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Genetic similarity among zebra mussel populations within North America and Europe

J. Ellen Marsden, Adrian Spidle, and Bernie May

Abstract: The zebra mussel *Dreissena polymorpha* has rapidly established both contiguous and disjunct populations during its spread through eastern North America. If new colonies are founded by small numbers of individuals, populations with markedly different genetic and phenotypic characters could arise (founder effect); this possibility could confound ecological comparisons of populations from different geographic locations. We analyzed genetic differentiation among 18 populations of mussels from the Great Lakes and seven populations from Europe using starch-gel electrophoresis. Analysis of 27 proteins yielded data from 15 polymorphic loci and one monomorphic locus. The data indicated that zebra mussels in North America have the same high genetic variability ($H_o = 0.30-0.43$) that is found in European populations ($H_o = 0.27-0.35$) and is typical of molluscs. Little variation appears to have been lost when zebra mussel were transported to North America. Nei's genetic distances between populations were small (0.004-0.028) compared with distances among populations of other mollusc species (0.023-0.181). Like populations from Europe, populations within North America were not highly differentiated, which suggests that founder populations have not been small and (or) frequent genetic mixing has occurred. European populations clustered separately from North American populations (Nei's distance = 0.058).

Résumé : La moule zébrée (*Dreissena polymorpha*) a rapidement constitué des populations contiguës et isolées, au cours de sa propagation dans l'est de l'Amérique du Nord. Si de nouvelles colonies sont fondées par de petites quantités d'individus, des populations présentant des caractères génétiques et phénotypiques radicalement différentes pourraient surgir (effet fondateur) ce phénomène pourrait confondre les comparaisons écologiques de populations provenant de différents secteurs géographiques. Nous avons analysé la différenciation génétique chez 18 populations de moules des Grands Lacs et de 7 populations d'Europe, par électrophorèse sur gel d'amidon. L'analyse de 27 protéines a fourni des données de 15 sites polymorphes et d'un site monomorphique. Ces données indiquent que la moule zébrée en Amérique du Nord présente la même variabilité génétique ($H_o = 0.30-0.43$) que les populations européennes ($H_o = 0.27-0.35$), laquelle est typique des mollusques. La variabilité perdue lors du transport des moules en Amérique du Nord semble minime. Les distances génétiques de Nei entre les populations étaient modestes (0,004-0,028), quand on les compare aux distances constatées chez les populations d'autres espèces de mollusques (0,023-0,184). Comme pour les populations européennes, les populations nord-américaines n'étaient pas hautement différenciées, ce qui laisse à penser que les populations fondatrices n'étaient pas petites ou encore qu'il y a eu de fréquentes combinaisons de populations. Les populations européennes étaient groupées différemment des populations nord-américaines (distances de Nei = 0,058).

[Traduit par la Rédaction]

Introduction

Zebra mussel (*Dreissena polymorpha*) populations in Europe are characterized by high variability in number, biomass, production, age structure, and individual size, weight, and color pattern (Stanczykowska 1977; Smirnova et al. 1993).

This variability is so striking that it is often difficult to find consistent agreement on the limits of life-history traits such as size and age at reproductive maturity, temperature limits for survival, and (or) growth rates. The populations that have developed in North America differ from their European counterparts in characteristics such as growth

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Table 1. Location of zebra mussel populations sampled for this study, with sample size (N) used for genetic analysis. Dates of collection are approximate, based on information from field teams.

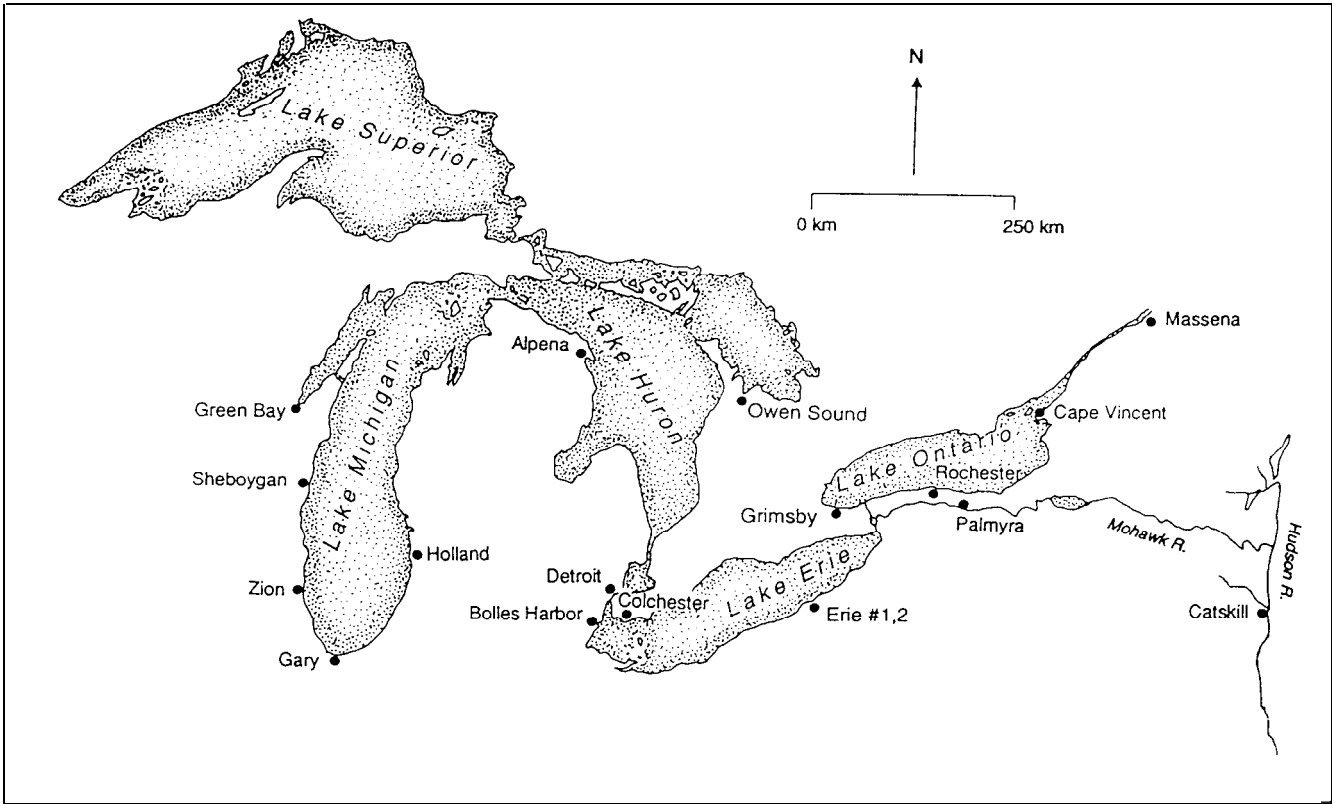
Collection date	Abbr.	Sample location	Body of water	N
June 1990	Clair	Detroit, Mich.	Lake St. Clair	45
Oct. 1991	Erie 1	Erie, Pa., inside Presque Isle	Lake Erie	80
Oct. 1991	Erie 2	Erie, Pa., outside Presque Isle	Lake Erie	40
June 1991	Boll	Bolles Harbor, Ohio	Lake Erie	40
June 1990	Colch	Colchester, Ont.	Lake Erie	43
June 1991	Grim	Grimsby, Ont., at 50-mile Point	Lake Ontario	40
Nov. 1991	Cape	Cape Vincent, N.Y., from Department of Environmental Conservation harbor	Lake Ontario	78
Nov. 1991	Roth	Rochester, N.Y., trawled approximately 2 km offshore	Lake Ontario	40
Nov. 1991	Holl	Holland, Mich., on buoy chain at mouth of L. Macatawa	Lake Michigan	80
Sept. 1991	Green	Green Bay, Wis.	Green Bay	80
Oct. 1991	Zion	Zion, Ill., at Zion-Benton Water Treatment Plant	Lake Michigan	80
Sept. 1991	Sheb	Sheboygan, Wis.	Lake Michigan	80
June 1991	Gary	Gary, Ind., at Union Carbide plant	Lake Michigan	39
July 1991	Owen	Owen Sound, Ont.	Lake Huron	80
July 1991	Alpe	Alpena, Mich.	Lake Huron	40
July 1991	Palm	Palmyra, N.Y.	Erie Canal	39
Sept. 1991	Cats	Catskill, N.Y., near mouth of Catskill Creek	Hudson River	40
Sept. 1991	Mass	Massena, N.Y.	St. Lawrence River	80
Oct. 1991	Polan	Wtoctawek Reservoir, Poland	Wtoctawek Reservoir	80
Oct. 1991	Hung	Budapest, Hungary	River Danube	40
Oct. 1991	Vuren	Vuren, Germany	River Rhine	40
Oct. 1991	Enkh	Enkhuizerzand, Netherlands	Ijsselmeer	40
Oct. 1991	Roer	Roerland, Netherlands	River Meuse	40
Oct. 1991	Peter	St. Petersburg, Russia	Neva River/Lake Ladoga	30
Oct. 1991	Volga	Russia - exact reservoir on Volga Rover unknown	Volga Reservoir	30

