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Population genetic history of the dreissenid mussel invasions: expansion patterns across North America

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Abstract This study tests population genetic patterns across the Eurasian dreissenid mussel invasions of North America—encompassing the zebra mussel *Dreissena polymorpha* (1986 detection) and the quagga mussel *D. rostriformis bugensis* (detected in 1990, which now has largely displaced the former in the Great Lakes). We evaluate their source-spread relationships and invasion genetics using 9–11 nuclear microsatellite loci for 583 zebra mussels (21 sites) and 269 quagga mussels (12 sites) from Eurasian and North American range locations, with the latter including the Great Lakes, Mississippi River basin, Atlantic coastal waterways, Colorado River system, and California reservoirs. Additionally, mtDNA cytochrome *b* gene sequences are used to verify species identity. Our results indicate that North American zebra mussels originate from multiple non-native northern European populations, whereas North American quagga mussels trace to native estuaries in the Southern Bug and Dnieper Rivers. Invasive populations of both species show considerable genetic diversity and structure (zebra $F_{ST} = 0.006$–0.263, quagga $F_{ST} = 0.008$–0.267), without founder effects. Most newer zebra mussel populations have appreciable genetic diversity, whereas quagga mussel populations from the Colorado River and California show some founder effects. The population genetic composition of both species changed over time at given sites; with some adding alleles from adjacent populations, some losing them, and all retaining closest similarity to their original composition. Zebra mussels from Kansas and California appear genetically similar and assign to a possible origin from the St. Lawrence River, whereas quagga mussels from Nevada and California assign to a possible origin from Lake Ontario. These assignments suggest that overland colonization pathways via recreational boats do not necessarily reflect the most proximate connections. In conclusion, our microsatellite results comprise a valuable baseline for resolving present and future dreissenid mussel invasion pathways.

Keywords *Dreissena* · Invasion genetics · Long-distance dispersal · Microsatellites · Quagga mussel · Zebra mussel

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Introduction

Genetic studies using high-resolution microsatellite DNA loci offer means to elucidate the pathways and vectors of invasive species, and assess their temporal population dynamics. This is the first comprehensive population genetic analysis of the Eurasian dreissenid mussel (Mollusca: Bivalvia: Dreissenidae) invasion

across North America, which has been one of the most ecologically and economically important aquatic introductions. The zebra mussel *Dreissena polymorpha* appeared in the Great Lakes in 1986, where it was introduced via ballast water (Hebert et al. 1989; Carlton 2008), followed by the quagga mussel *D. rostriformis bugensis* in ~1989 (May and Marsden 1992; Mills et al. 1996). The zebra mussel had previously considerably expanded its original Ponto-Eurasian range via central European canals in the Black Sea and Baltic Sea drainages (Morton 1993; Bij de Vaate et al. 2002), whereas the Great Lakes was one of the quagga mussel's first documented expansions (Pligin 1979; Karatayev et al. 2008). The zebra mussel also spread from the Baltic Sea drainage to Italy (1960s), Ireland (1997), and Spain (2001) by recreational boats (Bij de Vaate et al. 2002; Aldridge et al. 2004; Gosling et al. 2008).

Dreissenid mussels widely disperse via their planktonic larvae, whereas juveniles and adults adhere to hard substrates with byssal threads (summarized by Ackerman et al. 1994). These life history traits distinguish dreissenids from most native North American freshwater bivalves, which lack a planktonic larval stage and do not attach to hard substrates (Johnson and Padilla 1996). The spread of dreissenids was facilitated by their life history traits, including unintentional transport of larvae in ships' ballast and bilge water, and by attachment of their juveniles and adults to engines, hulls, and anchors (Ricciardi et al. 1995; Mackie and Schloesser 1996; Johnson et al. 2001). As they have spread, dreissenid mussels have exerted both economic and ecological impacts. Notably, their fouling of power generation and water treatment facilities in the United States and Canada has cost $161–$467 million/year (Connelly et al. 2007). They filter feed on phytoplankton with high efficiency that has shifted the basis of Great Lakes' aquatic food chains from pelagic to benthic (Berg et al. 1996; Zhu et al. 2006). Spread of dreissenid "mats" on the lake floors—where they host an associated community of plants and invertebrates in their shells and interstices—has further increased benthification, markedly changing the ecosystem (Zhu et al. 2006; Hebert et al. 2009; Stewart et al. 2009). Dreissenids likely facilitated the invasion of the round goby *Neogobius melanostomus* (Vanderploeg et al. 2002), their natural predator that became established throughout the Great Lakes, whose populations have high genetic diversity and show no founder effect (Stepien and Tumeo 2006; Brown and Stepien 2009).

