Genetic evidence for canal-mediated dispersal of Mapleleaf, *Quadrula quadrula* (Bivalvia:Unionidae) on the Niagara Peninsula, Canada

Jordan R. Hoffman[1,3], Todd J. Morris[2,4], and David T. Zanatta[1,5]

[1]Institute for Great Lakes Research, Biology Department, Central Michigan University, Mount Pleasant, Michigan 48859 USA
[2]Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, Ontario, Canada L7S 1A1

Abstract: Alterations to watercourses affect connectivity in aquatic systems and can influence dispersal of aquatic biota. Dams fragment populations and act as isolating barriers, but canals create connections between waterbodies that can be used as corridors for dispersal by opportunistic invaders. The Niagara Peninsula of Ontario, Canada, has a 200-y history of canal operation, resulting in major modification of the watercourses in the region. This modification allowed numerous invasive species to enter the upper Great Lakes (e.g., sea lamprey) and probably has facilitated dispersal in native species. The purpose of our study was to explore the effects of canal and dam construction on the genetic structure of Mapleleaf (*Quadrula quadrula*), a widespread and relatively common species in the central Great Lakes that has been found only recently in several western Lake Ontario harbors. Establishment of *Q. quadrula* in Lake Ontario may have been a recent event, facilitated by the Niagara Peninsula's history of canal operation. We used analyses of microsatellite DNA genotypes to examine the effect of canals on the genetic structure of mussel populations. Structure analysis revealed a pattern of gene flow between lakes that cannot be explained by watercourse connections prior to the creation of the Welland Canal. Evidence suggestive of historical bottlenecks at some Lake Ontario sites may indicate that these populations became established after canal creation. After considering genetic structure, hydrogeography and isolation-by-distance (IBD) analysis, the first iteration of the canal (1829–1833) is most supported as the configuration that facilitated colonization. However, weak IBD signals across canal models may signify continued gene flow across configurations. Our study demonstrates the connective effect of canals on freshwater mussel populations and has the potential to improve conservation strategies for this and other unionid species at risk.

Key words: canals, population genetic structure, gene flow, microsatellites, Laurentian Great Lakes, Welland Canal, Niagara Falls

North America has greater species richness of freshwater mussels than any other continent, with >300 species in the order Unionida (families Unionidae and Margaritiferidae), constituting 43% of the world's collective freshwater bivalve species (Graf and Cummings 2007, Haag 2012). Freshwater mussels also are prominent components of North American riverine communities, with mussel assemblages constituting a significant proportion of benthic biomass (Vaughn et al. 2004, 2008). Freshwater mussels are important ecosystem engineers that alter nutrient availability by filter-feeding phytoplankton from the water column and depositing feces and pseudofeces in the sediment (Strayer et al. 1999, Vaughn and Hakenkamp 2001, Vaughn 2017). Mussel beds and shells also provide habitat to other invertebrates and algae (Vaughn and Hakenkamp 2001).

The Laurentian Great Lakes and associated watersheds are habitats for diverse assemblage of unionids (Haag 2012). The Great Lakes and the biogeography of their aquatic community were shaped by the Laurentide ice sheet, isostatic rebound, and glacial meltwater (Larson and Schaetzl 2001, Borden and Krebs 2009, Hewitt et al. 2016, Mathias et al. 2016). For instance, Lake Erie and Lake Ontario historically have been hydrologically separated. After the recession of the Laurentide ice sheet after the last ice age, the lakes were isolated from each other and associated with different drainages, with early Lake Erie draining into the Mississippi and

E-mail addresses: [3]hoffm3jr@cmich.edu; [4]todd.morris@dfo-mpo.gc.ca; [5]zanat1d@cmich.edu

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early Lake Ontario draining into the Atlantic. The lakes were separated until ~11.8 kya, when the Niagara River formed (Larson and Schaetzl 2001). The Niagara Falls and the lower Niagara River rapids were probably a strong barrier to dispersal and gene flow for many aquatic species. Natural upstream dispersal by fish is impossible because of the size of the waterfall, and downstream dispersal is thought to be highly unlikely because of the perceived dangers of Niagara Falls and downstream rapids. Because of this historical isolation, the mussel species assemblages in the lakes were fairly distinct, with many Lake Erie species found in the Ohio and Mississippi drainages and Lake Ontario species in the Atlantic drainages (Haag 2012). Only Eastern Elliptio (*Elliptio complanata*) and the Eastern Pondmussel (*Ligumia nasuta*) are known to be widely distributed across regions associated with both Mississippi–Ohio and Atlantic drainages. Both *E. complanata* and *L. nasuta* exist today in Lake Ontario and parts of the upper Great Lakes, and probably attained their current distribution by dispersing from Atlantic coastal rivers (Haag 2012, Scott et al. 2014).

Lakes Erie and Ontario historically have remained effectively isolated from each other, but human influences have facilitated faunal connections and enabled dispersal between the lakes. Several dams, canals, and other watercourse alterations have been constructed in the Niagara Peninsula (Fig. 1) over the last 200 y to bring water to mills and to create a trade route between Lakes Erie and Ontario. Some of these canals, such as early Welland Canal and the Feeder Canal (connected the Grand River, Ontario, Canada, and the Welland Canal), remained active and in use for many decades (Daniels 2001), whereas others were abandoned (Ashworth 1986). For example, the Feeder Canal was abandoned after construction of the 3rd Welland Canal, is no longer operational, and does not provide any present-day hydrologic connection (Styran and Taylor 2011). The 4th configuration of the Welland Canal connects the lakes between Port Dal-

