

Genetic variability and phylogeographical patterns of a nonindigenous species invasion: a comparison of exotic vs. native zebra and quagga mussel populations

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nonindigenous species;
phylogeography;
quagga mussel;
zebra mussel.

Abstract

There have been few investigations of the number of founding sources and amount of genetic variability that lead to a successful nonindigenous species invasion, although genetic diversity is believed to play a central role. In the present study, population genetic structure, diversity and divergence patterns were analysed for the zebra mussel *Dreissena polymorpha* [$n = 280$ samples and 63 putative randomly amplified polymorphic DNA (RAPDs) gene loci] and the quagga mussel *D. bugensis* ($n = 136$ and 52 loci) from 10 nonindigenous North American and six Eurasian sampling sites, representing their present-day ranges. Results showed that exotic populations of zebra and quagga mussels had surprisingly high genetic variability, similar to those in the Eurasian populations, suggesting large numbers of founding individuals and consistent with the hypothesis of multiple colonizations. Patterns of genetic relationships indicate that the North American populations of *D. polymorpha* likely were founded by multiple source populations from north-western and northcentral Europe, but not from southcentral or eastern Europe. Sampling areas within North America also were significantly divergent, having levels of gene flow and migration about twice those separating long-established Eurasian populations. Samples of *D. bugensis* in Lakes Erie and Ontario were significantly different, with the former being more closely related to a native population from the Dnieper River, Ukraine. No evidence for a founder effect was discerned for either species.

Introduction

Many ecologists believe that introductions of exotic species pose one of the most serious threats to native ecosystems worldwide (Simberloff & Von Holle, 1999; Everett, 2000). The genetic composition of a founding population likely determines its ability to adapt to a new environment (Holland, 2000; Tsutsui & Case, 2001) and high genetic variability has been positively correlated with invasive success (Williamson, 1996). The initial

genetic structure of a successful invasive population depends on several factors, including the effective population size of the introduction event(s), the genetic diversity of the source population(s) and the number of founding sources. However, data concerning the molecular genetic structure of nonindigenous populations are rare (Ross *et al.*, 1996, 1999; Holland, 2000), and the present study offered a unique opportunity to examine the population genetics of a large, successful invasion and make comparisons with possible source populations. The initially established genotypes then may either pave the way for more successful later-arriving genotypes (Simberloff & Von Holle, 1999) or may persist over time and provide resistance against later-arriving genotypes (Hewitt, 2000, 2001).

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Of the many anthropogenic introductions to the North American Great Lakes, dreissenid mussels have exerted the most pronounced ecological consequences (Mills *et al.*, 1993; Ricciardi & MacIsaac, 2000). Since their accidental introduction in 1988, zebra mussel *Dreissena polymorpha* populations have expanded rapidly with some early populations exceeding 500 000 individuals m^{-2} in nearshore areas of the lower Great Lakes (Stewart & Haynes, 1994). Nonindigenous dreissenid populations have profound economic and environmental consequences because of their prodigious filtering settlement capacities. They alter food web pathways, convert soft benthos into hard substrate, bioamplify contaminants, lead to the decline of native bivalves, and seriously foul water intakes, ships and navigational buoys (Mills *et al.*, 1993; Morton, 1996). Considerable research effort, funded by a diversity of agencies, has been directed towards understanding the ecological and economic impacts of the dreissenid mussel invasion in the Great Lakes, and the means by which nonindigenous species arrive. However, there have been few studies of the population genetic structure of dreissenids and previous studies were unable to discern their possible founding sources (summarized in Marsden *et al.*, 1996; Stepien *et al.*, 1999).

History of the Dreissenid mussel invasion

The dreissenid mussel invasion of the Great Lakes began about 1988, with the successful establishment of the Eurasian zebra mussel *D. polymorpha* (Bivalvia:

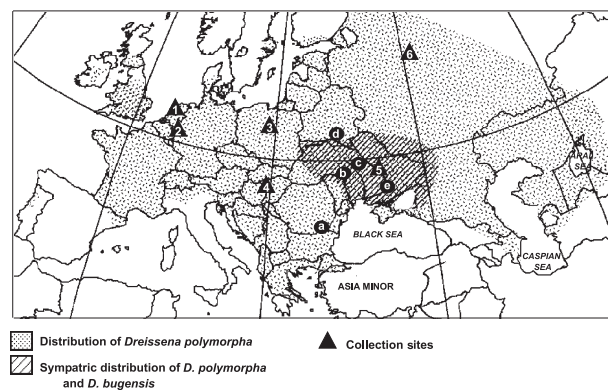


Fig. 1 Map showing respective Eurasian ranges of the zebra mussel *Dreissena polymorpha* (dots) and the quagga mussel *D. bugensis* (stripes), with triangles denoting locations of sampling sites. Collection locations were numbered, as follows: 1. Lake IJsselmeer, the Netherlands (zebra mussel, zm), 2. Rhine River, the Netherlands (zm), 3. Wtoctawek Reservoir, Poland (zm), 4. Danube River, Hungary (zm), 5. Dnieper River, Ukraine (both zm and the quagga mussel), and 6. Volga River, Russia (zm). Rivers discussed in the text are lettered: (a) Danube River, (b) Dniester River, (c) Bug River, (d) Pripjat' River, and (e) Dnieper River.

Dreissenidae) in Lake St Clair via ships' ballast water (Hebert *et al.*, 1989). *Dreissena polymorpha* is widespread throughout much of Eurasia (Fig. 1), including western Europe and the drainage basins of the Baltic, Black and Caspian Seas (Mills *et al.*, 1993; Rosenberg & Ludyanskiy, 1994; Valovirta & Porkka, 1996; Minchin & Moriarty, 1997). Its present North American range (Fig. 2) includes all of the Great Lakes and the Mississippi, Hudson and Ohio River basins (National *D. polymorpha* and Aquatic Nuisance Species Clearinghouse, 2002).

During the Quaternary Ice Ages, *D. polymorpha* is believed to have been largely restricted to pockets of southerly glacial refugia that were located primarily in central and eastern Europe (Kinzelbach, 1992; Morton, 1993). Populations isolated in separate refugia accumulated genetic differences because of random mutations and losses of genotypes, the latter resulting from alterations in effective population sizes (see Hewitt, 1996, 2000; Avise, 2000). During the interglacial periods and following the end of the Ice Ages, aquatic species surged northward from the refugia pockets along river drainage systems (Banarescu, 1992). Many temperate freshwater species today retain the divergent genetic characteristics of their glacial refugia ancestors, provided that separate watersheds have remained isolated (Hewitt, 1996, 2000; Stepien *et al.*, 1999). Construction of canals during the Industrial Revolution led to the anthropogenic expansion of *D. polymorpha* throughout most of Western Europe (Morton, 1993). *Dreissena polymorpha* thus has an extensive history as a successful invader and colonizer.

A second species of dreissenid, the quagga mussel *D. bugensis*, was found in the Erie Canal and Lake Ontario in 1991 (May & Marsden, 1992), and is now common in Lakes Erie and Ontario (Fig. 2). The quagga mussel recently invaded Lake Michigan – where it is still rare (Nalepa *et al.*, 2001). Recent field studies in the eastern, central and western basins of Lake Erie indicate that *D. bugensis* has greatly increased its abundance in what was formerly *D. polymorpha* habitat, resulting in the progressive displacement of the latter (Berkman *et al.*, 2000; Jarvis *et al.*, 2000).