Upon establishment in North America, the zebra mussel spread rapidly, expanding throughout the Great Lakes and into the Mississippi and Hudson River drainages within 5 years of detection (Nalepa and Schloesser 1993; Allen and Ramcharan 2001; Kelly et al. 2009). The quagga mussel spread more slowly (Kelly et al. 2009), gradually replacing zebra mussels in regions of the St. Lawrence River (Ricciardi and Whoriskey 2004) and the lower Great Lakes (Mills et al. 1999; Jarvis et al. 2000; Wilson et al. 2006), and is now doing the same in the upper Lakes (Nalepa et al. 2009). Researchers and managers attempted to stop westward spread at the 100th meridian, to prevent colonization of the heavily dammed and diverted waterways of the western United States (Mangin 2001; Britton and McMahon 2005; Bossenbroek et al. 2007, 2009). However, in 2007 the quagga mussel was detected in Lake Mead, Nevada—a reservoir on the Colorado River—and then discovered downstream (Stokstad 2007). Dreissenids since have spread into many southern California reservoirs via the extensive canal system radiating from the Colorado River (http://nas.er.usgs.gov/queries/FactSheet.asp?speciesID=95). In 2008, zebra mussels were detected in San Justo Reservoir in central California (http://nas.er.usgs.gov/queries/FactSheet.asp?speciesID=5). It is important that we understand the population sources and pathways of these recent expansions so that we can evaluate the efficacy of our control efforts and attempt to avoid further expansions.

Role of genetics

Modern genetics can play a crucial role to aid invasive species identification and evaluate their population linkages. Hebert et al. (1989), May and Marsden (1992), and Stepien et al. (1999) used genetics to determine the identity of dreissenid invaders in the Great Lakes. Additional genetic research has examined portions of the invasive ranges for zebra (Elderkin and Klerks 2001; Stepien et al. 2001, 2003, 2005; Gosling et al. 2008) and quagga mussels (Stepien et al. 2001, 2003, 2005), illustrating the need for more rapidly-evolving reproducible genetic markers to understand their origin and spread, compare genetic

variation, determine population genetic structure, and predict their expansion pathways.

The genetic composition of an introduced population can be substantially altered from its native source(s), including the amount of overall variation and its geographic partitioning. Genetic variation comprises the raw material for adaptive evolution, which can play a critical role governing invasive success (Blossey and Notzold 1995; Bossdorf et al. 2005). Selectively neutral molecular markers can serve as a proxy for estimating quantitative genetic variation in phenotypic traits that underlies adaptive evolution (Merila and Crnokrak 2001; Reed and Frankham 2001; Chun et al. 2009). Population genetic data also are useful for elucidating the number and sources of an introduction (Stepien et al. 2005; Rosenthal et al. 2008; Brown and Stepien 2009), as well as determining whether changes occur during the time course of its establishment and spread (Andreakis et al. 2009; Henry et al. 2009). Such information can aid control efforts. For example, determining the genetic origin of the invasive climbing fern *Lygodium microphyllum* led to a biological control agent that was matched to its native genotypes (Goolsby et al. 2004).