rate, age at reproductive maturity, and temperature tolerances (e.g., Schloesser et al. 1990). In part, these differences among zebra mussel population characteristics can be attributed to differences in their local environments. However, if North American populations of zebra mussels are genetically different from the principal populations studied in Europe, they may have different environmental tolerances. Such differences would exist if a group of founding individuals originated from an unusual population in Europe, or if its genetic character were altered owing to founder effect. Ecological studies tend not to regard genetic influences on the phenotype, i.e., for simplicity, they assume genetic uniformity among populations. However, genetically distinct subpopulations may respond differently to environmental variables as well as to control measures. Such differences could invalidate attempts to make inferences about populations. Zebra mussels have unusually high levels of genetic and morphological variability (Hebert et al. 1989; May and Marsden 1992; Boileau and Hebert 1993). Consequently, there may be large differences in the genetic and, therefore, phenotypic characters among zebra mussel populations. A prime example of this phenomenon was the recognition during this study of a second dreissenid species in the Great Lakes that we termed the quagga mussel (May and Marsden 1992) and that was subsequently

identified as *D. bugensis* (Rosenberg and Ludyanskiy 1994; Spidle et al. 1994).

The purpose of this study was to intensively and extensively examine the genetic structure of zebra mussel populations in North America using starch-gel electrophoresis, and to compare these populations with seven populations of mussels collected in Europe. Three alternate hypotheses are proposed. H_1 : the mussels represent a single panmictic population across the Great Lakes. In this case, phenotypic differences found between populations will be due to ecological rather than genetic effects. H_2 : the mussels have subdivided into genetically distinct subpopulations, as they spread across the Great Lakes, owing to founder effect and microevolution. Differences would be due to alterations in allelic frequencies among populations. H_3 : some populations are genetically distinct and may represent separate introductions from genetically distinct populations in Europe. Distinct introductions might be detectable by the presence of unique alleles in addition to widely differing allelic frequencies. Under H_2 , identification of source populations in Europe is important to understand and predict zebra mussel population behavior in North America. Under either H_2 or H_3 , researchers must consider genetic differences when generalizing observations from single populations to the zebra mussels in North America as a whole.

Fig. 1. Locations where zebra mussels were sampled in North America from 1990 to 1991