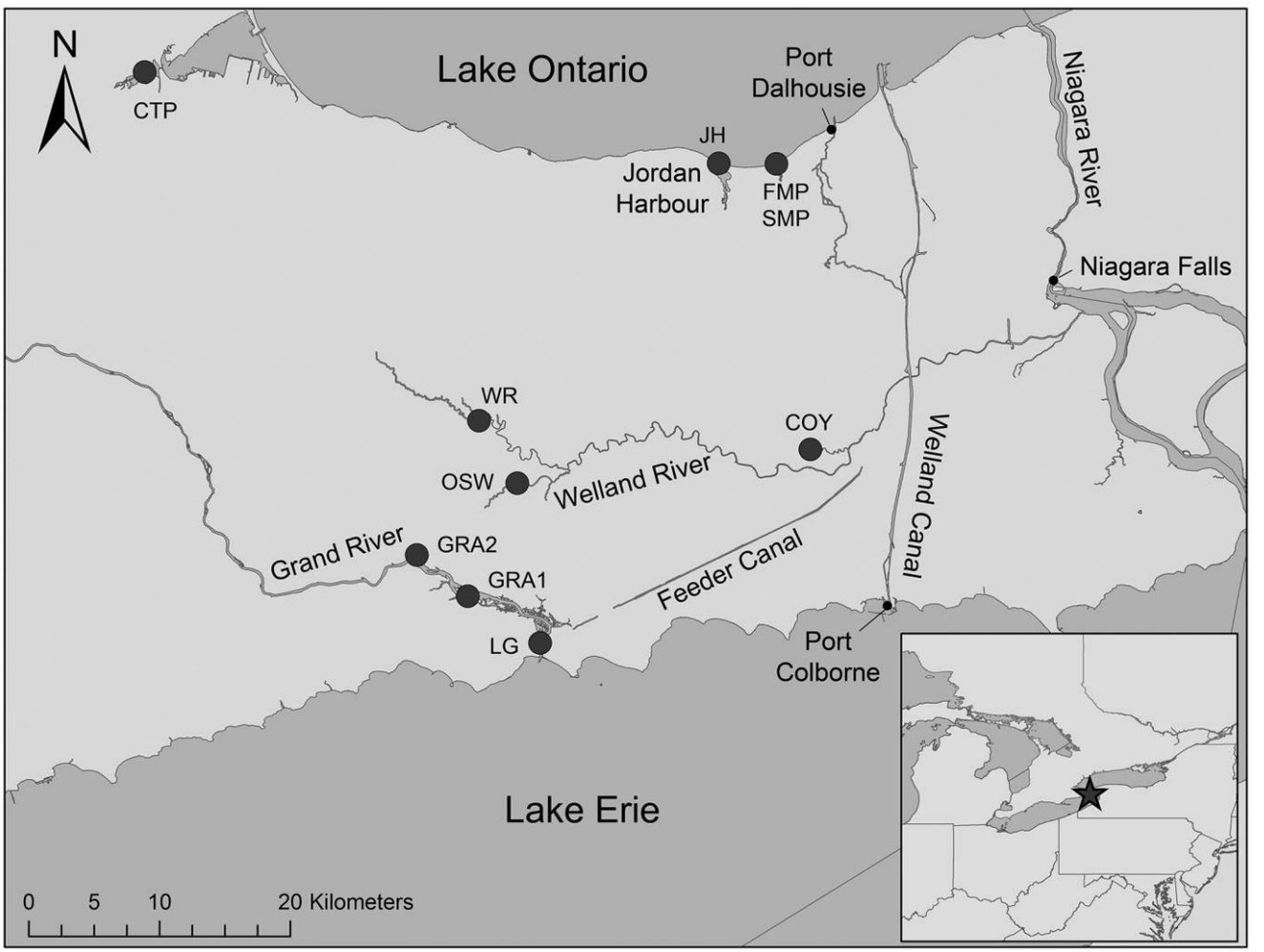

Figure 1. Locations on the Niagara Peninsula where tissue biopsies were collected from *Quadrula quadrula* in 2010, 2014, and 2015. Historical pathways of the Welland Canal and Feeder Canal are labeled accordingly. See Table 1 for site abbreviations.

housie (Lake Ontario) and Port Colborne (Lake Erie) (Ashworth 1986). The possibility for ship traffic and aquatic organisms (Kim and Mandrak 2016) to disperse easily between the lakes exists today as a result.

Canals have acted as dispersal corridors for other species in the past and enabled movement and homogenizing of populations via gene flow between previously isolated water bodies (Rahel 2007). Canals also facilitate invasion of new habitat by exotic species. A number of aquatic invasions between Lake Ontario and the rest of the Great Lakes have been facilitated by the Welland Canal: Sea Lamprey (*Petromyzon marinus*), Alewife (*Alosa pseudoharengus*), and American Eel (*Anguilla rostrata*) have all invaded Lake Erie, and then other Great Lakes, through the Welland Canal (Aron and Smith 1971, Byran et al. 2005). Rainbow Smelt (*Osmerus mordax*) followed the opposite pathway, having entered Lake Ontario from Lake Erie via the canal (Daniels 2001). A recent tagging study showed that several native and invasive fishes are capable of moving through the locks on the Welland Canal (Kim and Mandrak 2016).

Unionids are relatively sessile organisms that spend their adult lives burrowed in the sediment. Their larval host–parasite relationships with fish and other aquatic organisms enable them to disperse over long distances. Female unionids use various methods, including moving lures and release of glochidia packets (conglutinates) disguised as prey, to parasitize host fishes with their larval (glochidial) stage. Glochidia metamorphose on the gills of their hosts and drop off when they reach their juvenile stage (Haag 2012). During their parasitic stage, unionids are subject to the movement of the host. The dispersal a unionid can achieve depends on the behavior of its host species.

The Mapleleaf, *Quadrula quadrula*, is a unionid mussel native to the Great Lakes watershed. *Quadrula quadrula* has a wide distribution and occurs naturally throughout the Ohio–Mississippi River drainage, into the upper Great Lakes (except for Lake Superior) and into the Hudson Bay drainage (Red River of the North, Assiniboine River, and Lake Winnipeg drainages) (COSEWIC 2006, Mathias et al. 2016). The species is considered a habitat generalist, and often is found in a variety of substrates in rivers, streams, and shallow lakes (COSEWIC 2006). *Quadrula quadrula* is widespread and common throughout most of its range in the USA, but it is considered threatened in Ontario, Canada (SARA 2016).

The known host fishes of *Q. quadrula* include the Flathead Catfish (*Pylodictis olivaris*) and the Channel Catfish (*Ictalurus punctatus*) (Howard and Anson 1922, Schwebach et al. 2002, Watters et al. 2009). Both species are capable of dispersing over many kilometers of river (Stewart and Watkinson 2004). As parasites of these species, *Q. quadrula* probably is capable of distant dispersal. Of the 2 known host fishes, *I. punctatus* is native to Lake Erie, Lake Ontario, and the associated tributaries (COSEWIC 2006), whereas *P. olivaris* is not known in Ontario. As a result, *I. punctatus* is the most probable host for *Q. quadrula* in Lake Ontario. Other species, such as the Brown Bullhead (*Ameiurus nebulosus*), may be potential hosts but have not been tested for *Q. quadrula*. Channel Catfish and Brown Bullhead are capable of moving through some of the locks on the Welland Canal (Kim and Mandrak 2016), thus making dispersal of *Q. quadrula* feasible.

In 2010, *Q. quadrula* was discovered in a harbor in western Lake Ontario during mussel surveys (Reid et al. 2014, TJM, unpublished data). Additional populations in embayments were identified in 2015, but the species has never been reported from the USA side of the lake despite intensive sampling (Burlakova et al. 2014, DTZ, unpublished data). This discovery was surprising, considering the long history of separation between Lakes Erie and Ontario. The presence of *Q. quadrula* in Lake Ontario raises the possibility that the mussel was able to disperse into Lake Ontario recently through manmade canals. Movement of dispersing *I. punctatus* through one of the historical or current canals could have facilitated the dispersal of *Q. quadrula* and led to their subsequent establishment in Lake Ontario. Such an establishment would have occurred within the last 200 y, and probably would exhibit a genetic signature of bottlenecks and high similarities to their source population. A more ancient colonization that had occurred long before the construction of the canals would show that the Lake Ontario populations had become genetically distinct from Lake Erie populations because of their hydrologic isolation resulting from the Niagara Falls barrier. The first mussel survey records of *Q. quadrula* in Lake Ontario were in 2010, and the first records of the species in the Welland River date back only to 1983 (COSEWIC 2006). The first collection of Mapleleaf was made >150 y after the creation of the Welland Canal. Thus, the history and origins of *Q. quadrula* in the Welland River are currently unknown.

We built on recent studies of the genetic structure of *Q. quadrula* in the Great Lakes (Paterson et al. 2015, Mathias et al. 2016) to: 1) investigate the population genetic structure of *Q. quadrula* in the Niagara Peninsula, and 2) evaluate its structure in light of historical canal structure to learn the history and origins of *Q. quadrula* in Lake Ontario. We used microsatellite analyses to evaluate the population genetics and structure of *Q. quadrula* populations from the Niagara Peninsula and to answer the questions: were these colonizations recent events, facilitated by new or intermittent connections via canals, or did these mussels arrive long ago, after the end of the last ice age?

Microsatellites are variable, repeating sections of noncoding DNA that are not subject to selection. The high variability of these segments makes microsatellites useful for recent timescale analyses of population genetics (Freeland 2005). We amplified a suite of microsatellite loci for *Q. quadrula* collected from different sites in the Niagara Peninsula to analyze the population genetics of the species in Lakes Ontario and Erie. We compared genetic diversity and differentiation at each site and examined gene flow and structure. De-

tection of structure in Niagara Peninsula *Q. quadrula* populations will show whether differentiation exists among river sites or whether some sites are more genetically alike than others, which will help answer when and how the introduction of *Q. quadrula* to Lake Ontario occurred.