The genetic variation of an introduced population may be reduced, increased, or unchanged relative to native populations with reduction as the most common outcome (Nei et al. 1975; Ficetola et al. 2008; Henry et al. 2009). Three processes may reduce genetic diversity of the introduction and preclude significant variation among local population groups. First, most introductions are founded by only a few individuals that represent only a small part of native variation, and subsequent bottlenecks and Allee effects may further reduce it (DeWalt and Hamrick 2004; Grapputo et al. 2005; Griffen and Drake 2008). Second, introduction and spread likely lead to high gene flow that homogenizes population genetic structure (Viard et al. 2006; Kim et al. 2009). Lastly, a recent introduction may lack sufficient time for local adaptation or drift to generate local population genetic structure via stochastic processes in the new range (Maron et al. 2004; Whitney and Gabler 2008). Any new genetic variation would need to arise from mutations and recombinations, before population structure could develop (Perez et al. 2008). Selection also might lead to reduced genetic diversity; since when an introduced genotype is more fit, its numbers will increase relative to others, resulting in a net loss of genetic variability (Kliber and Eckert 2005). If these processes predominate during an introduction, the new population should be genetically depauperate in comparison to native populations (Amsellam et al. 2000; DeWalt and Hamrick 2004; Grapputo et al. 2005).

If an introduction is founded from multiple genetically differentiated native populations, its resultant genetic diversity can be higher than that characterizing native populations, therefore possibly increasing heterozygosity and/or generating novel allelic combinations (Ellstrand and Schierenbeck 2000). Several studies have shown patterns indicating multiple introductions and high genetic diversity in invaders (Kolbe et al. 2004; Williams et al. 2005; Stepien et al. 2005; Brown and Stepien 2009). Because increased genetic diversity likely also increases potential for adaptive evolution, an invader with high diversity may evolve quickly in its new range. Another possible evolutionary outcome for an introduction is no significant change in genetic diversity between the native and introduced ranges. This is possible if large population size is maintained during an invasion (Brown and Marshall 1981) or regained shortly following introductions (Zenger et al. 2003).

Understanding the origin of population genetic structure, or lack thereof, in an invasive species also can provide valuable information on transport pathways from source populations. Correctly identifying transport pathways and source populations is critical to interpreting an invader's relative ecological success in introduced versus native ranges and for targeting management efforts to shut down invasion pathways (Amsellam et al. 2000; Kang et al. 2007). Like the zebra and quagga mussels, 73% of Great Lakes invaders entered via ballast water exchange from oceanic vessels (Holeck et al. 2004; Ricciardi 2006; Kelly et al. 2009) and 70% of those from 1985 to 2000 traced to the Eurasian Ponto-Caspian region (Ricciardi and MacIsaac 2000). Three shipping routes extend from the Ponto-Caspian region, across the Atlantic, and into the Great Lakes, including: (1) from the Black Sea through the Mediterranean Sea, (2) from the Danube River into the North Sea, or (3) from the Dnieper River into the Baltic Sea (Ricciardi and MacIsaac 2000; Grigorovich et al. 2002). These pathways are evaluated in our study for the zebra and quagga mussel introductions.

Relatively few Great Lakes-introduced species have been genetically assessed to date. Both the

spiny water flea *Bythotrephes longimanus* (Berg et al. 2002) and the ruffe *Gymnocephalus cernuus* (Stepien et al. 1998, 2004, 2005) showed evidence of single-source colonization. The water flea originated from Lake Ladoga in Russia (Berg et al. 2002) and the ruffe from the Elbe River system in Germany (Stepien et al. 2004, 2005). All the Elbe River ruffe genotypes are present in the upper Great Lakes in similar frequencies, and likely were introduced into the upper Great Lakes via increased US shipping trade with Germany near the time of its reunification (Stepien et al. 2004, 2005). In contrast, Chinese mitten crab *Eriocheir sinensis* introductions into the Great Lakes traced to multiple European ports rather than native Asian populations (Tepolt et al. 2007); however, this species does not reproduce in freshwater and thus failed to establish (Herborg et al. 2007). The Great Lakes introduction of the freshwater tubenose goby *Proterorhinus semilunaris* has been characterized by levels of genetic diversity that are similar to native river source populations (Stepien et al. 2005; Stepien and Tumeo 2006; Neilson and Stepien 2009). Invasive Ponto-Caspian round goby *Neogobius melanostomus* populations in the Great Lakes have considerable genetic variation and population structure in comparison with native populations (Dillon and Stepien 2001; Stepien et al. 2005; Stepien and Tumeo 2006; Brown and Stepien 2008), and the Dnieper River population was identified as its primary source (Brown and Stepien 2009).

