outlet and Michigan's Lower Peninsula. The microsatellite data are consistent with both sole-source colonization from the Chicago–Illinois outlet and with initial colonization from the Chicago–Illinois outlet with subsequent access to Lake Huron and the lower Great Lakes via Glacial Lake Maumee (Larson & Schaetzl, 2001; Herdendorf, 2013). Thus, despite the genetic similarities between the upper Wabash River and the Maumee River populations, our data support the hypothesis that entry by *Q. quadrula* into the Great Lakes basin occurred solely through the Chicago–

Illinois outlet following the last glaciation. Rather, our data instead suggest that the genetic similarity between the upper Wabash River and Maumee River sites may have resulted from headwater capture following isostatic rebound, enabling *Q. quadrula* from early Lake Erie to enter the Ohio River watershed.

Conservation and Management Implications

Abundance and genetic diversity are both important when considering the status of unionid populations. As *Q. quadrula* is common and considered demographically stable at sampling localities in this study, it was an ideal candidate for assessing range-wide phylogeography with potential conservation and management implications. Using Moritz' (1994) definition of management units (MUs) as genetic groupings with significant differences in frequencies of mitochondrial and/or nuclear loci, our results were similar to those of Berg et al. (1998) in that there are at least two genetically divergent MUs for *Q. quadrula* detected within the Mississippi River drainage (upper Mississippi and Ohio River drainages). Likewise, the Great Lakes and Mississippi River drainages also had moderate genetic differentiation and could also warrant recognition as separate MUs despite their recent (postglacial) shared ancestry. Based on spatial patterns of multi-species genetic structure and diversity in southern Canadian watersheds, Galbraith et al. (2015) suggested that, if needed, translocating individuals and augmenting populations should be done within rather than between watersheds. The genetic structure and diversity documented in this study warrant a similar recommendation to avoid inadvertent mixing between divergent gene pools. Applying the results from *Q. quadrula*'s postglacial colonization into the Great Lakes to more imperiled unionid fauna with similar life histories (i.e., Haag 2012) could provide a template for conservation planning until species-specific data for at-risk taxa are developed.

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