The native distribution of *D. bugensis* is confined to the Bug, Dnieper, and Pripjat' Rivers in the Ukraine (Mills *et al.*, 1993; Marsden *et al.*, 1996), and thus encompasses significantly less geographical area than does *D. polymorpha* (Fig. 1). There are some reports that *D. bugensis* invaded the Volga River, dating to 1992 (M. Orlova, Russia, pers. commun.). The two species are distinguishable morphologically by shell characteristics (May & Marsden, 1992), including a pronounced angle between the dorsal and ventral surfaces in *D. polymorpha* vs. a rounded angle and flatter shape in *D. bugensis*. Analysis of mitochondrial DNA (mtDNA) sequence divergence in their 16S ribosomal DNA (rDNA) and COI genes, calibrated with the fossil record, suggests

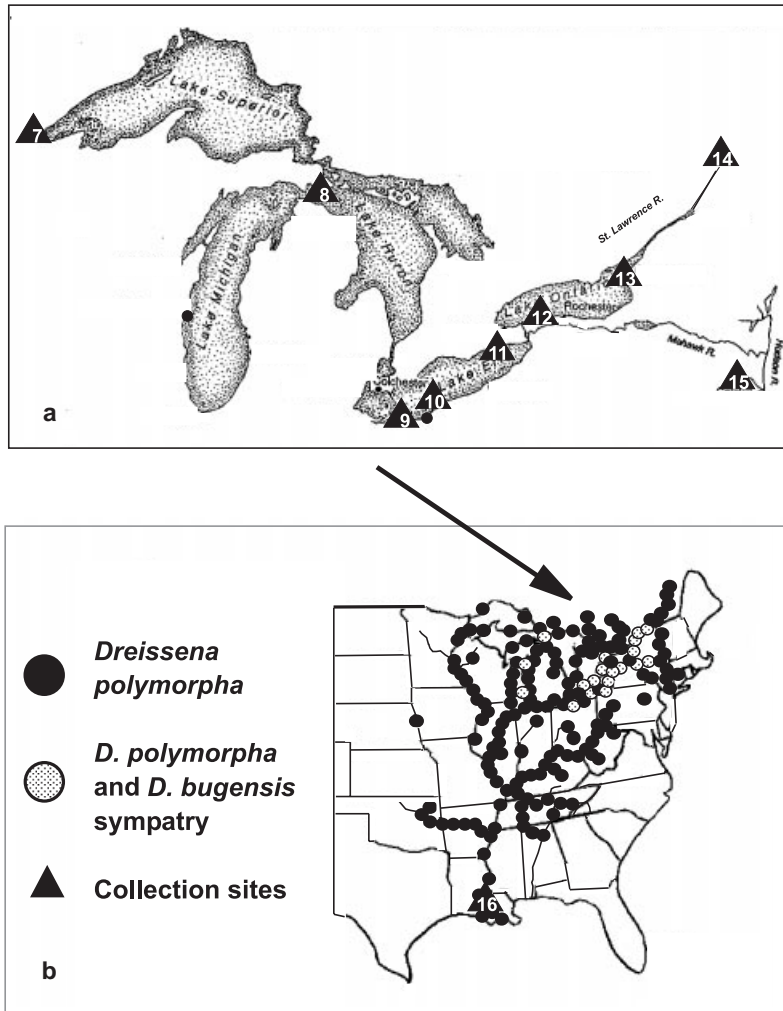


Fig. 2 Maps showing North American sampling sites (triangles) and the present respective ranges of the invasive populations of zebra mussel *Dreissena polymorpha* (closed circles on b) and the quagga mussel *D. bugensis* (open circles on b) in North America. Figure 2a shows the Great Lakes and 2b depicts the present-day ranges across North America. Collection locations were numbered as: 7. Duluth Harbor, Lake Superior (zebra mussel, zm), 8. Mackinac Straits, between Lakes Huron and Michigan (zm), 9. Put-in-Bay, western Lake Erie (both zm and quagga mussel, qm), Cleveland, 10. central Lake Erie (qm), 11. eastern Lake Erie (qm), 12. Olcott, western Lake Ontario (qm), 13. Cape Vincent, eastern Lake Ontario (zm and qm), 14. St Lawrence River, Quebec (zm), 15. Hudson River, New York (zm), and 16. Baton Rouge, lower Mississippi River (zm).

that *D. polymorpha* and *D. bugensis* diverged approximately 11–13 million years ago (Stepien *et al.*, 1999).

Dermott & Munawar (1993) described a morphologically distinguishable 'profundal' (deepwater) ecotype of *D. bugensis* from 10 to 30 m depths in eastern Lake Erie. The deepwater *D. bugensis* ecotype is characterized by its white colour, elliptical shell with a distinctive basal knob and a dorsal swelling, straight ventral shell margin, and more anterior location of the foot and byssal threads. The profundal variant often occurs individually, whereas *D. polymorpha* and the shallow water variant of *D. bugensis* are found in clusters (Dermott & Munawar, 1993; Berkman *et al.*, 2000). The question of whether the profundal mussel is genetically distinguishable from the quagga mussel has been controversial (Baldwin *et al.*, 1996; Claxton *et al.*, 1997), and was tested in the present study.

Objectives of the present study

The objective of the present study was to test for genetic relationships and divergence patterns among invasive and native populations of *D. polymorpha* and *D. bugensis*, using a sampling design that represented their present ranges in North America and Eurasia. Our study specifically focused on testing their possible variation across the Great Lakes, in order to provide a baseline data set for future comparisons during the course of the invasion. A primary goal was to evaluate the possible founding source(s) for the North American introductions, in order to facilitate correct ecological comparisons with Eurasian populations. A secondary goal was to compare the amount and distribution of genetic variability in the exotic and native populations, in order to determine whether a founder effect (loss of variation) may have occurred, and to what extent. It has been hypothesized that high genetic variab-

ility of invasive populations may be predictive of their relative successes (Nevo, 1983; Williamson, 1996). Results of the present study will aid our understanding of the pattern of distribution spread of *D. polymorpha* and *D. bugensis*, which may help to predict their invasion successes – and those of other exotic species.

Approach

The present investigation employed randomly amplified polymorphic nuclear DNA (RAPD) analysis (Welsh & McClelland, 1990; Williams *et al.*, 1990, 1993), with five primers and 52 presumptive gene loci for *D. bugensis* and 63 loci for *D. polymorpha*, to examine genetic variation patterns and divergences among population areas. RAPD assays utilize the polymerase chain reaction (PCR, Mullis & Faloona, 1987) DNA amplification, gel electrophoresis, and fluorescent visualization of the lengths of the amplified fragments. The technique requires only nanogram quantities of DNA and is applicable to small samples, including individual eggs and larvae (Williams *et al.*, 1993).

The RAPD analyses sample randomly across the genome and it is likely that every chromosome will be represented (Palumbi, 1996). Random assays have been shown in modelling experiments to closely approximate true phylogenies (Swofford *et al.*, 1996). RAPD assays are visualized as the presence or absence of bands on agarose gels. Because the distance between primer sites often varies among individuals in a population according to mutational changes, fragments of varying lengths are produced. Fragments that are present in some individuals, and absent in others, are identified as polymorphisms. These polymorphisms are inherited according to Mendelian genetics as dominant genetic markers (Williams *et al.*, 1993; Borowsky, 2001).

Difficulty in distinguishing polymorphisms from PCR artefacts, notably primer-derived amplification products, is one of the possible disadvantages of the RAPD technique. In order to correct for this possible problem, negative no-DNA controls were run and RAPDs products that comigrated with primer-induced bands were omitted from the analyses (Pan *et al.*, 1997). Optimization of the PCR parameters is vital for reducing the presence of PCR artefacts, and was practiced in the present study. Adjustments in the concentrations of template DNA, primers, dNTPs, and KlenTaq polymerase were made to determine the conditions yielding the strongest and most reproducible results (Haig *et al.*, 1994). Repeated assays (twice or more) and a random blind design were used to confirm the reproducibility of the RAPD fragment patterns.

Materials and methods

Zebra mussels *D. polymorpha* were sampled from six sites in Eurasia (Fig. 1), totalling 106 individuals. Sampling

sites included Lake IJsselmeer near Amsterdam, the Netherlands (site 1, 52.46°N, 5.14°E, $n = 24$), the Rhine River at Vuren, the Netherlands (site 2, 51.2°N, 4.24°E, $n = 21$), Wtactawek Reservoir, Poland (site 3, 52.5°N, 19.0°E, $n = 18$), the Danube River at Budapest, Hungary (site 4, 47.3°N, 19.0°E, $n = 19$), the Dnieper River in the Ukraine (site 5, 48.23°N, 34.0°E, $n = 19$), and the Volga River, Russia (site 6, 58.0°N, 42.0°E, $n = 5$) (Fig. 1). These were compared with 174 *D. polymorpha* samples from seven locations in North America (Fig. 2), including: Duluth Harbor, Minnesota, Lake Superior (site 7, 46.5°N, 92.07°W, $n = 24$), the Mackinac Straits between Lakes Huron and Michigan (site 8, 44.9°N, 85°W, $n = 28$), Put-in-Bay, Ohio, western Lake Erie (site 9, 41.5°N, 83°W, $n = 31$), Cape Vincent, New York, eastern Lake Ontario (site 13, 44.2°N, 76.1°W, $n = 21$), the St Lawrence River at Gently, Quebec (site 14, 43°N, 78.5°W, $n = 22$), the Hudson River at Stuyvesant, New York (sites 15, 42.2°N, 73.5°W, $n = 22$), and the lower Mississippi River at Baton Rouge, Louisiana (site 16, 30°N, 90°W, $n = 26$) (Fig. 2).