Illuminating the history of this introduction is important because *Q. quadrula* is a species at risk in Canada. If *Q. quadrula* was introduced into Lake Ontario recently, its ability to disperse and colonize new habitats probably is greater than estimated in the latest assessment by COSEWIC (2006), which could affect its threatened status. Preparation of more effective conservation approaches for this species requires identification of the locations where genetically distinct populations of *Q. quadrula* occur and assessment of gene flow among populations. Understanding the influence of anthropogenic changes in watercourse connectivity has implications for other species at risk. Many other unionids are endangered and threatened, and exploring how mussel dispersal and gene flow is affected by anthropogenic influences can lead to more informed plans for conservation of these species.

METHODS

We sampled *Q. quadrula* at rivers and embayments on the Niagara Peninsula of Ontario (Fig. 1). In 2014, we sampled mussels from Jordan Harbour (JH) in Lake Ontario, the upper reaches of the Welland River, which drains into the Niagara River, and the lower Grand River (a Lake Erie tributary), downstream of the Dunnville Dam. Following additional surveys of Niagara Peninsula waterways in 2015, we collected additional samples at Cootes Paradise (CTP) and Fifteen- and Sixteen-Mile Ponds (FMP and SMP, respectively) in Lake Ontario, and Oswego and Coyle Creeks on the Welland River. Other samples used in our study included collections from coastal wetlands and embayments in Lake Erie in 2011 and 2012 (Paterson et al. 2015) and from the Grand River, Ontario, upstream of the Dunnville Dam in 2008 (Galbraith et al. 2015, Mathias et al. 2016) (Table 1). We collected mussels at each site using tactile searches and mussel baskets in a sampling procedure described by Zanatta et al. (2015).

We collected mantle tissue biopsies from sampled mussels in accordance with nonlethal biopsy methods established by Berg et al. (1995). We attempted to collect a minimum of 30 tissue samples at each site to maintain a suitable level of power for analyzing population genetics (Hale et al. 2012). However, <30 mussels were found at some sites, despite >7 person-hours of search. We excluded sites with <20 individuals from all nonindividual-based tests and analyses. We collected tissue clippings in 1.5-mL microtubes in 95% ethanol and stored them at −80°C.

We used a modified alcohol extraction method (Sambrook et al. 1989) to extract DNA from the tissue samples. We clipped a small piece of each tissue sample (~0.25 cm^2 in size), lysed it in a 1.5-mL tube with 250 μL 1× lysis buffer and 15 μL proteinase K, and incubated it for ≥16 h at 37°C. After cell lysis, we added 500 μL 80% isopropanol and 10 μL of 5 M NaCl to each sample to precipitate the DNA. We centrifuged samples for 45 min at 13,300 rpm to collect DNA into a small, concentrated pellet. We poured off the supernatant and added a secondary wash of 1000 μL 70% ethyl alcohol to the sample. We vortexed samples and centrifuged them once again for 45 min to refine the DNA product further. We poured off the wash and dissolved the final pellet in 150 μL double-distilled (dd) H_2O for storage and use in amplification.

We amplified 6 microsatellite loci for *Quadrula quadrula* (C4, C114, A112, A130, R9, and D102) by polymerase chain reaction (PCR). Hemmingsen et al. (2009) developed the reaction primers for *Q. fragrosa* (Conrad 1835), and Paterson et al. (2015) and Mathias et al. (2016) optimized them for *Q. quadrula*. Each PCR reaction consisted of 1.0 μL of template DNA sample (extracted DNA diluted 1:10 in nanopure water) and 9.0 μL of PCR cocktail (1× Taq buffer, bovine serum albumin [BSA], deoxyribonucleotide phosphate [dNTP], forward and reverse primers, $MgCl_2$, and Taq DNA polymerase; Empirical Bioscience, Grand Rapids, Michigan) as in Paterson et al. (2015). We used an Eppendorf Mastercycler (Hauppauge, New York) with locus-specific settings for DNA amplification. To verify the success of each PCR, we stained reaction product with SYBR Green (Invitrogen, Eugene, Or-

Table 1. Sampling locations surveyed in 2008, 2011, 2012, 2014, and 2015, including site abbreviations (code) and the number of *Quadrula quadrula* tissue samples collected at each sampling location.

Sampling location	Site code	Number of genotyped samples
Jordan Harbor	JH	45
Fifteen- and Sixteen-Mile Ponds	FMP/SMP	31
Cootes Paradise	CTP	1
Upper Welland River	WR	46
Oswego Creek	OSW	23
Coyle Creek	COY	4
Lower Grand River (below dam)	LG	27
Lower Grand River (above dam)[a]	GRA1, GRA2	43, 20
North Maumee Bay[b]	NMB	24
Crane Creek Marsh[b]	CCR	21
Gath Kurdy Marsh[b]	GKU	24
Young Marsh[b]	YMC	25
Portage River[b]	POR	25
Muddy Creek Bay[b]	MCB	22
TOTAL 15 locations		381

[a] Collected in 2008 (Paterson et al. 2015, Galbraith et al. 2015)
[b] Collected in 2011 and 2012 (Paterson et al. 2015)

egon) and ran it on 1.5% agarose gel for visual confirmation of amplification.

We sent microsatellite product to the Natural Resources DNA Profiling and Forensic Centre at Trent University, where an Applied Biosystems (Foster City, California) 3730 Series DNA Analyzer was used for genotyping. Alleles were scored into size classes with the aid of GENEMARKER^{TM} software (SoftGenetics, State College, Pennsylvania). Samples previously analyzed by Paterson et al. (2015) were rescored to maintain consistency. Genotyping accuracy was assessed by examining the frequency of null alleles, a common type of amplification error, using the software MICROCHECKER (version 2.2.3; van Oosterhout et al. 2004). The Brookfield 1 method was used to calculate the frequency of null alleles in MICROCHECKER (Brookfield 1996). The genotypes of sampled mussels were grouped by sampling location and examined for deviations in Hardy–Weinberg Equilibrium (HWE) using GENALEX (version 6.5; Peakall and Smouse 2012). The probability of linkage disequilibrium between any 2 loci was estimated with GENEPOP (version 4.2; Raymond and Rousset 1995). GENALEX also was used to examine observed and expected heterozygosity (H_O and H_E) at each sampling location. Rarefacted allelic richness (A_r) was calculated in FSTAT (version 2.9.3; Goudet 1995) to adjust for variable sample sizes. Differences in A_r were examined with Kruskal–Wallis and Wilcoxon Sum Rank Tests on site- and genetic-based groupings. Genetic-based groupings were identified by analyses of genetic structure.

We analyzed the population genetic structure of *Q. quadrula* across Lake Erie and the Niagara Peninsula based on a Bayesian assignment test implemented in STRUCTURE (version 2.3.4; Pritchard et al. 2000). STRUCTURE assesses population genetic structure and estimates the most likely number of populations by grouping individuals into structured populations based on minimum deviations from HWE and linkage. Genotypes were analyzed without a priori geographic information, allowing for population admixture, and with a maximum number of populations (K) equal to the number of sampling locations + 2 additional populations ($K = 17$) to address the possibility of complex substructure. Following the complete structure analysis, we conducted additional substructure analyses of the Niagara Peninsula and the Grand River. All analyses used a standard set of parameters (Markov Chain–Monte Carlo [MCMC] = 100,000 iterations after a burn-in of 100,000 iterations to ensure stationarity in log-likelihood scores; 5 iterations per K). Mean log-likelihood (mean *LnPK*) and ΔK values from Evanno tables calculated in STRUCTURE HARVESTER (version 0.6.93; Earl and vonHoldt 2012) were used to determine the K that best fit STRUCTURE results via the Evanno method (Evanno et al. 2005).