Genetic studies of the dreissenid mussel invasion by Marsden et al. (1995) and Stepien et al. (1999, 2002, 2005) separately described relatively high levels of genetic diversity, suggesting little overall loss of genetic diversity in comparison with putative source populations. Allozyme markers by Marsden et al. (1995) appeared to suggest a single source for the zebra mussel invasion, yet a later allozyme study showed significant genetic heterogeneity among populations in the Great Lakes and a complex of inland lakes, suggesting an important role for stochastic processes governing their population genetic structure (Lewis et al. 2000). Subsequent genetic studies using mitochondrial and nuclear DNA markers similarly found high genetic diversity and implicated multiple founding sources, pointing to western central European sources for the zebra mussel (Stepien et al. 2002, 2005). In this new study, we analyze the invasion genetics of the zebra mussel and the quagga mussel in North America and Eurasia using high-resolution nuclear microsatellite markers for the first genetic study across their expanded geographic range.

Fig. 1 Maps showing current range and sampling locations for ▶ zebra mussels and quagga mussels. **A** North America (USGS; http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/), **B** Eurasia (DAISIE; http://www.europe-aliens.org/)

We test the following hypotheses underlying the genetic history of the dreissenid invasions in North America:

1. Each species was founded by a single versus multiple source populations.
2. The invasive populations are genetically homogenous/heterogeneous across their ranges.
3. Genetic diversity levels reflect overall founder effects/show no founder effects.
4. Populations outside the Great Lakes have reduced/similar/greater genetic diversity compared to those within the Great Lakes.
5. Once established, populations remain genetically static/change over time.

Materials and methods

Sampling, DNA extraction, and amplification

We sampled 24 zebra mussel ($N = 583$) and 13 quagga mussel ($N = 269$) population locations across North America and Eurasia that were collected from 1991 to 2008 (Fig. 1, Table 1). Five locations (three for zebra mussels and two for quagga mussels) had samples from more than 1 year, which were compared to test for temporal population changes. Samples were collected by hand or by fishery agency trawls, and were either immediately frozen, or placed in 95% ethanol and stored at room temperature. Genomic DNA was extracted from mantle tissue using Qiagen DNeasy kits (Qiagen, Inc.; Valencia, CA), eluted in 100 µl of water, stored at 4°C until used for amplification, and then archived at −80°C.

Genetic variation was assessed from eight microsatellite (µsat) loci for zebra mussels (identified as *Dpo*) and six for quagga mussels (*Dbu*) developed by Feldheim et al. (in review), and three additional previously published loci per species (Table 2; Wilson et al. 1999; Naish and Boulding 2001; Feldheim et al. in review). PCR amplifications were