Methods

Sample collection

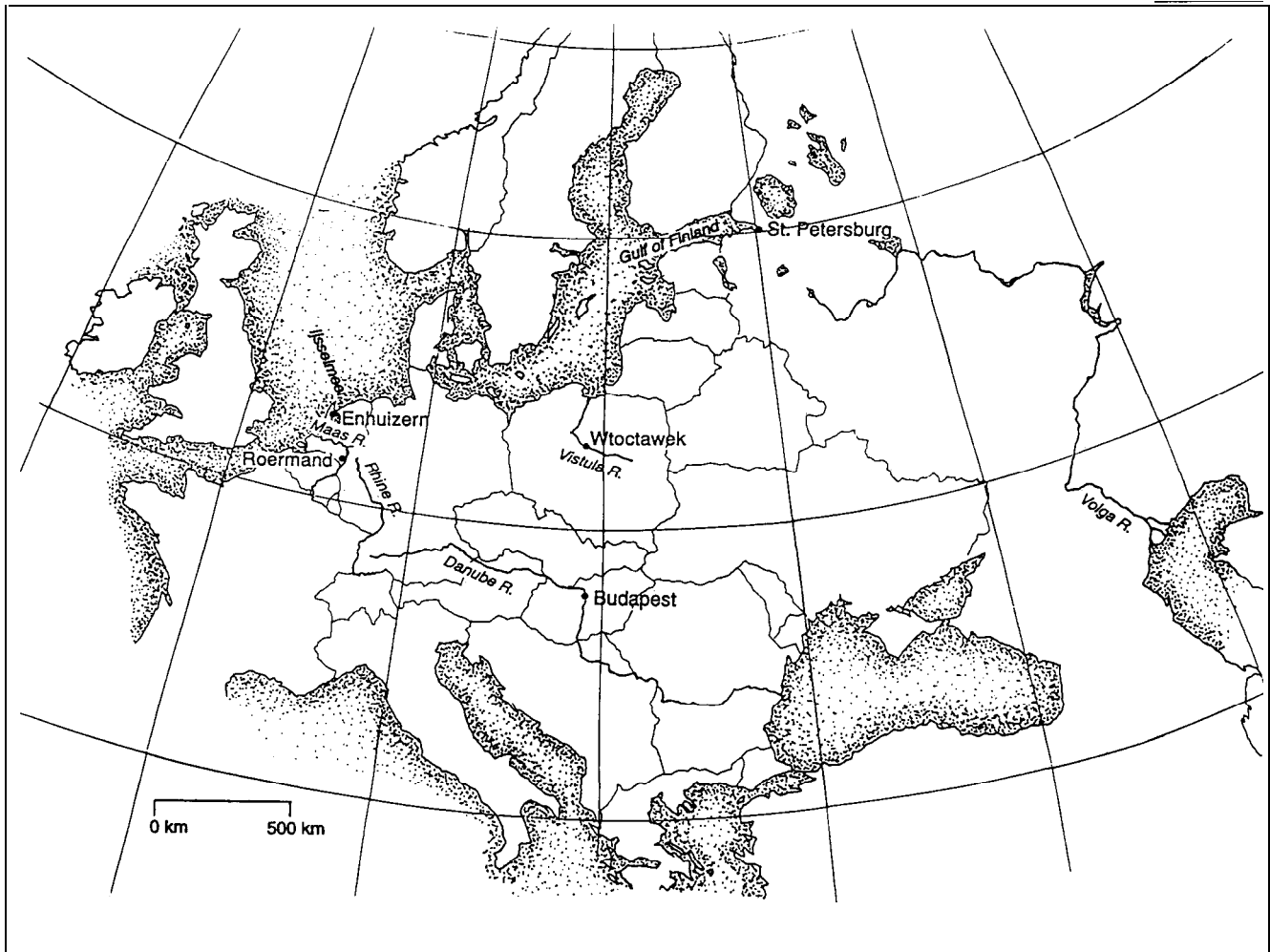
Up to several hundred zebra mussels were collected from a total of 18 locations in lakes Erie, Ontario, Huron, Michigan, and St. Clair, the Hudson and St. Lawrence rivers, and the Erie Canal (Table 1, Fig. 1). When sufficient mussels were available, 80 individuals were sampled from each location for analysis. At several sites insufficient animals could be found, and only 30-40 were available for analysis (Table 1). Larger mussels (>10 mm) were selected so that enough tissue would be available for electrophoretic analysis. All collections were made in the summer of 1990 and the summer and fall of 1991. Seven populations of mussels were sent to us from five European countries (Table 1, Fig. 2). Mussels were frozen on dry ice or in liquid nitrogen within a few hours of collection in the field, shipped to Ithaca, N.Y., and stored at -70°C until processed.

Allozyme analysis

Extracts of whole mussels and one valve from each specimen were homogenized for analysis. Half shells from each mussel were saved for later morphological analysis; these shells will be archived in the Illinois Natural History Survey collections in Champaign, Ill. Twenty-seven proteins were screened for allozyme variability according to methods outlined in May (1992). Genetic and enzyme nomenclature was used according to Shaklee et al. (1990). Aldolase (*ALD**, E.C. 4.1.2.13), aspartate aminotransferase (*AAT**,

E.C. 2.6.1.1), glycerol-3-phosphate dehydrogenase (*G3PDH**, E.C. 1.1.1.8) and leucyl-glycyl-glycine peptidase (*PEP-LGG**, E.C. 3.4.11-13) were rejected because they showed limited activity. Adenylate kinase (*AK**, E.C. 2.7.4.3), fructose kinase (*FK**, E.C. 2.7.1.2), glutathione reductase (*GR**, E.C. 1.6.4.2), lactate dehydrogenase (*LDH**, E.C. 1.1.1.27) leucine aminopeptidase (*LAP**, E.C. 3.4.11.1) malic enzyme (*MEP**, E.C. 1.1.1.40), and L-idoitol-dehydrogenase (*sIDDH**, E.C. 1.1.1.14) resolved poorly. In many of these cases the number of alleles was too great to allow consistently correct interpretation of the genotypes. Fifteen polymorphic loci for the following proteins were found to be consistently interpretable and were scored for all populations: diaphorase (*DIA**, E.C. 1.6.2.2) esterase (*EST**, E.C. 3.1.1.-), formaldehyde dehydrogenase (*FDH**, E.C. 1.2.1.1), fumarase (*FH**, E.C. 4.2.1.2), glyceraldehyde phosphate dehydrogenase (*GAPDH**, E.C. 1.2.1.12), glucose phosphate isomerase (*GPI**, E.C. 5.3.1.9), isocitrate dehydrogenase (*IDH**, E.C. 1.1.1.42), malate dehydrogenase (*MDH**, E.C. 1.1.1.37), phenyl-alanyl-proline peptidase (*PEP-PAP*, E.C. 3.4.11-13), phosphoglucomutase (*PGM**, E.C. 5.4.2.2), phosphogluconate dehydrogenase (*PGD**, E.C. 1.1.1.44), inorganic pyrophosphatase (*PP**, E.C. 3.6.1.1), and triosephosphate isomerase (*TPI**, E.C. 5.3.1.1). An additional polymorphic locus for aconitase (*AC**, E.C. 4.2.1.3) was scored for all except four populations, and was not included in the genetic distance analyses. One monomorphic locus was found for xanthine dehydrogenase (*XDH**, E.C. 1.2.3.2). Color slides were taken of each gel and kept for archival purposes. Alleles were designated in terms of

Fig. 2. Locations where zebra mussels were sampled in Europe in 1991. Locations of the Vuren and Volga samples are not shown because we are not certain where these samples were taken.



mobility of their homomeric protein product relative to that of the most common allele across all populations at that locus.

Tests for deviation of genotype frequencies from expected Hardy-Weinberg proportions were made using the chi-square contingency test. Genetic differences among samples were compared using the G -test, F_{IS} and F_{ST} values (estimates of deviation from Hardy-Weinberg proportions due to differentiation within and among subpopulations), and genetic distance coefficients. Genetic distances (Nei 1972) were calculated using only the 15 polymorphic loci scored, and were subjected to unweighted pair-group method cluster analysis (UPGMA; Sneath and Sokal 1973). Analyses of genetic data were performed using GENES IN POPULATIONS, a computer program designed by B. May and C.C. Krueger and written in C by W. Eng.