We used analysis of molecular variance (AMOVA) to test for significant genetic divergence between *Q. quadrula* populations based on a permutational approach. AMOVAs provide information on genetic divergence on local (pairwise F_{ST}) and regional (global F_{ST}) scales (Allendorf and Luikart 2007). We ran 2 AMOVAs with GENALEX software; one on samples grouped by sampling location, the other on samples grouped on the basis of populations indicated by STRUCTURE analysis. To account for the influence of heterozygosity on pairwise distance, we also calculated Jost's D in GENALEX (Jost 2008). For all tests, we excluded samples from locations with >10 successfully amplified individuals. We constructed an unrooted neighbor-joining tree to examine genetic distance among sites, with 10,000 replicates for bootstrap support, in POPTREE2 (Takezaki et al. 2010) and Nei's genetic distance (D_A). We calculated bootstrap support by sampling loci with replacement, then constructing trees based on distance values from the same number of sampled loci as in the original data set.

Quadrula quadrula at sampled embayments in Lake Ontario were investigated for evidence of genetic bottlenecks using the program BOTTLENECK (version 1.2.02; Cornuet and Luikart 1996). The date on which these embayments were colonized is unknown, so a bottleneck at these sites could potentially be the result of a founder effect, colonization of a region by a small sample of individuals. A Wilcoxon signed-rank test, recommended for loci with >20 alleles (Piry et al. 1999), was used to compare observed to expected heterozygosity for the Stepwise Mutation Model (SMM). SMM is recommended for microsatellite data because of their potential to mutate by adding or deleting one or more motif repeats (Piry et al. 1999) and, thus, was used for our study. Heterozygosity significantly greater than expected ($p < 0.05$) would signify the residual effects of a genetic bottleneck, so we used a 1-tailed test.

To identify when and how the introduction of *Q. quadrula* into Lake Ontario occurred, we ran a series of Mantel tests (Mantel 1967) in GENALEX. Mantel tests assess the correlation between genetic and geographic distance to determine if populations exhibit isolation by distance (IBD), the null hypothesis for natural populations because as distance between organisms increases, genetic similarity tends to decrease (Wright 1943). Configurations of connections that fail to exhibit IBD signify the presence of barriers to gene flow. Because of this quality of natural populations, landscape configurations that show IBD are likely candidates for the conditions that facilitated colonization. In contrast, configurations that do not show IBD are unlikely to have allowed colonization of Lake Ontario. The timeframe for each canal configuration is known, so identifying a colonization route supported by significant IBD also would reveal when the colonization probably occurred.

Multiple Mantel tests were run to represent the distinct historical and current states of waterway connectivity in the Niagara Peninsula. We ran 2 Mantel tests for each landscape configuration, each based on a different measure of genetic distance, linearized F_{ST} and Nei's D_A, calculated in GENALEX. For each landscape configuration before and after the creation of the Welland Canal, we calculated pairwise hydrogeographic

distances between sampling locations to the nearest 0.1 km based on Google Earth™ (version 7.1.5.1557; Google Corporation, Menlo Park, California). Pairwise distances between locations separated by a portion of a lake were calculated by following the coastline. In instances of historical canal configurations that are no longer present, we followed the historical pathway present on the Niagara Peninsula landscape. In total, we calculated 6 matrices of pairwise distances, representing 4 distinct configurations of the canal and precanal conditions (dispersal between Lake Erie and Lake Ontario sites must pass over Niagara Falls; Fig. 2A–F), and 1 Euclidian distance matrix to represent the potential for the arbitrary translocation of infected host fish or adult mussels (across land via roadways) for purposes, such as sport fishing, of facilitating dispersal. The modern configuration of the Welland Canal (Fig. 2G), from 1973 to present day, was not tested because pairwise distances differed by <1 km from the previous configuration (1930–1973; Fig. 2F).

RESULTS

DNA from 381 individuals from the Niagara Peninsula (240 individuals) and western Lake Erie (141 individuals) was successfully amplified at ≥4 loci. Null alleles were predicted to have been present at all 6 loci at frequencies ranging from 0.09 to 7.45%. However, simulations indicate that null alleles at frequencies <20% minimally bias results in population-based analyses (Dakin and Avise 2004, Carlsson 2008). All loci in our study exhibited null allele frequencies lower than this threshold, so we made no changes to the data set based on null alleles.

Significant deviations from HWE were observed at 11 of 96 locus–sampling site combinations after Bonferroni correction ($\alpha = 0.00052$). No locus was out of HWE in >4 of 15 sites. Loci D102 and R9 were linked (test for linkage with Bonferroni correction, $\alpha = 0.00333$, $p = 0.002533$). This result was unusual. Previous microsatellite analysis of *Q. quadrula* populations with a sample size >3× the size of ours found no evidence of linkage between loci (DTZ, unpublished data), and Paterson et al. (2015) found no evidence of linkage in western Lake Erie *Q. quadrula* populations. Thus, the addition of individuals from the Niagara Peninsula to the data set led to the detection of linkage. Unlike in western Lake Erie *Q. quadrula*, loci D102 and R9 are fixed at most and all sites, respectively, in the Niagara Peninsula. This allele fixation is the probable cause of linkage detection, and our result probably does not reflect true linkage between loci. Therefore, we included all 6 loci for further analyses.

Overall, observed heterozygosity from Lake Erie tributaries was generally greater than that from Lake Ontario tributaries, with values ranging from 0.415 to 0.537 (Table 2). The 2 lowest observed heterozygosities were found in Lake Ontario embayments. Twenty-one private alleles were observed across the 13 sampling sites. Eighteen private alleles were found in Lake Erie tributaries, whereas Lake Ontario tributaries contained only 3. No private alleles were observed in Lake Ontario embayments. Only data from JH indicated a genetic bottleneck ($p = 0.03125$).

Across sampling locations in Lake Erie tributaries and the Niagara Peninsula, *Q. quadrula* populations exhibited moderate genetic structure (Fig. 3A). STRUCTURE identified 2 highly mixed populations ($K = 2$, mean $LnPK = -4448.40$, $\Delta K = 39.669$). Tributaries of Lake Erie primarily constituted one cluster, whereas the other cluster was mainly composed of Lake Ontario embayments and tributaries. Both Lake Erie and Lake Ontario sites possessed individuals assigned to the other cluster (likelihood > 80%). Thirty-one of 231 individuals from Lake Erie sites were assigned to the Lake Ontario cluster. However, only 1 individual of 150 genotyped from Lake Ontario sites was assigned to the Lake Erie cluster. Substructure analysis of Lake Erie tributaries revealed no structure ($K = 1$, mean $LnPK = -2904.36$; Fig. 3B), but analysis of Lake Ontario tributaries and embayments found 3 populations ($K = 3$, mean $LnPK = -1380.02$; Fig. 3C) with a ΔK of 39.50. Welland River sites (WR and OSW) formed one cluster, another was made up of FMP and SMP (FSMP), and JH alone constituted a third.

Sites belonging to the Lake Ontario drainage exhibited relatively low rarefied allelic richness. Fifteen-Mile and Sixteen-Mile Ponds (FSMP) and JH exhibited the 1st- and 3rd-lowest richness out of all sites. Allelic richness did not differ significantly on a site-by-site basis or by substructure when Lake Ontario was separated into 3 distinct clusters. However, differences were significant when grouped by genetic clusters in Lake Erie and Lake Ontario. Allelic richness was significantly lower in Lake Ontario than in Lake Erie (1-tailed Wilcoxon rank sum test, $W = 851.5$, $p = 0.01365$).