Eurasian samples of quagga mussels *D. bugensis* were obtained from the Dnieper River in the Ukraine (site 5, $n = 25$, Fig. 1). North American samples of *D. bugensis* totaled 111 individuals from five locations in North America (Fig. 2), including Put-in-Bay, Ohio, western Lake Erie (site 9, $n = 30$), Cleveland, Ohio, central Lake Erie (site 10, 41.3°N, 81.42°W, $n = 19$), the eastern basin of Lake Erie (site 11, 42.26°N, 79.5°W, $n = 20$), Olcott, New York, western Lake Ontario (site 12, 43°N, 79°W, $n = 22$), and Cape Vincent, New York, eastern Lake Ontario (site 13, $n = 20$). All specimens of *D. bugensis* assayed from site 11 comprised the profundal variant, which were collected and identified by R. Dermott (who described the ecophenotype in Dermott & Munawar (1993)).

Mussels were either frozen live in liquid nitrogen or on dry ice and stored at -80°C , or were placed directly in 95% ethanol while alive and then stored at room temperature. The gonads were removed and sexes were determined by microscopic examination, as described in Stepien *et al.* (1999). Samples analysed included approximately equal numbers of males and females for each site. The shells were archived, and are available by request. Guts were removed and the remaining tissue was ground in liquid nitrogen using a cylindrical stainless steel mortar-and-pestle (Stepien, 1995). DNA was extracted and purified, following Stepien *et al.* (1999).

RAPD assays were performed utilizing five 10 base pair oligonucleotide primers (Quiagen Operon Technologies, Valencia, CA, USA), including OPD-1 (5'-ACCGGAAGG-3'), OPB-13 (5'-TT CCCCCGCT-3'), OPB-14 (5'-TCCGCTCTGG-3'), OPF-6 (5'-GGGAATTCGG-3'), and OPK-11 (5'-AATGCCCCAG-3'). PCR reactions used 0.25 μL of KlenTaq DNA polymerase (six units; Ab Peptides, St Louis, MO, USA, #1001), 0.25 μL 10 mM

deoxynucleotide mix (Boehringer Mannheim, Mannheim, Germany, #1-969-064), 30 μM of one of the primers, 2.5 μL of 10X PC2 buffer (Ab Peptides, #1001), and 0.5 μL of the purified target DNA (about 25 ng). A negative no-DNA control was run with all of the ingredients except DNA for each reaction. The amplification profile was an initial denaturation for 2 min at 96 °C, followed by 45 cycles of 94 °C denaturation for 1 min, 38 °C annealing for 2 min, and polymerization at 71 °C for 2 min.

Amplification products were visualized on 2% agarose gels with 0.1% ethidium bromide in 0.5X TBE [Tris–Borate–ethylene diamine tetraacetic acid (EDTA), pH 8.0] buffer, which were electrophoresed at 150 V for 1.5 h (HE 99X Max Submarine Unit, Amersham Biosciences, Sunnyvale, CA, USA, #80-6061-57). Amplification bands ranged from 2 kb to 200 base pairs (bp) in length. Migration distances were compared among individual samples and calibrated using the DNA marker VI (Boehringer Mannheim, #1-062-590). All experiments were repeated two to three times, in separate runs conducted several weeks apart to verify results. Sample order and inclusion were randomized among the separate runs. Bands (corresponding to dominant alleles at specific loci) were scored as present (1) or absent (0) for data that were congruent between two separate trials. In cases when the scoring was inconsistent, the samples were run a third time. Bands that were inconsistent in resolution, present in no-DNA controls, or not repeatable between the runs were discarded from the analysis.

Nucleotide diversity (π), defined as 'the average number of nucleotide differences per site between two randomly chosen DNA sequences' in a population (Nei & Li, 1979), and its standard error were estimated from RAPDs band survey data following Borowsky (2001). Corrections were made for the number of RAPDs primers that were employed (we selected five of the 19 that were tested in a pilot survey, having the greatest number of resolvable and repeatable bands), the number of individuals sampled, and the estimated population size (Borowsky, 2001).

Allelic frequency differences among the locations were analysed with modified χ^2 tests and Bonferroni corrections were made for multiple comparisons (Sokal & Rohlf, 1995). Gene diversity (H), genetic distances (including Nei's, 1972 D), F -statistics, G -statistics, and Nm migration estimates and their standard errors were employed to evaluate the distribution of variability within vs. among sampling sites and population subdivisions, as implemented by POPGENE (vers. 2.3, Yeh & Yang, 1997) and following recommendations by Nei (1987).

Nei's (1972) genetic distances between pairs of sampling sites and for population groups were clustered with the neighbour-joining algorithm (NJ, Saitou & Nei, 1987) in MEGA (Molecular Evolutionary Genetics Analysis, vers. 2.1, Kumar *et al.*, 2001). Frequency parsimony trees (Swafford & Berlocher, 1987) also

analysed the relationships among the samples, as implemented in PAUP* (Phylogenetic Analysis Using Parsimony, vers. 4.0b5, Swofford, 2001). Branch-and-bound (zebra mussel) and exhaustive (quagga mussel) maximum parsimony (MP) searches were conducted and support of the data sets for relationships were determined from 1000 bootstrap replications (Swofford *et al.*, 1996). In addition, NJ trees were constructed from the allelic frequency data and 1000 bootstrap replications were conducted in PAUP* (Swofford, 2001).

Results

Primer survey

A total of 63 putative gene loci consistently were resolved for 280 *D. polymorpha* samples, and 52 loci were analysed for 136 *D. bugensis*. Nineteen bands were resolved using the primer OPD-1 in *D. polymorpha* (ranging from 1800 – 234 bp) and 17 in *D. bugensis* (having lengths from 1300 – 234 bp). Sixteen bands were resolved for the primer OPB-13 in *D. polymorpha* (with lengths from 1632 – 234 bp) and 10 in *D. bugensis* (with estimated sizes of 938–322 bp). Using OPB-14, 11 bands were scored in *D. polymorpha* (with lengths approximating 1000–260 bp) and nine in *D. bugensis* (having estimated sizes from 750 – 260 bp). There were 10 bands analysed from OPF-6 in *D. polymorpha* (with lengths from 1025 – 232 bp) and five in quagga mussels (having approximate sizes from 505 – 239 bp). Seven putative loci were resolved for the primer OPK-11 in *D. polymorpha* (with lengths of 640–220 bp) and 11 in *D. bugensis* (having sizes estimated from 640 – 230 bp).

Population genetic variability in *D. polymorpha*

The numbers of zebra mussel individuals having dominant vs. homozygous recessive RAPD alleles per population location varied significantly at 16 loci in contingency table χ^2 tests. The proportion of polymorphic loci ranged from 58.7% of the zebra mussels in the Volga River to 87.3% in the St Lawrence River, and the mean was 77.2% for all populations (Table 1A). Nei's (1972) heterozygosity estimates ranged from 0.10 ± 0.02 in the Rhine River to 0.22 ± 0.2 in Lake Erie, with a mean of 0.18 per population site. Thus, population sites in North America and Eurasia exhibited similar levels of heterozygosity. Estimates of nucleotide diversity (π) in zebra mussel populations ranged from 0.009 to 0.042, and averaged 0.012 (Table 1A).

Genetic divergence patterns among populations of *D. polymorpha*

The mean numbers of loci exhibiting significantly different allelic frequencies (i.e. numbers of individuals

Table 1 Genetic variability measures, including the number of polymorphic loci, Nei's (1972) heterozygosity, and nucleotide diversity (Borowsky, 2001) ± SE for zebra mussel *Dreissena polymorpha* (A) and quagga mussel *D. bugensis* (B) population sampling sites, determined from 63 and 52 presumptive RAPDs loci, respectively. Sampling sites are numbered according to the maps on Figs 1 and 2.