Results

All populations of zebra mussels examined in this study had high levels of heterozygosity, varying between 0.27 and 0.43 (Table 2). The European populations had a slightly lower range of average heterozygosities (0.27-0.35) than the

North American populations (0.30-0.43; $P < 0.05$ using the Mann-Whitney rank test). However, the range of Nei's genetic distance coefficients was higher among European populations than among North American populations (0.007-0.139 versus 0.005-0.025; Table 3, Fig. 3). Most of the differences between populations were due to differences in allelic frequencies at a number of loci, rather than the presence or absence of a rare allele at a single locus. The European populations (except Hungary) and the North American populations clustered separately with a distance between the clusters of 0.058.

F-statistics show that there was less differentiation among populations within North America than within Europe ($F_{ST} = 0.019$ vs. 0.07; Table 4). However, differentiation among populations within geographic units, i.e., each of the Great Lakes in North America and adjacent populations in Europe (Netherlands, Russia), was similar (0.07-0.021 vs. 0.015; Table 4). Significant differences were detected among all of the comparisons that we made, summed over all loci (Table 4).

Rare alleles were found at *DIA**, *GAPDH**, *GPI** (4 alleles), *MDH-I**, and *TPI** in North American populations that were not present in European populations, and at

Table 2. Allelic frequencies at 15 polymorphic loci in 25 populations of zebra mussels from North America and Europe. Allele

Locus/ allele	Alpe	Boll	Cape	Cats	Clair	Colch	Erie 1	Erie 2	Gary	Green	Grim	Holl	Mass	Owen
DIA														
0	0.03	-	0.03	-	-	-	0.03	0.01	0.01	0.02	-	0.01	-	-
100	0.69	0.67	0.69	0.63	0.63	0.50	0.62	0.68	0.55	0.68	0.69	0.71	0.67	0.58
150	0.18	0.05	0.13	0.21	0.21	0.23	0.14	0.21	0.22	0.15	0.16	0.14	0.16	0.19
160	0.01	0.03	0.03	0.01	0.01	0.05	0.02	0.01	0.03	0.03	-	0.01	0.01	0.02
190	0.01	0.07	-	0.04	0.04	0.05	0.05	0.01	0.05	0.03	-	0.01	0.01	0.03
200	0.08	0.18	0.12	0.10	0.12	0.18	0.14	0.08	0.14	0.09	0.14	0.12	0.15	0.19
N	39	38	73	35	43	42	74	39	39	76	34	76	69	72
EST														
100	0.68	0.73	0.65	0.80	0.66	0.63	0.70	0.61	0.75	0.73	0.66	0.69	0.70	0.69
105	0.33	0.27	0.36	0.20	0.34	0.37	0.30	0.39	0.25	0.27	0.34	0.31	0.30	0.32
N	40	39	76	40	44	42	80	40	26	75	35	80	71	65
FDH														
86	0.09	0.04	0.12	0.11	0.16	0.11	0.17	0.12	0.13	0.20	0.11	0.12	0.08	0.14
100	0.91	0.96	0.88	0.89	0.84	0.89	0.83	0.88	0.87	0.80	0.89	0.88	0.92	0.86
N	40	27	65	40	44	31	78	34	38	72	36	73	60	70
FH														
63	0.35	0.30	0.26	0.18	0.28	0.33	0.38	0.25	0.23	0.26	0.27	0.32	0.32	0.25
69	0.10	0.03	0.03	0.01	0.05	0.04	0.04	-	-	0.04	0.01	0.01	0.05	0.02
100	0.55	0.68	0.72	0.80	0.67	0.63	0.59	0.75	0.77	0.69	0.72	0.68	0.63	0.73
N	40	37	74	38	44	42	80	36	31	72	37	74	77	77
GAPDH														
97	—	—	—	0.03	0.03	0.01	-	-	-	-	-	-	-	-
100	0.73	0.45	0.58	0.53	0.56	0.74	0.86	0.69	0.65	0.53	0.54	0.72	0.52	0.69
123	0.28	0.55	0.42	0.45	0.41	0.25	0.14	0.31	0.36	0.47	0.46	0.28	0.48	0.31
N	40	39	74	40	45	42	80	35	31	77	39	79	76	74
GPI														
21	-	—	0.02	-	-	-	0.01	-	0.01	-	0.01	-	-	-
50	0.04	-	0.01	0.01	-	0.02	0.03	0.03	-	0.03	0.03	0.03	0.05	0.02
83	-	-	-	-	-	-	0.01	0.01	-	-	-	-	0.01	-
100	0.34	0.31	0.31	0.30	0.39	0.27	0.33	0.23	0.37	0.35	0.39	0.36	0.31	0.31
121	-	0.04	-	0.01	0.01	-	0.01	-	-	-	-	-	-	-
146	0.45	0.54	0.44	0.50	0.48	0.50	0.44	0.51	0.53	0.46	0.41	0.39	0.49	0.50
188	0.01	-	0.01	-	-	-	0.01	0.01	-	-	-	-	-	-
196	0.16	0.12	0.20	0.15	0.12	0.20	0.16	0.21	0.09	0.13	0.15	0.23	0.14	0.17
208	-	-	0.01	0.03	-	-	0.01	-	-	0.03	0.01	-	0.01	-
N	40	39	78	40	45	42	80	40	38	80	39	79	80	80
IDH														
91	0.33	0.27	0.31	0.27	0.27	0.35	0.32	0.32	0.42	0.39	0.28	0.24	0.29	0.34
100	0.68	0.73	0.69	0.73	0.73	0.66	0.68	0.68	0.58	0.61	0.73	0.76	0.70	0.67
114	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	40	39	72	39	44	42	80	39	37	79	40	80	80	79
MDHI														
77	—	—	0.01	-	-	-	0.01	-	-	0.01	-	-	-	0.01
100	0.60	0.63	0.56	0.55	0.50	0.52	0.61	0.64	0.62	0.58	0.61	0.65	0.60	0.53
120	0.40	0.38	0.43	0.45	0.50	0.48	0.39	0.36	0.38	0.41	0.39	0.35	0.40	0.47
N	40	40	77	40	45	42	80	40	38	79	32	80	78	78
MDH2														
100	0.86	0.91	0.91	0.95	0.87	0.80	0.87	0.85	0.86	0.89	0.94	0.89	0.85	0.94
140	0.14	0.10	0.09	0.05	0.13	0.20	0.13	0.15	0.14	0.11	0.06	0.11	0.15	0.06
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	40	37	74	40	45	42	80	40	39	80	40	80	78	78

mobilities are given in the left-hand column. Abbreviations for sampling locations are given in Table 1