AMOVA results were consistent with those acquired from structure and substructure analyses of Lake Erie and Lake Ontario mussels. When grouped by sampling site, Lake Erie and Lake Ontario *Q. quadrula* showed significant differentiation among groups (global $F_{ST} = 0.078$, $p = 0.0001$). Within the Niagara Peninsula, FSMP (2 adjacent Lake Ontario embayments) exhibited higher pairwise F_{ST} values between sites than with JH (a neighboring site) (Table 3). FSMP and JH also exhibited moderate, but significant, differentiation ($F_{ST} = 0.059$, $p = 0.0001$). Similar results were observed with pairwise Jost's *D* values. *Quadrula quadrula* exhibited low and nonsignificant differentiation among Lake Erie sites but significant differentiation from Lake Ontario sites (Table 3). Lake Ontario sites differed moderately, with significant Jost's *D* values ranging from 0.038 to 0.108. Jost's *D* values between WR and OSW were not significant.

The unrooted neighbor-joining tree constructed in POPTREE2 revealed clustering consistent with results from STRUCTURE analysis (Fig. 4). Lake Erie tributaries were

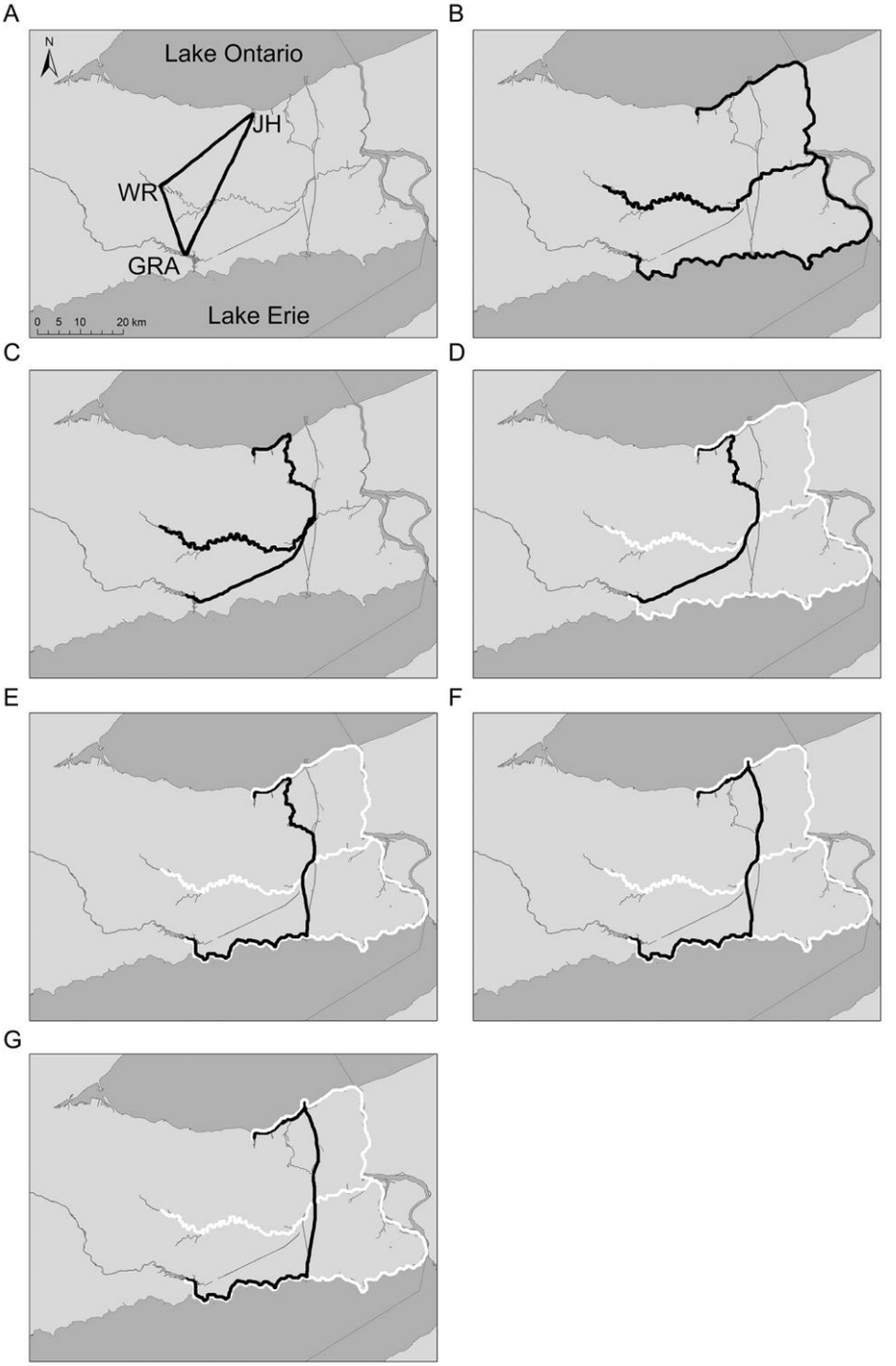

Figure 2. Models of routes of dispersal and gene flow across the Niagara Peninsula based on Euclidean distances (A), pre-canal conditions (B), first canal configuration, 1829–1833 (C), installation of the Welland River aqueduct, 1833–1908 (D), disconnection of the Feeder Canal, 1908–1930 (E), connection with Port Weller, 1930–1973 (F), and map of the modern-day watercourse configuration (G), which was not tested due to its high degree of similarity to model F. The white line in D–G represents a dispersal route from Welland River sites to sites in Lake Ontario and Lake Erie after installation of the Welland River aqueduct.

Table 2. The number of *Quadrula quadrula* genotyped (*N*), mean rarefied allelic richness (*Ar*), total number of private alleles (*PA*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) by sampling site. *N*, *Ar*, *Ho*, and *He* are also listed per locus per site. Sample site codes as in Table 1. Bold = deviation from Hardy–Weinberg Equilibrium.

		Lake Erie									Lake Ontario			
		NMB	CCR	GKU	YMC	POR	MCB	LG	GRA1	GRA2	WR	OSW	FMP/SMP	JH
Overall	*N*	24	21	24	25	25	22	27	43	20	46	23	31	45
	Ar	4.202	3.402	4.206	4.838	4.153	3.214	3.621	3.645	3.829	3.044	2.749	2.508	3.200
	PA	5	0	2	6	2	0	1	0	2	3	0	0	0
	Ho	0.476	0.489	0.503	0.415	0.434	0.537	0.441	0.508	0.539	0.391	0.411	0.340	0.329
	He	0.578	0.440	0.530	0.604	0.534	0.448	0.452	0.478	0.511	0.421	0.408	0.329	0.401
A112	*N*	22	21	20	22	23	22	19	43	19	38	19	31	40
	Ar	5.897	5.250	5.156	5.834	4.783	5.024	5.060	4.338	4.565	4.257	4.056	3.395	3.967
	Ho	0.636	0.714	0.800	0.682	0.609	0.818	0.895	0.791	0.947	0.763	0.842	0.581	**0.450**
	He	0.742	0.721	0.756	0.752	0.724	0.747	0.690	0.677	0.741	0.701	0.654	0.527	0.623
A130	*N*	19	19	24	19	16	17	17	43	20	42	21	29	34
	Ar	5.687	6.565	6.708	7.549	5.971	4.746	6.829	6.245	6.055	5.458	3.356	4.163	5.479
	Ho	0.895	0.789	0.750	0.632	0.875	0.824	0.588	0.837	0.850	0.643	0.476	0.621	0.529
	He	0.748	0.760	0.787	0.817	0.801	0.670	0.804	0.785	0.665	0.740	0.616	0.668	0.750
C4	*N*	23	11	22	19	22	11	27	43	19	41	23	31	45
	Ar	6.147	3.714	6.149	5.110	6.240	3.662	4.942	6.139	6.077	4.554	5.083	4.225	5.237
	Ho	0.696	0.727	0.636	0.632	0.591	0.818	0.704	0.744	0.684	0.659	0.696	0.806	0.733
	He	0.773	0.579	0.724	0.708	0.720	0.591	0.727	0.804	0.785	0.711	0.738	0.750	0.759
C114	*N*	23	20	20	20	23	22	26	43	20	46	20	30	42
	Ar	2.696	2.884	3.683	4.738	3.412	2.849	2.894	2.962	2.995	1.994	2.000	1.267	2.517
	Ho	0.522	0.700	**0.700**	**0.500**	**0.435**	0.636	0.462	0.651	0.600	0.283	0.450	0.033	0.262
	He	0.533	0.580	0.583	0.651	0.604	0.562	0.489	0.580	0.649	0.375	0.439	0.033	0.274
D102	*N*	19	10	23	22	21	8	27	43	20	46	19	29	45
	Ar	1.986	1.000	2.542	3.419	2.868	2.000	1.000	1.186	2.284	1.000	1.000	1.000	1.000
	Ho	**0.053**	0.000	**0.130**	**0.045**	**0.095**	0.125	0.000	0.023	0.150	0.000	0.000	0.000	0.000
	He	0.301	0.000	0.328	0.468	0.259	0.117	0.000	0.023	0.224	0.000	0.000	0.000	0.000
R9	*N*	18	21	22	24	20	20	25	43	20	42	23	31	39
	Ar	2.799	1.000	1.000	2.375	1.646	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Ho	**0.056**	0.000	0.000	**0.000**	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	He	0.369	0.000	0.000	0.226	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