Genetic variability measure	14														Mean for all sites
	1	2	3	4	5	6	7	8	9	13	14	15	16		
	Netherlands	Rhine River	Poland	Danube River	Dnieper River	Dnieper River	Volga River	Lake Superior	Lake Huron	Lake Erie	Lake Ontario	St Lawrence River	Hudson River	Mississippi River	
(A) <i>D. polymorpha</i>															
N Polymorphic loci	46 (73.02%)	43 (68.2%)	52 (82.54%)	51 (80.95%)	40 (63.49%)	37 (58.73%)	52 (82.54%)	51 (80.95%)	51 (80.95%)	52 (82.54%)	53 (84.13%)	55 (87.30%)	48 (76.19%)	52 (82.54%)	43.7 (70.45%)
Nei's (1972) <i>H</i>	0.154 ± 0.021	0.128 ± 0.020	0.196 ± 0.022	0.186 ± 0.023	0.178 ± 0.026	0.181 ± 0.025	0.171 ± 0.021	0.193 ± 0.023	0.222 ± 0.024	0.211 ± 0.024	0.211 ± 0.024	0.181 ± 0.021	0.174 ± 0.024	0.161 ± 0.020	0.189 ± 0.022
π (Borowsky, 2001)	0.011 ± 0.004	0.009 ± 0.003	0.015 ± 0.003	0.012 ± 0.004	0.012 ± 0.004	0.042 ± 0.005	0.013 ± 0.004	0.009 ± 0.004	0.009 ± 0.004	0.015 ± 0.003	0.012 ± 0.004	0.012 ± 0.003	0.011 ± 0.004	0.011 ± 0.004	0.012 ± 0.004
(B) <i>D. bugensis</i>															
N Polymorphic loci	50 (96.15%)	46 (88.46%)	44 (84.62%)	51 (98.08%)	48 (92.31%)	47 (90.38%)	48 (91.67%)	47 (90.38%)	48 (91.67%)	47 (90.38%)	47 (90.38%)	47 (90.38%)	47 (90.38%)	47 (90.38%)	47 (90.38%)
Nei's (1972) <i>H</i>	0.269 ± 0.019	0.192 ± 0.021	0.228 ± 0.023	0.266 ± 0.019	0.278 ± 0.022	0.217 ± 0.021	0.242 ± 0.021	0.266 ± 0.019	0.278 ± 0.022	0.217 ± 0.021	0.242 ± 0.021	0.266 ± 0.019	0.278 ± 0.022	0.217 ± 0.021	0.242 ± 0.021
π (Borowsky, 2001)	0.015 ± 0.004	0.009 ± 0.004	0.016 ± 0.002	0.017 ± 0.002	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004	0.015 ± 0.004

having dominant vs. homozygous recessive alleles, using contingency table tests and Bonferroni corrections; Sokal & Rohlf, 1995) were 5.6 between pairs of locations in North America, 7.0 in Eurasia, and 6.5 between North America and Eurasia. There were no significant differences between zebra mussels from eastern Lake Ontario and the St Lawrence River, and samples from the other locations differed at two (e.g. between the Netherlands vs. Poland and the Hudson Rivers, between the Rhine River vs. Lake Superior and the Mississippi River, and between Poland and eastern Lake Ontario) to 18 loci (e.g. between the Danube and Dnieper Rivers). Sampling locations that diverged by the greatest number of significantly different loci included the Volga (a mean of 6.2 differences from other sites), Dnieper ($X = 8.0$), and the Danube Rivers ($X = 9.5$).

Nei's (1972) genetic distances between *D. polymorpha* from pairs of sampling sites are given in Table 2A (below the diagonal), and ranged from $D = 0.006$ between the Rhine and Mississippi Rivers to 0.126 between the Volga River and the St Lawrence River and 0.122 between the Volga River and the site in Poland. F_{ST} values (Table 2A, above the diagonal) revealed analogous patterns, ranging from 0.018 between samples in the Rhine and Mississippi Rivers to 0.212 in the Volga and the St Lawrence Rivers and 0.198 between the Volga River and Poland. The Volga River samples were the most divergent from others, averaging $D = 0.101$ and $F_{ST} = 0.183$. The Danube River was the second most divergent population site, with mean $D = 0.049$ and $F_{ST} = 0.101$. The sample from the Dnieper River had the third greatest mean divergence, with $D = 0.042$ and $F_{ST} = 0.086$.

The overall amount and distribution of genetic variation within and between sampling sites in Eurasia and North America are compared in Table 3A. Mean levels of genetic variation were analogous in North America and Eurasia, with heterozygosities estimated as 0.21. There was more variation among sampling locations (H_c and G_{ST}) in Eurasia than in North America. Sampling sites in Eurasia were differentiated by about twice the levels ($G_{ST} = 0.19$) found among North American locations ($G_{ST} = 0.10$). Estimated migration among sites in Eurasia was about half ($Nm = 2.2$) that estimated for North America ($Nm = 4.8$). Similarly, mean genetic distances were about twice among Eurasian locations ($D = 0.06$) than in North America ($D = 0.03$) (Table 3A).

Genetic relationships among Eurasian populations of *D. polymorpha*

Phylogenetic relationships among the Eurasian locations of *D. polymorpha* are shown in Fig. 3, and were analogous in the NJ and MP analyses. A single most-parsimonious tree was obtained from an exhaustive search in PAUP* (Swofford, 2001), which had a length of 26 steps and a consistency index excluding uninformative characters of

Table 2 Genetic distances and divergences between pairs of sampling sites for the zebra mussel *Dreissena polymorpha* (A) and the quagga mussel *D. biguttata* (B). Below the diagonal: Nei's (1972) genetic distances $D \pm$ SE. Above the diagonal: F_{ST} divergences \pm SE. Sampling sites are numbered according to the maps on Figs. 1 and 2.

Sampling site	1	2	3	4	5	6	7	8	9	13	14	15	16	
	The Netherlands	Rhine River	Poland	Danube River	Dnieper River	Volga River	Lake Superior	Lake Huron	Lake Erie	Lake Ontario	St Lawrence River	Hudson River	Mississippi River	
(A) <i>D. polymorpha</i>														
1	X	0.069 ± 0.002	0.045 ± 0.005	0.101 ± 0.011	0.075 ± 0.003	0.177 ± 0.019	0.056 ± 0.003	0.059 ± 0.006	0.044 ± 0.003	0.065 ± 0.006	0.050 ± 0.009	0.059 ± 0.002	0.054 ± 0.003	
2		X	0.105 ± 0.007	0.063 ± 0.012	0.058 ± 0.004	0.197 ± 0.021	0.031 ± 0.003	0.151 ± 0.016	0.037 ± 0.004	0.093 ± 0.007	0.110 ± 0.008	0.050 ± 0.002	0.018 ± 0.001	
3			X	0.108 ± 0.011	0.098 ± 0.006	0.198 ± 0.023	0.069 ± 0.005	0.053 ± 0.003	0.068 ± 0.004	0.043 ± 0.003	0.030 ± 0.004	0.094 ± 0.009	0.090 ± 0.004	
4				X	0.105 ± 0.011	0.194 ± 0.021	0.066 ± 0.011	0.061 ± 0.009	0.062 ± 0.010	0.091 ± 0.011	0.126 ± 0.014	0.086 ± 0.013	0.149 ± 0.016	
5					X	0.171 ± 0.024	0.054 ± 0.004	0.090 ± 0.008	0.070 ± 0.004	0.094 ± 0.007	0.099 ± 0.007	0.071 ± 0.004	0.045 ± 0.002	
6						X	0.158 ± 0.018	0.183 ± 0.022	0.185 ± 0.025	0.184 ± 0.021	0.212 ± 0.023	0.159 ± 0.012	0.181 ± 0.021	
7							X	0.047 ± 0.001	0.059 ± 0.005	0.059 ± 0.005	0.169 ± 0.017	0.164 ± 0.017	0.029 ± 0.003	
8								X	0.029 ± 0.005	0.047 ± 0.003	0.064 ± 0.004	0.151 ± 0.017	0.049 ± 0.007	
9									X	0.055 ± 0.004	0.071 ± 0.006	0.045 ± 0.005	0.046 ± 0.007	
13										X	0.043 ± 0.004	0.061 ± 0.009	0.074 ± 0.006	
14											X	0.089 ± 0.011	0.091 ± 0.008	
15												X	0.045 ± 0.002	
16													X	
Mississippi River														X

Table 2 (Contd.)

Sampling site	5 Dnieper River	9 Western Lake Erie	10 Central Lake Erie	11 Eastern		13 Eastern Lake Ontario
				Lake Erie (profoundal ecotype)	Western Lake Ontario	
(B) <i>D. bugensis</i>						
5 Dnieper River	X	0.048 ± 0.017	0.087 ± 0.035	0.034 ± 0.011	0.063 ± 0.023	0.052 ± 0.020
9 Western Lake Erie	0.030 ± 0.001	X	0.077 ± 0.040	0.038 ± 0.016	0.072 ± 0.027	0.066 ± 0.024
10 Central Lake Erie	0.064 ± 0.001	0.045 ± 0.001	X	0.053 ± 0.021	0.053 ± 0.018	0.067 ± 0.023
11 Eastern Lake Erie	0.026 ± 0.001	0.023 ± 0.001	0.037 ± 0.001	X	0.034 ± 0.012	0.035 ± 0.011
12 Western Lake Ontario	0.062 ± 0.001	0.047 ± 0.001	0.038 ± 0.001	0.027 ± 0.001	X	0.057 ± 0.019
13 Eastern Lake Ontario	0.035 ± 0.001	0.037 ± 0.001	0.042 ± 0.001	0.023 ± 0.001	0.040 ± 0.001	X

0.77. The g^{-1} skewness for the distribution of 105 trees was -0.47 . These relationships show that the population from the Volga River, eastern Europe was the most divergent, forming the sister group to samples from central and western Europe. Zebra mussels from the Dnieper River, Ukraine then comprised the sister taxon to the locations to the west. The northernmost sites from the Netherlands and Poland were most closely related. This northern clade formed the sister group to the clade from central Europe that included the Rhine and Danube Rivers. However, the Danube River population was quite genetically divergent from that in the Rhine River, as well as from the sites in the Netherlands and Poland in NJ trees based on the genetic distances and the allelic frequencies (Fig. 3, Table 2A).