Palm	Roth	Sheb	Zion	Hung	Enkh	Peter	Roer	Volga	Vuren	Polan
—	—	—	—	—	—	—	—	0.02	—	—
0.73	0.66	0.63	0.68	0.99	0.97	0.98	0.97	0.82	0.91	0.85
0.15	0.19	0.24	0.18	—	—	—	—	0.03	—	0.03
0.03	—	0.02	—	—	—	—	—	—	—	—
0.03	0.01	0.04	0.04	—	—	—	—	0.02	—	—
0.06	0.14	0.08	0.10	0.01	0.03	0.02	0.03	0.12	0.10	0.12
39	35	72	74	40	37	29	32	30	37	78
0.63	0.89	0.60	0.72	0.64	0.89	0.91	0.69	0.74	0.75	0.76
0.37	0.11	0.40	0.28	0.36	0.11	0.09	0.30	0.26	0.25	0.24
39	14	79	79	28	35	29	29	29	36	76
0.14	0.08	0.10	0.16	0.65	0.11	0.11	0.18	0.09	0.07	0.18
0.86	0.92	0.90	0.84	0.35	0.89	0.89	0.82	0.91	0.93	0.82
39	26	77	70	13	35	22	37	28	35	76
0.39	0.29	0.28	0.25	0.36	0.25	0.07	0.15	0.13	0.27	0.32
—	0.01	0.03	0.05	—	—	—	—	—	—	0.02
0.61	0.70	0.69	0.70	0.65	0.75	0.93	0.85	0.87	0.73	0.66
36	35	77	77	31	38	27	39	30	37	80
—	—	—	0.01	—	—	—	—	—	—	—
0.63	0.61	0.70	0.61	0.75	0.36	0.36	0.24	0.27	0.30	0.30
0.37	0.39	0.30	0.38	0.25	0.64	0.64	0.76	0.73	0.70	0.70
39	35	68	78	38	39	28	39	26	38	76
0.01	—	—	—	—	—	—	—	—	—	—
0.04	0.01	0.02	0.01	—	0.01	—	—	0.05	0.01	—
—	—	—	—	—	—	—	—	—	—	—
0.30	0.31	0.35	0.30	0.14	0.33	0.38	0.40	0.27	0.20	0.39
—	—	0.05	—	—	—	—	—	—	—	—
0.47	0.44	0.48	0.51	0.73	0.45	0.33	0.42	0.54	0.58	0.51
—	—	—	0.01	—	—	—	0.01	—	—	0.01
0.18	0.21	0.11	0.17	0.14	0.21	0.28	0.17	0.14	0.21	0.09
—	0.03	—	0.01	—	—	—	—	—	—	—
39	40	78	77	40	38	30	28	28	40	80
0.23	0.29	0.50	0.30	0.36	0.69	0.67	0.67	0.50	0.67	0.54
0.77	0.70	0.50	0.69	0.64	0.31	0.33	0.33	0.47	0.33	0.46
—	0.01	—	0.01	—	—	—	—	0.02	—	—
—	—	—	—	—	—	—	—	0.02	—	—
39	38	74	69	40	39	29	39	29	39	75
—	0.01	—	0.01	—	—	—	—	—	—	—
0.54	0.63	0.59	0.59	0.47	0.68	0.70	0.81	0.82	0.81	0.55
0.46	0.36	0.41	0.41	0.53	0.32	0.30	0.19	0.18	0.19	0.45
39	40	80	80	39	39	30	39	30	39	80
0.89	0.81	0.89	0.92	0.80	0.90	0.85	0.94	0.84	0.95	0.89
0.12	0.19	0.11	0.08	0.20	0.10	0.12	0.06	0.11	0.05	0.11
—	—	—	—	—	—	0.03	—	0.05	—	—
39	37	78	78	40	39	30	39	28	37	80

Table 2 (concluded).

Locus/ allele	Alpe	Boll	Cape	cats	Clair	Colch	Erie 1	Erie 2	Gary	Green	Grim	Holl	Mass	Owen
PAP														
100	0.65	0.75	0.69	0.53	0.54	0.58	0.67	0.59	0.59	0.66	0.59	0.67	0.69	0.69
114	0.35	0.25	0.31	0.47	0.46	0.42	0.33	0.41	0.41	0.34	0.41	0.33	0.31	0.31
N	39	34	74	37	41	26	79	38	34	74	39	72	74	75
PGD														
100	0.95	1.00	0.83	0.93	0.94	0.92	0.91	0.90	0.91	0.91	0.93	0.89	0.91	0.92
108	-	-	0.12	-	-	-	-	-	0.06	-	-	-	-	0.01
123	0.05	-	0.05	0.07	0.06	0.08	0.08	0.10	0.01	0.09	0.08	0.12	0.09	0.07
130	-	-	-	-	-	-	0.01	-	0.01	-	-	-	0.01	-
N	40	37	74	36	45	42	77	34	39	78	40	74	79	77
PGMI														
88	-	0.01	-	-	-	-	-	-	-	-	-	0.01	-	0.01
96	-	-	-	-	-	-	0.01	-	-	-	0.01	-	-	-
100	0.90	0.91	0.91	0.86	0.88	0.87	0.87	0.96	0.90	0.88	0.89	0.93	0.82	0.88
107	-	-	0.01	0.01	-	0.01	-	-	-	-	-	-	0.02	-
111	0.10	0.08	0.08	0.13	0.13	0.12	0.13	0.04	0.10	0.12	0.10	0.06	0.16	0.12
N	40	37	73	40	44	41	80	40	30	76	39	80	78	72
PGM2														
95	0.13	-	-	0.04	0.10	0.06	0.03	-	0.04	0.07	0.03	0.05	-	0.01
96	-	-	-	0.03	0.02	0.02	0.01	-	0.04	-	0.02	0.01	-	0.05
100	0.63	0.64	0.64	0.60	0.53	0.54	0.69	0.71	0.72	0.73	0.59	0.62	0.80	0.73
108	0.18	0.36	0.23	0.21	0.26	0.31	0.28	0.26	0.19	0.19	0.33	0.23	0.19	0.19
113	0.08	-	0.14	0.13	0.09	0.07	-	0.03	-	0.01	0.03	0.09	0.01	0.03
N	40	36	74	40	45	41	72	40	34	71	33	80	74	74
PP														
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N	40	40	78	40	45	43	80	40	39	80	40	80	80	80
TPI														
69	0.05	-	0.22	0.01	0.04	0.04	0.03	0.04	0.01	0.03	0.04	0.03	-	0.03
100	0.69	0.77	0.61	0.61	0.59	0.70	0.74	0.60	0.65	0.60	0.51	0.73	0.55	0.65
112	-	-	-	0.01	-	-	0.04	-	-	-	-	-	-	0.01
115	0.21	0.23	0.17	0.36	0.36	0.24	0.18	0.34	0.28	0.36	0.41	0.24	0.43	0.29
121	0.05	-	-	-	0.01	0.02	0.02	0.03	0.06	0.01	0.04	-	0.02	0.03
N	40	39	76	40	45	42	80	40	36	80	39	79	80	79
Mean HS	0.38	0.33	0.39	0.37	0.40	0.40	0.37	0.36	0.37	0.38	0.37	0.36	0.37	0.37
Mean SE	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05
Mean H_0	0.39	0.31	0.35	0.37	0.40	0.43	0.37	0.35	0.36	0.37	0.36	0.33	0.33	0.35
SE	0.06	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.04	0.05