separated from Lake Ontario tributaries and embayments. Low bootstrap values (0.08–0.61) for the relationships among Lake Erie sites lend support to the high degree of similarity among sites observed in STRUCTURE. Coastal Lake Ontario sites (JH and FSMP) were more closely related to each other than to sites in the Welland River drainage (WR and OSW). Sites WR and OSW showed a high degree of similarity, but JH and FSMP exhibited greater differentiation. Both relationships were supported by high bootstrap values (0.97 and 0.87, respectively), and were similar to what was found in the STRUCTURE analysis.

We ran Mantel tests for 7 different waterscape configurations, representing various stages of development on the Welland Canal, pre-canal conditions, and Euclidean distances, neglecting watercourse connections (Fig. 2A–G). Of all historical and current watercourse configurations examined, 3 Mantel tests were significant for IBD. Analyses based on linearized F_{ST} and Nei's distance (D_A), Euclidean distances between sites, pre-canal conditions, and the first configuration of the Welland Canal from 1829 to 1833 exhibited significant IBD (Table 4). Mantel tests for all configurations of the canal after 1833 were not significant.

DISCUSSION

Analyses of the genetic structure of *Q. quadrula* across Lake Erie and Lake Ontario revealed 2 populations with evidence of considerable gene flow between them. Overall,

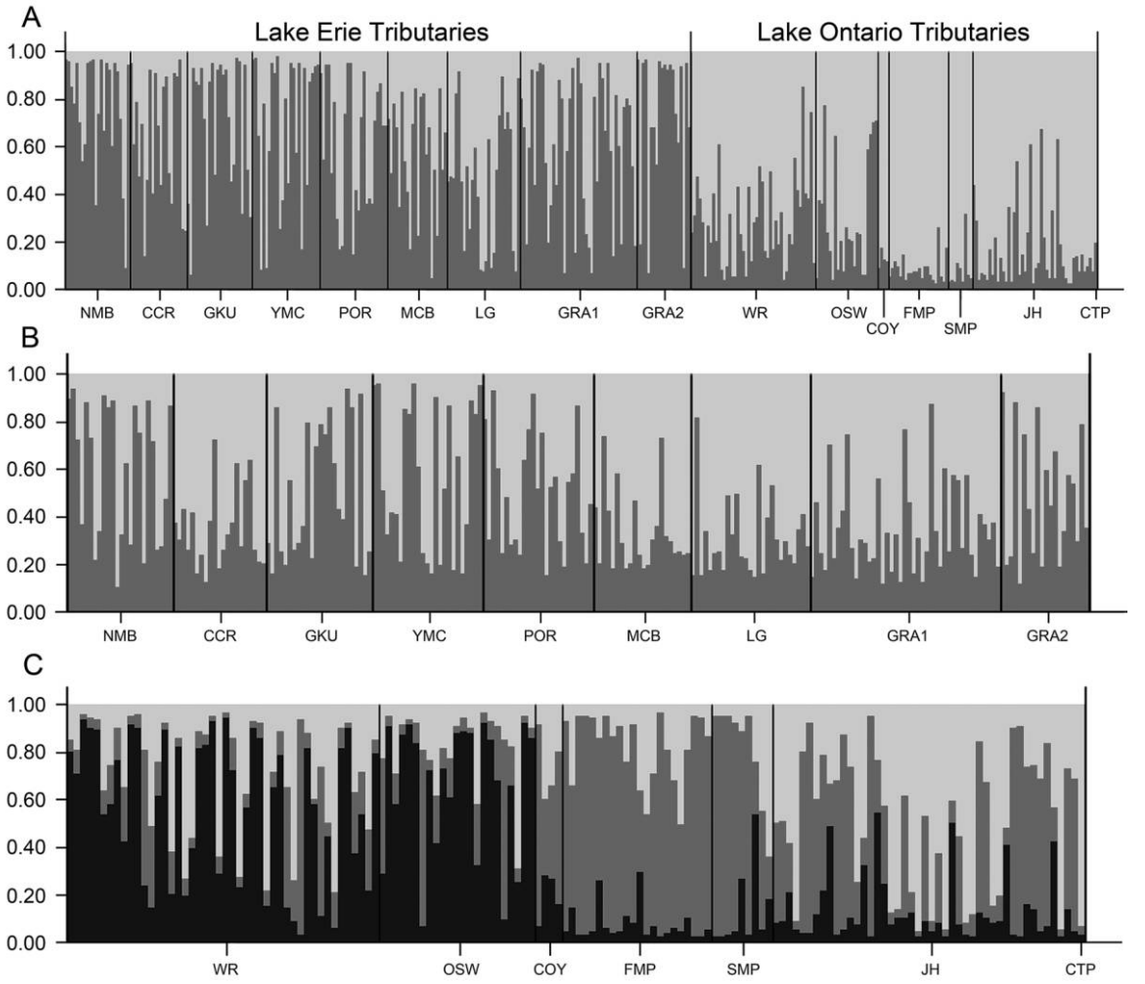

Figure 3. A.—Summary of STRUCTURE (Pritchard et al. 2000) analyses for *Quadrula quadrula* from Lake Erie and Lake Ontario tributaries and embayments ($K = 2$) without a priori geographic information. B.—Substructure of Lake Erie tributaries ($K = 1$). C.—Substructure analysis of Lake Ontario tributaries and embayments ($K = 3$). Sampling locations are separated by black lines. See Table 1 for site abbreviations.

Lake Erie and Lake Ontario formed distinct clusters, but a number of individuals from Lake Erie and Lake Ontario sites exhibited variable likelihood of belonging to one population or another. However, the difference in opposite-assigned samples suggested the gene flow is mostly unidirectional, favoring upstream movement from Lake Ontario into Lake Erie. The function of Niagara Falls as a downstream barrier is questionable, but it functions as a powerful upstream barrier to dispersal and gene flow, preventing host-fish movement upstream. As such, upstream gene flow under pre-canal conditions was not possible without facilitation by humans (e.g., translocation of infected hosts or individual mussels between waterbodies). These results support the hypothesis that Welland Canal connections have facilitated gene flow between lakes.