Comparisons between the Eurasian and North American sites for *D. polymorpha*

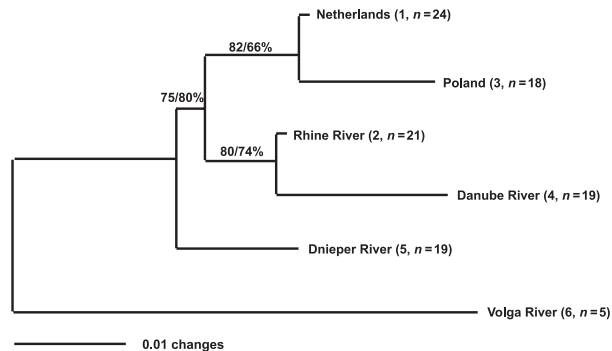
A single most-parsimonious tree was obtained from the MP search, which was congruent with the relationships depicted by the NJ trees. The most-parsimonious tree had a consistency index excluding uninformative characters of 0.49 and a length of 49 steps. The Volga and Dnieper Rivers were the most divergent from the other locations, and were very different from the North American populations (Table 2A, Fig. 4). The Hudson River sample fell outside the clade containing the remaining North American populations (clade c on Fig. 4), however, genetic distances (Table 2A) appeared to link it closer to the Rhine River location. The discrepancy apparently is because of its closer genetic similarity to the population from Lake Superior, than to other North American locations.

There were two primary clades for the remaining samples (a and b on Fig. 4), with group a being most closely related to founding sources from northern Europe (the Netherlands and Poland locations) and group b from central Europe (the Rhine River). In clade a, zebra mussels from Lake Superior appeared to be closest to a possible founding source near the Rhine River. The sample from the lower Mississippi River also resembled the Rhine River, but was considerably divergent from the Lake Superior sample (Fig. 4).

Samples from the Great Lakes region other than Lake Superior and the Hudson River clustered together in clade a (Fig. 4). Zebra mussels from Lakes Huron and Erie appeared most similar to founding sources from Poland, the Netherlands and the Rhine River, according to the genetic distances (Table 2A). The phylogenetic tree places the populations from Lakes Huron and Erie between the potential Eurasian founding sources of the Netherlands and Poland, and outside the clade containing Lake Ontario and the St Lawrence River. Samples from Lake Ontario and the St Lawrence River were very similar to each other and to a potential founding population from Poland (Fig. 4).

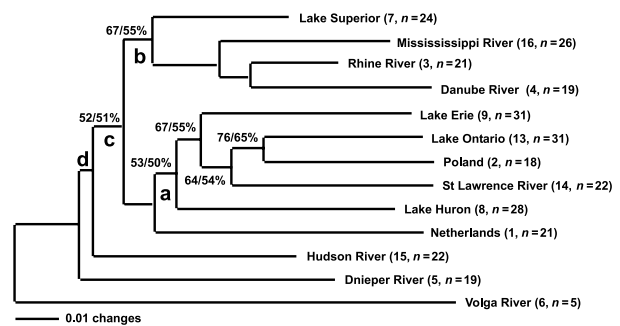
Table 3 Comparisons of measures of genetic variation \pm SE within and between North American and Eurasian population sampling sites for the zebra mussel *Dreissena polymorpha* (A) and the quagga mussel *D. bugensis* (B).

Genetic variation measures	North American populations	Eurasian populations	Between North America and Eurasia	Overall
<i>(A) D. polymorpha</i>				
<i>Ht</i> (total variation)	0.207 \pm 0.003	0.211 \pm 0.003	0.198 \pm 0.003	0.211 \pm 0.002
<i>Hc</i> (variation between groups)	–	–	0.197 \pm 0.002	0.180 \pm 0.001
<i>Hs</i> (variation within sites)	0.188 \pm 0.002	0.171 \pm 0.002	0.197 \pm 0.002	0.209 \pm 0.003
G_{ST} (divergence among sites)	0.095 \pm 0.009	0.189 \pm 0.020	0.065 \pm 0.022	0.070 \pm 0.032
<i>Nm</i> (migration)	4.78 \pm 1.87	2.15 \pm 0.40	3.46 \pm 0.19	5.66 \pm 0.51
Total <i>N</i> of significantly different loci among sites and pairwise mean	20, \bar{X} = 5.6	20, \bar{X} = 7.0	11, \bar{X} = 6.5	20, \bar{X} = 6.4
Mean Nei's (1972) <i>D</i>	0.029 \pm 0.012	0.060 \pm 0.026	0.032 \pm 0.024	0.032 \pm 0.023
<i>(B) D. bugensis</i>				
<i>Ht</i> (total variation)	0.257 \pm 0.052	0.269 \pm 0.052	0.265 \pm 0.050	0.265 \pm 0.051
<i>Hc</i> (variation between groups)	–	–	0.241 \pm 0.047	0.241 \pm 0.003
<i>Hs</i> (variation within sites)	0.236 \pm 0.049	0.269 \pm 0.052	0.259 \pm 0.049	0.241 \pm 0.048
G_{ST} (divergence among sites)	0.084 \pm 0.036	–	0.021 \pm 0.013	0.089 \pm 0.034
<i>Nm</i> (migration)	5.407 \pm 0.697	–	8.674 \pm 0.744	5.106 \pm 0.450
Total <i>N</i> of significantly different loci among sites and pairwise mean	20, \bar{X} = 3.6	–	11, \bar{X} = 5.2	22, \bar{X} = 4.1
Mean Nei's (1972) <i>D</i>	0.037 \pm 0.013	–	0.035 \pm 0.018	0.037 \pm 0.015

**Fig. 3** Neighbour-joining tree (from MEGA Kumar *et al.*, 2001 using Nei's, 1972 genetic distances) of relationships among zebra mussel *Dreissena polymorpha* populations in Eurasia. This tree was analogous to the most-parsimonious maximum parsimony tree and the neighbour-joining tree from allelic frequencies in PAUP* 4.0b5 (Swofford, 2001) analyses. Numbers beside location names refer to the map of Fig. 1. Bootstrap support values above 50% from 1000 replications are indicated at nodes, with those from neighbour-joining above the slash, and those from maximum parsimony below.

Genetic relationships among North American sampling sites for *D. polymorpha*

Genetic distance comparisons within North America (Table 2A) revealed that samples from the Lake Superior location appeared most similar to those from the Hudson and Mississippi Rivers. Zebra mussels from Lake Huron more closely resembled those from Lake Superior. Samples from Lake Erie were most like those from Lake Huron, Lake Ontario and the St Lawrence River. Samples

**Fig. 4** Neighbour-joining tree and most-parsimonious maximum parsimony tree from PAUP* 4.0b5 (Swofford, 2001) analyses showing relationships among zebra mussel *Dreissena polymorpha* populations from North America and Eurasia. This tree had congruent relationships to the neighbour-joining tree from Nei's (1972) genetic distances in a MEGA analysis (Kumar *et al.*, 2001). Numbers beside location names refer to the maps of Figs 1 and 2. Nodes discussed in text are lettered 'a' through 'd'. Bootstrap support values above 50% from 1000 replications are indicated at nodes, with those from neighbour-joining above the slash, and those from maximum parsimony below.

from eastern Lake Ontario and the St Lawrence River appeared very similar (Table 2A).

Population genetic variability in *D. bugensis*

The number of individuals having dominant vs. homozygous recessive RAPD alleles per population site varied significantly at 12 loci. The proportion of polymorphic loci per sampling site was higher in the quagga mussel

than in the zebra mussel. The number of polymorphic loci for *D. bugensis* ranged from 84.6% in central Lake Erie to 98.1% in eastern Lake Erie, and was similar in the native Dnieper River, Ukraine site (96.2%) (Table 1B). Nei's (1972) heterozygosity estimates were also higher in the quagga mussel populations, ranging from $H = 0.19 \pm 0.02$ in western Lake Erie to 0.28 ± 0.2 in western Lake Ontario, and averaged 0.24 per sampling site. Populations from North America and Eurasia exhibited similar levels of genetic variability (Table 1B), as found for the zebra mussel. Estimates of nucleotide diversity (π) from the RAPDs data ranged from 0.009 to 0.017 for *D. bugensis*, and averaged 0.015 (Table 1B). The quagga mussel thus was somewhat more genetically variable than the zebra mussel.