*IDH**, *MDH-2**, and *PP** we found rare alleles that were present in Europe but not in North America. We were unable to determine whether these alleles are the same as those detected by Hebert et al. (1989) and Boileau and Hebert (1993) as they did not record relative mobilities of allelic products in their studies. All of the rare alleles were present at such low frequencies that the likelihood of missing them if they were present in any one of the other populations was greater than 0.95 (Gregorius 1980). However, the probability that we missed all eight North American rare alleles if they were all present in at least one of the European populations is close to zero ($<10^{-7}$), i.e., the product of the probabilities of missing each allele separately in all samples.

Tests for deviations from Hardy-Weinberg expected genotype frequencies using $\alpha = 0.05$ revealed a high number of deviations (13%). Note that the frequency of such deviations can be reduced by using larger sample sizes. Using Bonferroni's procedure to correct for multiple tests made simultaneously, only two tests were significant: *PGD** in the population from IJsselmeer in the Netherlands and *DZA** in the population from Grimsby, Ont., on Lake Ontario (Snedecor and Cochran 1980; see also Cooper 1968). However, as Bonferroni's procedure is very conservative, it was worth looking for patterns in the tests that were significant at the 0.05 level. Using this criterion, eight deviations from Hardy-Weinberg expected genotype frequencies were found at *PGM-2** and nine were

Palm	Roth	Sheb	Zion	Hung	Enkh	Peter	Roer	Volga	Vuren	Polan
0.60	0.53	0.69	0.71	0.68	0.60	0.57	0.50	0.73	0.63	0.61
0.41	0.47	0.31	0.29	0.32	0.40	0.43	0.50	0.27	0.38	0.39
37	30	77	70	28	36	27	37	30	36	71
1.00	0.92	0.94	0.86	0.82	0.67	0.67	0.55	0.83	0.69	0.74
	0.06	-	0.13	—	0.13	—	—	-	0.19	0.01
	0.03	0.06	0.02	0.18	0.20	0.27	0.45	0.10	0.12	0.25
	-	-	-	—	-	0.06	—	0.08	-	0.01
38	36	72	64	39	38	24	38	26	37	72
—	—	—	—	—	—	0.09	—	—	—	—
		—	0.01	-	—	—	—	—	—	0.01
0.93	0.92	0.88	0.87	0.96	0.95	0.83	0.95	0.93	0.96	0.76
		0.02	—	—	—	—	—	—	—	0.01
0.07	0.08	0.10	0.13	0.04	0.05	0.09	0.05	0.07	0.04	0.21
38	30	77	78	40	39	29	38	30	39	70
0.07	0.04	0.01	0.02	0.14		—	0.01	-	-	0.02
		0.12			0.01			0.12		0.01
0.71	0.83	0.66	0.71	0.73	0.88	0.97	0.82	0.80	0.93	0.79
0.20	0.11	0.18	0.22	0.06	0.11	0.03	0.12	0.08	0.07	0.14
0.03	0.01	0.03	0.05	0.06			0.04	-		0.03
38	35	73	58	39	37	29	37	30	38	75
—	-	-	-	-	0.01	—	—	-	-	-
1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
39	40	80	80	40	40	30	40	30	40	80
0.03	0.01	0.03	0.04		-	-	-			-
0.69	0.54	0.71	0.68	0.80	0.51	0.18	0.55	0.47	0.58	0.41
-	0.01	0.01	-		0.01	-	-	0.05	0.01	
0.28	0.42	0.24	0.27	0.20	0.47	0.82	0.45	0.48	0.41	0.57
	0.01	0.01	0.01				-			0.02
39	37	80	80	38	39	30	39	30	39	73
0.35	0.35	0.37	0.37	0.34	0.32	0.29	0.32	0.33	0.31	0.38
0.05	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.04
0.33	0.30	0.35	0.35	0.28	0.30	0.27	0.31	0.33	0.29	0.35
0.05	0.05	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.05	0.04

found at *DIA**. Given the high numbers of alleles found at many loci, including those that could not be resolved owing to crowding of the bands, it is likely that null alleles could have been present at these two loci, giving the appearance of deviations from expected Hardy-Weinberg genotype frequencies (heterozygote deficiencies). The majority (30%) of the deviations detected at all loci at the 0.05 level were due to a deficiency of the common heterozygote. This is confirmed by the F_{IS} values, that are all positive (Table 4). Heterozygote deficiency has been frequently noted in bivalve molluscs, but no consensus explanation for this aberration has been proposed (Zouros and Foltz 1984; Mallet et al. 1985).

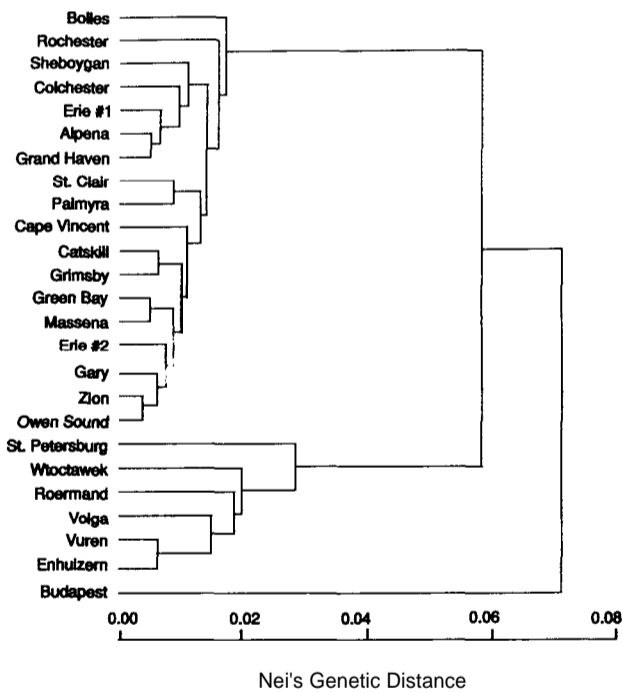
Discussion

Our data indicate that the zebra mussel populations within the Great Lakes and adjacent waters are not highly differentiated. The most genetically similar populations should be those from adjacent areas or areas linked by water flow. However, populations that were widely separated geographically, such as those from Massena, N.Y. and Green Bay, Wis. ($D = 0.005$), had genetic distances comparable with those from adjacent populations such as Gary, Ind., and Zion, Ill., in Lake Michigan ($D = 0.006$). The range of genetic distances among the North American populations (0.004-0.028) was smaller than has been detected in other mollusc species, for example, among 16 populations of two

Table 3. Nei's genetic distance coefficients calculated in 25 populations of zebra mussels using 15 polymorphic allozyme loci.