The results of our study are suggestive of historical bottlenecks in *Q. quadrula* populations in coastal Lake Ontario. However, JH did not show evidence of a significant bottleneck effect after Bonferroni correction nor did the other Lake Ontario sites show a significant bottleneck effect under any of the models used. However, a bottleneck at these sites may have been too recent to present distinguishable genetic cues (Rowe and Zanatta 2015). Alternatively, the genetic evidence of a bottleneck may have been concealed by rescue effects. Significantly lower rarefied allelic richness than that found in Lake Erie *Q. quadrula* was observed in all 4 Lake Ontario populations. Moreover, all Lake Ontario sites were fixed at 2 loci, and, with the exception of the upper Welland River (WR), Lake Ontario sites exhibited no private alleles. These results are consistent with ≥1 bottlenecked populations that have experienced recent gene flow with neighboring populations. Historical bottlenecks also may explain the moderate differences in genetic structure between Niagara Peninsula populations, particularly JH and FSMP, neighbor-

Table 3. Pairwise F_{ST} values (below diagonal) and Jost's D (above diagonal) for *Quadrula quadrula* derived from 6 microsatellite loci. Bold = significant following Bonferroni correction ($\alpha = 0.0006$).

	NMB	CCR	GKU	YMC	POR	MCB	LG	GRA1	GRA2	WR	OSW	FMP/SMP	JH
NMB		0.019	0.025	0.001	0.009	0.021	0.044	**0.038**	0.018	**0.086**	**0.062**	**0.167**	**0.142**
CCR	**0.061**		0.003	0.011	0.008	−0.007	0.002	0.008	0.017	**0.060**	**0.045**	**0.115**	**0.091**
GKU	**0.031**	**0.064**		−0.016	0.009	−0.002	0.015	0.009	0.017	**0.062**	**0.051**	**0.102**	**0.086**
YMC	0.014	0.030	0.000		0.000	0.009	0.024	0.026	0.014	**0.063**	0.043	**0.118**	**0.092**
POR	0.000	**0.056**	0.025	0.000		0.009	0.040	**0.029**	0.024	**0.053**	0.043	**0.130**	**0.096**
MCB	**0.071**	0.000	**0.095**	**0.049**	**0.067**		0.009	0.011	0.018	**0.061**	**0.047**	**0.090**	**0.092**
LG	**0.064**	**0.131**	**0.038**	**0.041**	**0.047**	**0.160**		−0.001	0.032	**0.040**	0.038	**0.062**	**0.039**
GRA1	**0.077**	**0.110**	0.024	**0.059**	**0.071**	**0.156**	**0.045**		0.022	**0.054**	**0.047**	**0.080**	**0.069**
GRA2	**0.048**	**0.088**	0.015	0.034	**0.050**	**0.129**	**0.063**	0.022		**0.083**	**0.049**	**0.164**	**0.122**
WR	**0.090**	**0.142**	**0.057**	**0.068**	**0.070**	**0.173**	**0.050**	**0.060**	**0.077**		0.002	**0.095**	**0.043**
OSW	**0.059**	**0.092**	0.030	0.031	**0.047**	**0.124**	**0.058**	**0.056**	0.042	0.015		**0.108**	**0.062**
FMP/SMP	**0.167**	**0.197**	**0.115**	**0.126**	**0.150**	**0.212**	**0.109**	**0.092**	**0.168**	**0.116**	**0.136**		**0.038**
JH	**0.109**	**0.171**	**0.079**	**0.077**	**0.080**	**0.192**	**0.034**	**0.076**	**0.110**	**0.046**	**0.074**	**0.059**	

ing sites that organize into fairly distinct populations according to STRUCTURE. Some or all of these populations could be the result of recent (post-canal construction, 1829) colonizations that experienced bottlenecks via the founder effect.

Tests for IBD in past and present watercourse configurations in the Niagara Peninsula identified 3 configurations that fit the IBD model (Fig. 2A–C). Euclidean distances, pre-canal conditions (prior to 1829), and the first configuration of the Welland Canal (1829–1833) showed significant IBD, whereas all configurations of the canal after 1833 did not. The 3 configurations with significant IBD fit the stepping-stone model of gene flow. *Quadrula quadrula* from Lake Ontario embayments are more closely related to those in the nearby Welland River than those in the more distant Grand River, fitting the 1-dimensional stepping-stone model described by Kimura and Weiss (1964). The Euclidean distance model does not follow hydrology, but pre-canal conditions and the 1829–1833 canal model exhibit pathways dispersing individuals must follow to move between watercourses. Geographic distances vary across these 3 models, but the general relationships of genetic to geographic distances are roughly preserved. The Grand River and Lake Ontario embayments show greater pairwise geographic distances than do Grand-to-Welland and Lake Ontario-to-Welland pairings, matching relationships in genetic distance. However, after 1833, watercourse configuration models exhibited a key alteration, in the form of an aqueduct that physically separated the Welland River from the Welland Canal. After this alteration, the geographic distance between the furthest watercourses was minimized, whereas the distance between nearby watercourses was maximized. These configurations did not fit the IBD model, so we conclude that the colonization of Lake Ontario did not occur via these connections and, therefore, probably occurred prior to the creation of the Welland River aqueduct.

The Euclidean distance model possesses the lowest p-value, but it is the least likely to have facilitated the colonization of Lake Ontario. This model is a representation of the potential for these colonizations to have occurred via human-facilitated movement of mussels or infected fishes between watercourses, effectively disregarding hydrogeographic distance. Fish stocking is not uncommon, but the most likely host for *Q. quadrula*, *I. punctatus*, is common throughout Lake Erie and Lake Ontario tributaries (COSEWIC 2006). *Ictalurus punctatus* is an important sport fish that is frequently targeted by anglers in the lower Great Lakes (ODW 2017), but its natural abundance in the region makes extra stocking unlikely. Rather, the significant IBD exhibited by this model may be an indicator of the extreme genetic similarity among Niagara Peninsula sites and may lend additional support to the hypothesis that the Welland Canal has facilitated gene flow between the 2 Great Lakes.

The pre-canal configuration represents a colonization of Lake Ontario hundreds to thousands of years before the creation of the canal. This configuration shows significant IBD but is not supported by the population genetic structure analyses, which provide evidence of recent dispersal and gene flow. Moreover, geographic distribution of *Q. quadrula* in Lake Ontario does not support post-glacial colonization, despite intense survey efforts in Lake Ontario coastal habitat (DTZ, unpublished data; Burlakova et al. 2014). *Quadrula quadrula* has been found in only a few locations along the southern and western coastline, primarily localized near the mouth of the Welland Canal in western Lake Ontario. This high degree of localization is consistent with recent establishment, where range expansion has been limited in Lake Ontario. Instead, population genetic and distributional