Genetic divergence patterns among populations of *D. bugensis*

Pairs of quagga mussel sampling sites significantly differed at two (e.g. between sites in the Dnieper River and western Lake Erie, between central and eastern Lake Erie, and between eastern Lake Erie and Lake Ontario and the St Lawrence River) to nine loci (between the Dnieper River and central Lake Erie). Quagga mussels from locations in North America diverged at an average of 3.6 loci and differed from Dnieper River specimens by a mean of 5.2 loci.

Nei's (1972) genetic distances between sites are given in Table 2B (below the diagonal), ranging from $D = 0.023$ between western and eastern Lake Erie and between eastern Lake Erie and eastern Lake Ontario to 0.064 between the Dnieper River and central Lake Erie. F_{ST} values (Table 2B, above the diagonal) for these locations ranged from 0.034 to 0.087. Comparisons between samples of quagga mussels revealed moderate genetic divergences compared with values for the zebra

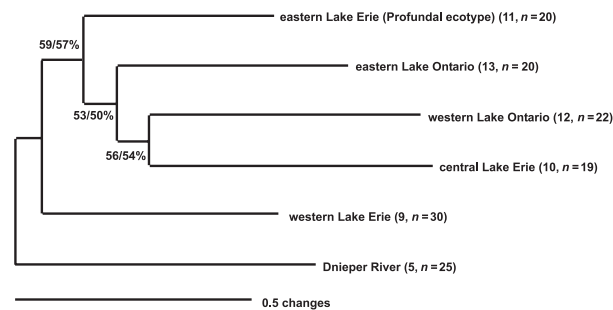


Fig. 5 Neighbour-joining and most-parsimonious maximum parsimony tree from PAUP* 4.0b5 (Swofford, 2001) analyses showing relationships among quagga mussel *Dreissena bugensis* populations. Numbers beside location names refer to the maps of Figs 1 and 2. Bootstrap support values above 50% from 1000 replications are indicated at nodes, with those from neighbour-joining above the slash, and those from maximum parsimony below.

mussel (i.e. the most similar sites were more distantly related and the least similar sites were more closely related).

The topology and relationships depicted by the NJ and MP trees were identical and are shown in Fig. 5. A single most-parsimonious tree was obtained from an exhaustive MP search, which had a length of 197 steps and a consistency index of 0.71 excluding uninformative characters. The frequency index of tree scores from 105 trees was significantly skewed, with a g_1 of -0.014 . The phylogenetic tree showed that the Dnieper River population was most similar to the sample from western Lake Erie (Fig. 5). The patterning among populations did not exhibit an isolation-by-distance correspondence, with samples from western and eastern Lake Erie (profundal variant) appearing more closely related than either was to central Lake Erie. The central Lake Erie and western Lake Ontario populations were more similar genetically (Fig. 5).

Shallow and deep ecophenotypes of *D. bugensis*

Samples of the profundal deep-water variant of *D. bugensis* from eastern Lake Erie were not significantly divergent from shallow water *D. bugensis* (Table 2B, Fig. 4). The eastern Lake Erie samples were more similar to those from western Lake Erie than to quagga mussels from central Lake Erie (Fig. 4).

Comparison of nonindigenous and native population sites for *D. bugensis*

The single Eurasian sampling location from the Dnieper River, Ukraine housed slightly greater heterozygosity than did the North American sites (Table 3B). The mean genetic distance separating the North American sites of the quagga mussel (Table 3B) was greater than that for the zebra mussel (Table 3A), despite their being more closely spaced. F_{ST} values among the North American sites were somewhat less for the quagga than for the zebra mussel, and migration values were slightly greater. This may reflect the smaller geographical range of the quagga mussel and our more closely spaced sampling locations in the Great Lakes (Fig. 2).

Discussion

Divergences among population sites of dreissenid mussels

The present study discerned significant genetic divergences among samples of both zebra and quagga mussels across their native and invasive ranges. The distribution of RAPD alleles at many loci significantly differed among sampling locations, and F_{ST} and Nei's (1972) genetic distance (D) values were relatively high for both species (Nei, 1987; Hartl, 2000). Zebra mussels markedly

diverged among Eurasian sites, reaching a level of great genetic differentiation between the Volga River and the location in Poland, according to guidelines for the interpretation of F_{ST} by Hartl (2000). In comparison, locations in North America showed moderate levels of genetic divergence. This difference was predictable because of the greater evolutionary times for the evolution of population divergences in their native distribution. Differences between western and eastern European locations for the zebra mussel likely arose during their isolation in separate glacial refugia (see Stepien *et al.*, 1998; Hewitt, 2000). Anthropogenetic factors, including shipping and the construction of canals, led to the later mixing of some populations in north-western Europe (Morton, 1993).

An allozyme study of zebra mussels by Marsden *et al.* (1995) based on 15 polymorphic loci detected relatively high genetic diversity but less divergence among sample sites within both Europe and North America than was found in the present study. The discrepancy between the allozyme study and the present results may be because of the lower number of polymorphic loci sampled in the former, and merits further investigation.

Differentiation levels among samples of quagga mussels from F_{ST} estimates in our study ranged from small (0.034) to moderate (0.089), according to guidelines described by Hartl (2000). Samples diverged by moderate levels of mean genetic differentiation within North America (0.084) and showed little difference between the sites in the native Ukraine vs. North America (0.021). Divergence levels were higher in the zebra mussel, averaging moderate differentiation among sites in North America (0.095) and great differentiation in Eurasia (0.189).

Genetic diversity of invasive vs. native population sites

Overall levels of genetic variability of exotic zebra mussel populations in North America were similar to those in the Eurasian population areas surveyed. Our results suggest that there were multiple founding source populations and/or that founding events comprised a large number of individuals. High shipping traffic in the Great Lakes to and from foreign ports likely would facilitate the transport and dispersal of multiple source populations via frequent ballast water exchanges in single vessels, as well as among different vessels (Mills *et al.*, 1993; Ricciardi & MacIsaac, 2000).

Levels of genetic variability also were high in the quagga mussel, and were slightly less in North America than in the native Ukrainian population site. This finding contrasted with an earlier allozyme study by Marsden *et al.* (1995), who found that the quagga mussel was less genetically variable than the zebra mussel. The disparity may be because of differences

in allozymes vs. RAPDs loci, but alternatively, may indicate that the populations in North America experienced additional founding events (since the time of the Marsden samples vs. the present study) and/or are undergoing rapid selection. A study of genotype frequencies during the life history of the zebra mussel by Haag & Garton (1995) found significant differences at an allozyme locus at the western Lake Erie location examined in our study, implicating selection. As found in our study of dreissenids, mtDNA control region sequences of the round goby *Neogobius melanostomus* invasion in the Great Lakes by Dillon & Stepien (2001) revealed considerable genetic variability (and significant differences among sampling sites), with levels about half that of a native population in the Black Sea. Our laboratory is presently testing whether the genetic compositions of dreissenid and goby populations have changed over the time course of their invasions.

Estimated nucleotide diversity (π) from the RAPDs data averaged 0.012 for populations of zebra mussels and 0.015 for quagga mussels, placing them near the upper range of eukaryotes (Borowsky, 2001). It is possible that as we selected for RAPDs primers that yielded many bands, these estimates remained biased towards the high end despite the corrections that were employed (see Methods). Zebra mussels from the Volga River site in eastern Europe had the greatest genetic diversity and appeared the most genetically differentiated from other populations, but were not found as divergent using allozymes (Marsden *et al.*, 1995). As our sample size was smaller than those for the other locations, this may be the result of error; however, the Volga River site in eastern Europe may be geographically closer to the ancestral location for the species, which would be predicated to house the most variation (Avice, 2000).

Genetic divergences among North American sites were appreciable, and were about half the level of those among Eurasian locations. Greater geographical distances separated the Eurasian samples, which had considerably longer times to evolve in isolated river systems. The significant differences among sites in North America suggest that there were multiple founding sources for the invasions. In addition, it is possible either that there are some barriers to dispersal and gene flow, or that selection has occurred – as was found for life history stages of zebra mussels (Haag & Garton, 1995). A recent paper by Hewitt (2000) postulated that the dynamics of colonization would remodel the colonizing population's genetic architecture and compound divergence between population genomes. An investigation of the round goby *N. melanostomus* invasion of the Great Lakes by Dillon & Stepien (2001) similarly found considerable genetic differentiation among sampling sites in the lower Great Lakes, which appears analogous to our findings here for dreissenid mussels.