	Erie 1	Erie 2	Boll	Colch	Grim	Cape	Roth	Holl	Green	Zion	Sheb	Gary	Owen	Alpe	Palm	
Clair	0.020	0.011	0.017	0.009	0.005	0.012	0.017	0.012	0.009	0.010	0.016	0.011	0.010	0.011	0.007	
Erie 1		0.013	0.024	0.009	0.022	0.018	0.024	0.007	0.018	0.011	0.012	0.014	0.009	0.006	0.012	
Erie 2			0.018	0.010	0.009	0.010	0.014	0.007	0.010	0.008	0.010	0.008	0.007	0.009	0.007	
Boll				0.022	0.013	0.014	0.024	0.015	0.012	0.010	0.020	0.017	0.015	0.017	0.014	
Colch					0.016	0.016	0.023	0.010	0.018	0.013	0.011	0.011	0.009	0.008	0.011	
Grim						0.011	0.015	0.011	0.007	0.010	0.017	0.012	0.010	0.014	0.010	
Cape							0.021	0.009	0.010	0.005	0.014	0.013	0.009	0.011	0.011	
Roth								0.017	0.011	0.013	0.025	0.010	0.015	0.017	0.016	
Holl									0.012	0.007	0.012	0.012	0.008	0.004	0.007	
Green										0.005	0.011	0.007	0.007	0.011	0.010	
Zion											0.010	0.007	0.004	0.008	0.008	
Sheb												0.008	0.007	0.008	0.014	
Gary													0.006	0.011	0.013	
Owen														0.008	0.009	
Alpe															0.006	
Palm																
Cats																
Mass																
Polan																
Hung																
Vuren																
Enkh																
Roer																
Peter																
Volga																

Fig. 3. Relative genetic distance dendrogram generated by UPGMA analysis of Nei's (1972) genetic distance coefficients calculated in 25 populations of zebra mussels using 15 polymorphic allozyme loci.



species of North American unionid bivalves (0.023-o. 184; Davis 1983). Therefore, in the 6 years since the zebra mussels entered North America, they have not undergone any genetic differentiation, microevolution, or natural selection for traits linked to protein-coding loci. This result suggests that, as the mussels expand their range, any differentiation due to small founder populations has been overwhelmed by frequent genetic mixing with new immigrants. This result is not surprising. The mussels spread throughout the Great Lakes in 4 years, and throughout the major river drainages within 6 years of their introduction. This rate of spread suggests that mussels are translocated very easily, particularly downstream of established populations owing to the passive movement of veligers. Consequently, the likelihood of reintroduction to many of these locations on a regular basis is quite high. The size of many zebra mussel vectors (ballast tanks, intra- and inter-lake currents) suggests that each new introduction probably comprises a large number of animals. Thus, founder effect is unlikely to occur, and continual genetic mixing of established populations ensures homogeneity among populations. The effects of natural selection, which would have to be quite strong to have occurred within 6 years (a hypothetical maximum of 12 zebra mussel generations), would also be lost owing to such mixing. This being the case, the only areas where we would be likely to find genetically differentiated populations would be in isolated bodies of water that have low probabilities for repetitive

These numbers were used to generate the dendrogram in Fig. 3.

Cats	Mass	Polan	Hung	Vuren	Enkh	Roer	Peter	Volga
0.006	0.012	0.038	0.061	0.063	0.058	0.074	0.090	0.050
0.026	0.021	0.067	0.050	0.075	0.073	0.099	0.118	0.071
0.012	0.011	0.044	0.054	0.047	0.049	0.063	0.079	0.039
0.016	0.012	0.043	0.075	0.048	0.053	0.073	0.097	0.035
0.017	0.020	0.062	0.062	0.076	0.075	0.097	0.119	0.069
0.007	0.008	0.031	0.073	0.048	0.047	0.058	0.071	0.035
0.014	0.014	0.043	0.059	0.048	0.050	0.066	0.088	0.041
0.011	0.010	0.036	0.074	0.043	0.037	0.065	0.058	0.036
0.014	0.014	0.054	0.054	0.059	0.058	0.075	0.097	0.051
0.009	0.005	0.021	0.050	0.033	0.030	0.047	0.059	0.025
0.010	0.008	0.038	0.050	0.043	0.044	0.066	0.082	0.037
0.020	0.016	0.046	0.059	0.049	0.050	0.069	0.090	0.044
0.010	0.012	0.039	0.061	0.043	0.042	0.063	0.075	0.036
0.010	0.009	0.042	0.055	0.053	0.053	0.075	0.086	0.044
0.017	0.013	0.052	0.054	0.060	0.059	0.083	0.102	0.055
0.013	0.010	0.046	0.048	0.058	0.059	0.080	0.099	0.051
	0.012	0.035	0.070	0.051	0.047	0.063	0.073	0.039
		0.023	0.067	0.039	0.039	0.059	0.064	0.028
			0.083	0.022	0.015	0.024	0.027	0.021
				0.094	0.090	0.106	0.139	0.098
					0.007	0.017	0.034	0.013
						0.015	0.018	0.018
							0.028	0.024
								0.031

reinfestation. If any of these isolated populations are found to have quite different characteristics from the Great Lakes populations, investigators would be advised to examine the genetic structure of the population before concluding that such differences are due only to environmental factors. Relatively isolated populations are likely to differentiate from source populations over time. Thus, the data presented here serve as baseline data to which similar studies can be compared in several years to determine whether genetic divergence of populations has taken place.

The closest genetic relationships among populations we examined were between the two populations from Lake Huron and between the two from the Netherlands. Lake Huron may have only been invaded once, as the lake is upstream of all other populations except the tenuous colony in Duluth Harbor, and there are few major ports on Lake Huron relative to the other lakes. The Netherlands populations are geographically close, but one would expect this busy shipping area to receive multiple introductions from different locations in Europe.