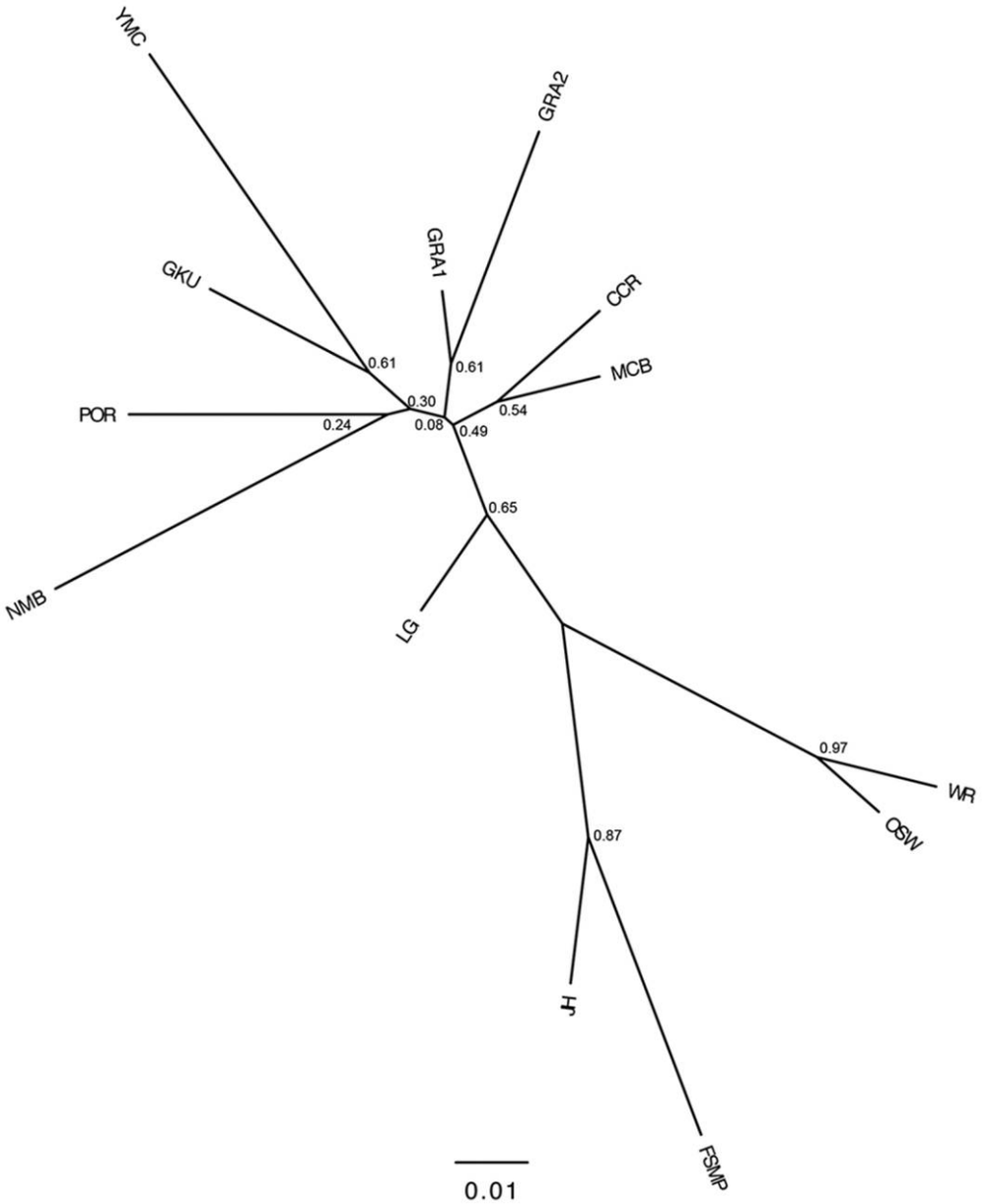

Figure 4. Neighbor-joining tree of Nei's genetic distances (D_A) between *Quadrula quadrula* collection sites in Lake Erie, the Niagara Peninsula, and Lake Ontario. Nodal bootstrap support values are displayed. See Table 1 for site abbreviations.

evidence suggest a canal-facilitated introduction, lending further support for the first configuration of the Welland Canal between 1829 and 1833.

That the configurations of the canal since 1833 do not fit the IBD model indicates either that the modern configuration of the canal may not facilitate dispersal and gene flow, or that the more recent changes are too recent for IBD to be reflected in the genetic structure observed. The latter hypothesis seems probable given the long estimated lifespans and overlapping generation times for *Q. quadrula* (COSEWIC 2006). All tested watercourse configurations, including those significant for IBD, exhibited low slope, indicating the signal for IBD is weak (Table 4). This result may be caused by the considerable dispersal ability of the host-fish species *Q. quadrula* parasitizes, or continuous dispersal occurring over multiple watercourse configurations throughout the life of the Welland Canal could reduce the strength of IBD in a single model.

The Welland Canal has been incriminated in facilitation of the introduction of a number of invasive species, particularly fishes (Aron and Smith 1971, Byran et al. 2005, Daniels 2001). Fish, including Channel Catfish and Brown Bullhead

Table 4. Watercourse configurations, approximate date ranges, and corresponding Pearson's *r*, *p*-value, and slope for Mantel test models run for linearized F_{ST} and Nei's distance (D_A). Bold = $p < 0.05$. Models are labeled A–F as they appear in Fig. 2.

			Linearized F_{ST}			Nei's D_A		
Model	Model conditions	Date range	*r*	*p*	Slope	*r*	*p*	Slope
A	Control (Euclidean distances)	N/A	**0.666**	**0.006**	**0.0022**	**0.647**	**0.007**	**0.0018**
B	Pre-canal conditions (following Niagara River)	Pre-1829	**0.335**	**0.038**	**0.0002**	**0.499**	**0.006**	**0.0003**
C	First configuration of Welland Canal	1829–1833	**0.378**	**0.036**	**0.0005**	**0.539**	**0.008**	**0.0007**
D	Construction of aqueduct disconnecting Welland River from the canal	1833–1908	0.147	0.244	0.0001	0.318	0.079	0.0002
E	Feeder canal disconnected	1908–1930	0.147	0.240	0.0001	0.318	0.074	0.0002
F	Modern canal	1930–present	0.174	0.190	0.0001	0.346	0.061	0.0002

(probable and possible hosts for *Q. quadrula* in the Great Lakes), are capable of passing through the Welland Canal locks in both the down- and upstream directions, but dispersal may be limited (Kim and Mandrak 2016). Our study provides new evidence of dispersal-related genetic exchange for *Q. quadrula*, an obligate symbiont of catfish, between Lakes Erie and Ontario across the canal-modified waterscape. Our study also suggests that mussels in Lake Ontario coastal embayments probably arose from a recent introduction via the canal rather than as an artifact of post-glacial (ancient) colonization.

The observed patterns of dispersal in *Q. quadrula* are a reminder that the dispersal patterns and range expansion of a species can be reflected in their symbionts. These results also demonstrate how canals may affect other unionid species. Native unionids, such as *Lasmigona subviridis*, and potential invasive species including *Sinanodonta woodiana*, currently inhabit man-made canals or waterways connected by them (Kraszewski 2007, USGS 2016). By dispersing on the gills of their host species, these and other unionids have the potential to move between waterbodies via these man-made corridors and colonize previously unoccupied habitats. The dispersal ability of the species and, therefore, the gene flow among its populations depends on the dispersal ability of its hosts. We showed that *Q. quadrula*, which parasitizes highly vagile catfish species, displays a high degree of connectivity among populations connected by the Welland Canal. However, mussels dependent on dispersal-limited fishes may not use the waterway connections created by canals. An example is the Snuffbox mussel (*Epioblasma triquetra*), which is thought to parasitize the common Logperch (*Percina caprodes*). The movement of *P. caprodes* suggests high site fidelity in the species, indicating a degree of dispersal limitation (Schwalb et al. 2011). Active canals, such as the Welland Canal, are generally regarded as poor habitat for fishes because of high shipping traffic and pollution in the habitat (e.g., Delft Canal in The Netherlands, Kelderman et al. 2000; Venice canals, Wetzel and Van Vleet 2004; Suez Canal, Zaki et al. 2014) and may offer little refuge for sensitive species dispersing through it. For species, such as *P. caprodes* and its symbiont *E. triquetra*, which rarely disperse >100 m from their native habitat, a kilometers-long stretch of poor-quality canal habitat may be a barrier to dispersal. However, the capacity for introduction into nonnative habitat should not be overlooked or dismissed, even for species suspected to be incapable of it.

Our study also has provided important information on the dispersal history and population structure of a threatened freshwater mussel that will prove valuable to conservation strategies for the species. Over the course of our study, several new populations of *Q. quadrula* were identified, expanding our understanding of the distribution of the species in the Niagara Peninsula and Lake Ontario. In addition, the evidence that populations in Lake Ontario embayments can be the result of a recent introduction poses an interesting situation, in which a threatened species is capable of rapidly expanding its range into previously unoccupied regions and, thus, is potentially capable of genetic rescue. The dispersal ability of *Q. quadrula* implied by this evidence and the dispersal abilities of the probable hosts will prove useful to conservation managers when developing conservation strategies for *Q. quadrula* or re-evaluating its conservation status in the future.

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