Possible Eurasian founding sources for *D. polymorpha* in North America

The invasion of *D. polymorpha* in North America appears to have been founded from multiple population sources in north-western and northcentral Europe. The closest genetic matches were with zebra mussels from the Netherlands, the Rhine River and Poland. In contrast Marsden *et al.* (1995) found greater allozymic similarity among all North American locations than was found in the present study. In our study, samples from central, southern and eastern Europe encompassing the Danube, Dnieper and Volga Rivers, were very divergent from zebra mussels in North America, suggesting that they did not found the invasive populations. Smirnova *et al.* (1993) analysed the shell coloration patterns of *D. polymorpha* and concluded that the Volga River was not the colonizing source for North America, which is supported by the present results.

Divergence among sampling sites in North America and their differential close relationships to some of the northern and northcentral western European sites support the multiple founding source hypothesis. Lake Superior had the closest genetic correspondence to founding population from the Rhine River. Sampling locations in Lakes Huron and Erie appeared to be intermediately related to those from the Netherlands and Poland. Zebra mussels from Lake Ontario and the St Lawrence River showed clear closest genetic relationship to the sample from Poland. A sample from the southern Mississippi River had closest locus frequency relationships to a possible founding source from the vicinity of the Rhine River. Zebra mussels from the Hudson River showed a less clear relationship, appearing most similar to the Rhine River and the Netherlands sites, but were markedly divergent from all North American population locations except Lake Superior.

Genetic divergences among populations of *D. polymorpha* and *D. bugensis*

Grant's (1987) molecular clock calibration for Nei's (1972) *D* suggests that the average population differentiation for the zebra mussel in Eurasia is about $316\,000 \pm 137\,000$ years, congruent with the hypothesis of Pleistocene divergences among glacial refugia. The Volga River sample of *D. polymorpha* differed from other sites by about 532 000 years, analogous to the primary population divergence of the ruffe *Gymnocephalus cernuus* from eastern vs. western Europe (Stepien *et al.*, 1998; Stepien & Dillon, 2002). This estimate of half a million years corresponds to the longest prolonged cold period in the European Pleistocene epoch (Hewitt, 1996). The Dnieper River population of zebra mussels diverged from other locations by an average of 258 000 years and the Danube River by about 221 000 years, reflecting their more intermediate locations.

Sampling sites for *D. bugensis* appear diverged by an estimated mean of $221\,000 \pm 78\,000$ years, suggesting their differentiation during a similar time period (despite their more circumscribed range in Europe) and, again, that there was likely more than one founding source for North America. Sites in North America showed some significant differences in allelic frequencies and did not correspond to an isolation-by-distance pattern, supporting the multiple colonization hypothesis, or alternatively, selection. Similarly, Wilson *et al.* (1999) found significant differences among sites in North America for quagga mussels from six polymorphic microsatellite markers that did not correspond to an isolation-by-distance pattern.

Relationship of shallow-water *D. bugensis* and the profundal ecophenotype

The divergences distinguishing the profundal ecophenotype (eastern Lake Erie site) from the shallow water quagga mussel samples in the Great Lakes were not significant, as in an allozyme study by Spidle *et al.* (1994). The profundal ecophenotype thus does not appear genetically different from the shallow ecotype and is not a separate species or a unique population. The native sampling location in the Dnieper River appeared most closely related to invasive samples from western and eastern Lake Erie, and was more divergent from those in Lake Ontario and central Lake Erie. The Lake Ontario and central Lake Erie sites may have been founded by one or more different sources, or alternatively, these differences may be the result of selection. Additional samples from other areas in the relatively restricted native range of *D. bugensis* in the Ukraine (Fig. 1) are necessary to address these hypotheses. Samples of the zebra mussel from Lakes Erie and Ontario also were significantly divergent, mirroring the quagga mussel. Thus, populations of each species in the two lakes and different lake basins may have had separate founding sources.

Conclusions

The primary differentiations of *D. polymorpha* populations in Eurasia appear to have resulted from periods of divergence during isolation in glacial refugia and subsequent restricted colonization patterns (Hewitt, 1996, 2000), as characterize the phylogeographical patterns of other temperate aquatic taxa (Banarescu, 1992; Stepien & Dillon, 2002). Both *D. polymorpha* and *D. bugensis* have high genetic variability in native and introduced populations, an attribute that may aid colonization success (Nevo, 1983; Williamson, 1996). Genetic relationships among sampling areas suggest that North America was colonized in the mid-1980s to early 1990s by a variety of genotypes of *D. polymorpha* originating from several locations in north-western and northcentral Europe. Local exotic populations display a relatively high degree

of genetic divergence from each other, and it will be interesting for future investigations to determine whether their genetic compositions remain stable over time. The 'ecological facilitation' hypothesis of Simberloff & Von Holle (1999) suggests that the establishment of the zebra mussel in the North American Great Lakes may have aided other coevolved invaders, such as the quagga mussel and the round goby. Recent studies show that *D. bugensis* now is replacing *D. polymorpha* in the lower Great Lakes (Berkman *et al.*, 2000; Jarvis *et al.*, 2000), and it may be out competing the latter. Ecological studies, coupled with morphological and genetic fitness measures, may address the question of whether and how such facilitation occurs. Genetic studies in the future should be 'on the lookout' for colonizations by other species in the genus *Dreissena*. Molecular genetics offer tools for understanding the dynamics of phylogeographical restructuring in an invasive community. Genetic diversity and divergence likely play important roles in the ecological sweepstakes governing the establishment and persistence of exotic species.

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References

Avice, J.C. 2000. *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, MA.

- Baldwin, B.S., Black, M., Sanjur, O., Gustafson, R., Lutz, R.A. & Vrijenhoek, R.C. 1996. A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial COI. *Mol. Mar. Biol. Biotechnol.* **5**: 9–14.
- Banarescu, P. 1992. *Zoogeography of Fresh Waters*, Vol. 2. *Distribution and Dispersal of Freshwater Animals in North America and Eurasia*. AULA-Verlag, Weisbaden, Germany.
- Berkman, P.A., Garton, D.W., Haltuch, M.A., Kennedy, G.W. & Febo, L.R. 2000. Habitat shift in invading species: Zebra and quagga mussel population characteristics on shallow soft substrates. *Biol. Invas.* **2**: 1–6.
- Borowsky, R.L. 2001. Estimating nucleotide diversity from random amplified polymorphic DNA amplified fragment length polymorphism data. *Mol. Phyl. Evol.* **18**: 43–148.
- Claxton, W.T., Martel, A., Dermott, R.M. & Boulding, E.G. 1997. Discrimination of field-collected juveniles of two introduced dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) using mitochondrial DNA and shell morphology. *Can. J. Fish. Aquat. Sci.* **54**: 1280–1288.
- Dermott, R. & Munawar, M. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. *Can. J. Fish. Aquat. Sci.* **50**: 2298–2304.
- Dillon, A.K. & Stepien, C.A. 2001. Genetic and biogeographic relationships of the invasive round (*Neogobius melanostomus*) and tubenose (*Proterorhinus marmoratus*) gobies in the Great Lakes versus Eurasian populations. *J. Great Lakes Res.* **27**: 267–280.
- Everett, R.A. 2000. Patterns and pathways of biological invasions. *TREE* **15**: 177–178.
- Grant, W.S. 1987. Genetic divergence between congeneric Atlantic and Pacific Ocean fishes. In: *Population Genetics and Fishery Management* (N. Ryman & F. Utter, eds), pp. 225–246. University of Washington Press, Seattle, WA.
- Haag, W.R. & Garton, D.W. 1995. Variation in genotype frequencies during the life history of the bivalve, *Dreissena polymorpha*. *Evolution* **49**: 1284–1288.
- Haig, S.M., Rhymer, J.M. & Heckel, D.G. 1994. Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. *Mol. Ecol.* **3**: 581–595.
- Hartl, D.L. 2000. A primer of population. *Genetics*, 3rd edn. Sinauer Associates, Inc., Sunderland, MA.
- Hebert, P.D.N., Muncaster, B.W. & Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* **43**: 1587–1591.
- Hewitt, G.M. 1996. Some genetic consequences of Ice Ages, and their role in divergence and speciation. *Biol. J. Linn. Soc. Lond.* **58**: 247–272.
- Hewitt, G.M. 2000. The genetic legacy of the quaternary ice ages. *Nature* **405**: 907–913.
- Hewitt, G.M. 2001. Speciation, hybrid zones and phylogeography- or seeing genes in space and time. *Mol. Ecol.* **10**: 547–549.
- Holland, B.S. 2000. Genetics of marine bioinvasions. *Hydrobiologia*. **420**: 63–71.
- Jarvis, P., Dow, J., Dermott, R. & Bonnell, R. 2000. Zebra (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) distribution and density in Lake Erie, 1992–1998. *Can. Tech. Rept. Fish. Aquat. Sci.* **2304**: 1–46.
- Kinzelbach, R. 1992. The main features of the phylogeny and dispersal of the zebra mussel *Dreissena polymorpha*. In: *The*