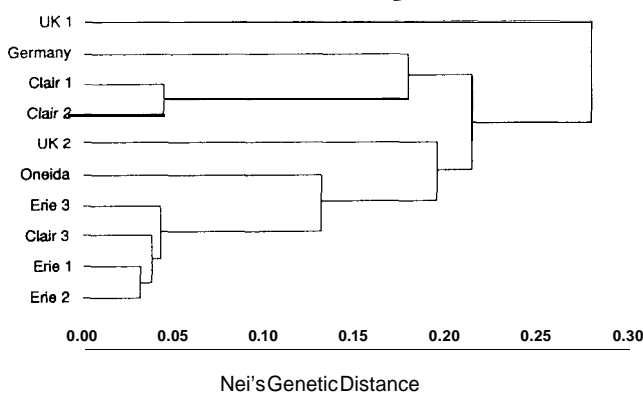
Boileau and Hebert (1993) observed much higher allelic frequency differences among populations than found in this study. A dendrogram that we constructed from Boileau and Hebert's data shows large genetic differences even between geographically adjacent populations (Fig. 4). For example, populations from within Lake St. Clair are separated by a genetic distance of 0.215, which is considerably

greater than the maximum distance we observed between any populations, even between continents. These differences can only partly be explained by the small sample sizes used by Boileau and Hebert ($N = 24$ in most cases), which will increase the variance on the allelic frequency estimates and increase the probability of missing alleles present in low frequencies. Similar numbers of loci were used in both studies (15 in our study, 11 in theirs), although we found more alleles, on average, at each locus (4.2 vs. 3.3). However, none of these factors explains the large differences between populations detected in Boileau and Hebert's study, such as the frequency of *LG3*1* found in the three populations of Lake St. Clair (0.04, 0.27, and 0.65, respectively). Most of the mussels in both studies were collected during the same time period (summer 1990 through fall 1991), so differences are not likely to reflect new introductions or temporal changes in the populations. The extreme genetic distances noted by Boileau and Hebert, which are larger than species differences found in some taxonomic studies, could be explained in part if some of their populations were inadvertently comprised of a mixture of zebra and quagga mussels (*Dreissena bugensis*; Spidle et al. 1994). We unfortunately cannot resolve this discrepancy because Boileau and Hebert did not record allele mobilities nor preserve a record of their gels (M.G. Boileau, Department of Biology, University of Guelph, Guelph, ON N1G 2W1, personal communication).

Table 4. F- and G-test statistics calculated over 15 polymorphic allozyme loci observed in 25 populations of zebra mussels. N is the number of groups compared in a test.

	N	G	df	P	F_{IS}	F_{IT}	F_{ST}
Lakes St. Clair and Erie	5	299.191	160	$<1 \times 10^{-9}$	0.000	0.020	0.021
Lake Ontario	3	165.61	74	$<1 \times 10^{-8}$	0.100	0.115	0.018
Lake Michigan	5	365.058	160	$<1 \times 10^{-17}$	0.060	0.072	0.012
Lake Huron	2	65.275	36	0.002	0.001	0.006	0.007
Lakes Ontario, Erie, Huron, and Michigan	3	260.737	123	$<1 \times 10^{-11}$	0.054	0.058	0.004
North American riverine populations	3	141.349	74	$<1 \times 10^{-5}$	0.060	0.073	0.014
Riverine and Great Lakes populations	2	97.787	41	$<1 \times 10^{-5}$	0.072	0.074	0.003
All North American populations	18	1395.01	697	$<1 \times 10^{-48}$	0.043	0.061	0.019
Netherlands	2	55.054	28	0.0017	0.050	0.065	0.015
Russia	2	83.656	35	$<1 \times 10^{-5}$	0.030	0.062	0.015
Eastern Europe	3	325.884	64	$<1 \times 10^{-36}$	0.088	0.159	0.077
Eastern Europe, Netherlands, and Russia	3	263.108	80	$<1 \times 10^{-20}$	0.089	0.106	0.018
All European populations	7	727.661	240	$<1 \times 10^{-49}$	0.062	0.128	0.070
North American and European populations	2	931.991	44	$<1 \times 10^{-166}$	0.091	0.115	0.027

Fig. 4. Relative genetic distance dendrogram generated by UPGMA analysis of Nei's (1972) genetic distance coefficients calculated using data from Boileau and Hebert (1993) for 11 polymorphic allozyme loci. Abbreviations refer to sites described in Boileau and Hebert (1993): ER, Lake Erie; ON, Oneida Lake; SC, Lake St. Clair; UK, United Kingdom.



Genetic distances among the European populations examined in this study, with the exception of the population from Hungary, were similar to distances among the North American populations. Thus, either the European populations are still exchanging individuals with sufficient regularity to prevent divergence of allelic frequencies, or these populations have not been separated for sufficient time for genetic drift and consequent divergence to occur. The relatively minor differences in the average heterozygosity between European and North American populations suggest that little variation was lost when zebra mussels invaded North America, and therefore, we can conclude that the founder population comprised a relatively large number of individuals. We cannot eliminate the possibility

that one or more of the populations we examined from Europe contributed to the populations in North America. However, the presence of eight unique alleles in North America and three in Europe, coupled with the difference observed between the North American and European population groupings, suggests that at least one major contributor to the invader population has yet to be found.

Detection of a rare allele that is present in North America but absent in a European population cannot be used to eliminate the European population as a potential Invader, as inferred by Boileau and Hebert (1993), owing to the large probability of missing the allele with the sample sizes used in both our and their study (see Gregorius 1980). Although we found higher genetic distances between North America and Europe than among populations from these two areas, and we also found allelic frequency differences among populations from the two continents, these differences are not sufficiently large to eliminate the possibility of relatedness. The extremely high levels of genetic variability in zebra mussels, also noted by Hebert et al. (1989), Boileau and Hebert (1993), and others, are not unusual for molluscs, which tend to be highly variable at allozyme loci (e.g., Nevo 1983). However, these levels of heterozygosity require that large sample sizes be used for adequate estimation of allelic frequencies, average heterozygosity, and genetic distances when only a few loci are examined (i.e., less than 30; e.g., Nei 1978).

We may never be able to absolutely identify the founder populations from Europe, even with genetic data, if more than one population contributed to the invasion. However, a complete survey of possible founder populations may permit estimation of the relative probabilities that each population could be a founder. A logical place to look for possible founder populations is the Dnieper and Bug drainages of the Black Sea, which is the source of the quagga mussel (Spidle et al. 1994).

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