- Zebra Mussel Dreissena polymorpha* (D. Neumann & H. A. Jenner, eds), pp. 5–17. Gustav Fisher Verlag, New York.
- Kumar, S., Tamura, K., Jacobson, I.B. & Nei, M. 2001. *MEGA: Molecular Evolutionary Genetics Analysis*, version. 1.02. Arizona State University, Tempe, AZ
- Marsden, J.E., Spidle, A.P. & May, B. 1995. Genetic similarity among zebra mussel populations within North America and Europe. *Can. J. Fish. Aquat. Sci.* **52**: 836–847.
- Marsden, J.E., Spidle, A.P. & May, B. 1996. Review of genetic studies of *Dreissena* spp. *Am. Zool.* **36**: 259–270.
- May, B. & Marsden, J.E. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. *Can. J. Fish. Aquat. Sci.* **49**: 1501–1506.
- Mills, E.L., Leach, J.H., Carlton, J.T. & Secor, C.L. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. *J. Great Lakes Res.* **19**: 1–54.
- Minchin, D. & Moriarty, C. 1997. Distribution of the zebra mussel *Dreissena polymorpha* (Pallas) in Ireland, 1997. *Irish Naturalists' J.* **26**: 38–42.
- Morton, B. 1993. The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenoida. In: *Zebra Mussels: Biology, Impacts, and Control* (T. F. Nalepa & D. W. Schloesser, eds), pp. 185–215. Lewis Publishers, Boca Raton, FL.
- Morton, B. 1996. The aquatic nuisance species problem: a global perspective and review. In: *Zebra Mussels and Other Aquatic Nuisance Species* (F. D'Itri, ed.), pp. 1–69. Ann Arbor Press Inc., Ann Arbor, MI.
- Mullis, K.B. & Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase chain reaction. *Meth. Enzymol.* **155**: 335–350.
- Nalepa, T.F., Schloesser, D.W., Pothover, S., Hondorp, D., Fanslow, D.L., Tuchman, M. & Fleischer, G. 2001. First finding of the amphipod *Echinogammarus ischnus* and the mussel *Dreissena bugensis*. Lake Michigan. *J. Great Lakes Res.* **27**: 384–391.
- National Zebra Mussel and Aquatic Nuisance Species Clearinghouse 2001. *New York Sea Grant Extension Program*. [WWW document]. URL <http://cce.cornell.edu/seagrant/nansc/zmaps.html>
- Nei, M. 1972. Genetic distance between populations. *Am. Nat.* **106**: 283–292.
- Nei, M. 1987. Genetic distance and molecular phylogeny. In: *Population Genetics and Fishery Management* (N. Ryman & F. Utter, eds), pp. 193–223. University of Washington Press, Seattle, WA.
- Nei, M. & Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Nat. Acad. Sci. USA.* **76**: 5269–5273.
- Nevo, E. 1983. Adaptive significance of protein variation. In: *Protein Polymorphism: Adaptive and taxonomic Significance* (G. S. Oxford & D. Rollinson, eds), pp. 239–282. Academic Press, New York.
- Palumbi, S.R. 1996. Molecular systematics: context and controversies. In: *Molecular Systematics* (D. M. Hillis, C. Moritz & B. K. Mable, eds), pp. 218–219. Sinauer Associates, Inc, Sunderland, MA.
- Pan, Y.B., Burner, D.M., Ehrlich, K.C., Grisham, M.P. & Wei, Q. 1997. Analysis of primer-derived, nonspecific amplification products in RAPD-PCR. *Biotechniques* **22**: 1071–1077.
- Ricciardi, A. & MacIsaac, H.J. 2000. Recent mass invasion of the North American Great lakes by Ponto-Caspian species. *TREE* **15**: 62–65.
- Rosenberg, G. & Ludyanskiy, M.L. 1994. A nomenclatural review of *Dreissena* (Bivalvia: Dreissenidae), with identification of the quagga mussel as *Dreissena bugensis*. *Can. J. Fish. Aquat. Sci.* **51**: 1474–1484.
- Ross, K.G., Shoemaker, D.D., Krieger, M.J.B., DeHeer, C.J. & Keller, L. 1999. Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Mol. Biol. Evol.* **16**: 525–543.
- Ross, K.G., Vargo, E.L. & Keller, L. 1996. Social evolution in a new environment: the case of introduced fire ants. *Proc. Nat. Acad. Sci. USA* **93**: 3021–3025.
- Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstruction phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
- Simberloff, D. & Von Holle, B. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invas.* **1**: 21–32.
- Smirnova, N.R., Biochino, G.I. & Vinogradov, G.A. 1993. Some aspects of the zebra mussel (*Dreissena polymorpha*) in the former European USSR with morphological comparisons to Lake Erie. In: *Zebra Mussels: Biology, Impacts, and Control* (T. F. Nalepa & D. W. Schloesser, eds), pp. 217–226. Lewis Publishers, Boca Raton, FL.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. W.H. Freeman, Inc., New York.
- Spidle, A., Marsden, J.E. & May, B. 1994. Identification of the Great Lakes quagga mussel as *Dreissena bugensis* from the Dnieper River, Ukraine, on the basis of allozyme variation. *Can. J. Fish. Aquat. Sci.* **51**: 1485–1489.
- Stepien, C.A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. In: *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*. American Fisheries Society Symposium 17 (J. L. Nielsen, ed.), pp. 263–287. American Fisheries Society, Bethesda, MD.
- Stepien, C.A. & Dillon, A.K. 2002. Phylogeographic patterns, glacial refugia, and nonindigenous population origins of the ruffe *Gymnocephalus cernuus* from mitochondrial and nuclear DNA sequences. *Molecular Ecology*, (in review).
- Stepien, C.A., Dillon, A.K. & Chandler, M.D. 1998. Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* the North American Great Lakes and Eurasia. *J. Great Lakes Res.* **24**: 361–378.
- Stepien, C.A., Hubers, A.N. & Skidmore, J.L. 1999. Diagnostic genetic markers and evolutionary relationships among invasive dreissenoid and corbiculoid bivalves in North America: Phylogenetic signal from mitochondria 16S rDNA. *Mol. Phyl. Evol.* **13**: 31–49.
- Stewart, T.W. & Haynes, J.M. 1994. Benthic macroinvertebrate communities of southwestern Lake Ontario following invasion of *Dreissena*. *J. Great Lakes Res.* **20**: 479–493.
- Swofford, D.L. 2001. *PAUP (Phylogenetic Analysis Using Parsimony) vers. 4.0b5*. Sinauer Associates, Sunderland, MA.
- Swofford, D.L. & Olse, S.H. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. *Syst. Zool.* **36**: 293–325.
- Swofford, D.L., Olsen, G.J., Wadell, P.J. & Hillis, D.M. 1996. Phylogenetic Inference. In: *Molecular Systematics* (D. M. Hillis,

- C. Moritz & B. K. Mable, eds), pp. 407–514. Sinauer Associates, Sunderland, MA.
- Tsutsui, N.D. & Case, T.J. 2001. Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution* **55**: 976–985.
- Valovirta, I. & Porkka, M. 1996. The distribution and abundance of *Dreissena polymorpha* (Pallas). *Mem. Soc. Pro Fauna Flora Fenn.* **2**: 63–78.
- Welsh, J. & McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* **18**: 7213–7218.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* **18**: 6531–6535.
- Williams, J.G.K., Hanafey, M.K., Rafalski, J.A. & Tingey, S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Meth. Enzymol.* **218**: 704–740.
- Williamson, M. 1996. *Biological Invasions*. Chapman & Hall, New York.
- Wilson, A.B., Naish, K.-A. & Boulding, E.J. 1999. Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis. *Can. J. Fish. Aquat. Sci.* **56**: 2248–2261.
- Yeh, F.C. & Yang, R.-C. 1997. *POPGENE, Version 1.21*. University of Alberta, Alberta, Canada.

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