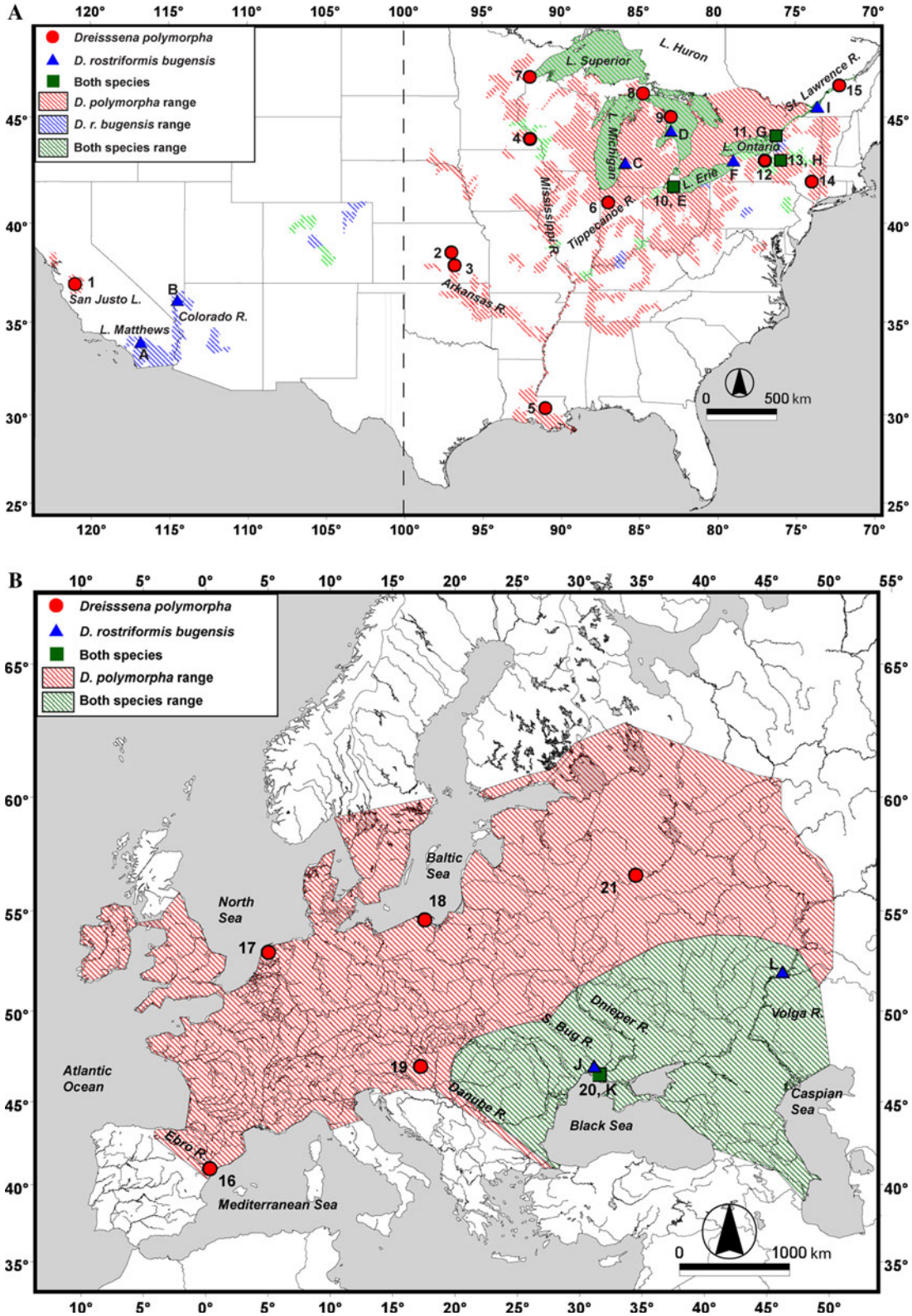
A
Dreisssena polymorpha
D. rostriformis bugensis
Both species
D. polymorpha range
D. r. bugensis range
Both species range
L. Superior
L. Huron
St. Lawrence R.
L. Michigan
L. Ontario
L. Erie
Mississippi R.
Tippecanoe R.
Arkansas R.
San Justo L.
Colorado R.
L. Matthews
0 500 km
B
Dreisssena polymorpha
D. rostriformis bugensis
Both species
D. polymorpha range
Both species range
Baltic Sea
North Sea
Atlantic Ocean
Mediterranean Sea
Black Sea
Caspian Sea
Dnieper R.
S. Bug R.
Danube R.
Ebro R.
Volga R.
0 1000 km

Table 1 Locations of (A) zebra mussel and (B) quagga mussel samples, with latitude, longitude, date of first sighting, sample size (N), microsatellite number of alleles (N_A), heterozygosity measures (H_E, expected; H_O observed), F_{IS}

Watershed/region	Sampling location	Latitude	Longitude	Year discovered	N	N_A	H_E	H_O	F_{IS}
(A) Zebra mussel									
Western expansion	1. San Justo L., CA	36.822987	−121.441801	2008	48	114	0.70	0.74	0.055
Miss. R. drainage	2. Walnut River, KS	37.891516	−96.800098	~2003	24	101	0.54	0.71	0.024
	3. Timber Creek, KS	37.353850	−96.898361	2006	24	104	0.51	0.73	0.030
Upper Miss. R.	4. Lake Pepin, WI	44.418687	−92.120517	1991	24	94	0.44	0.74	0.040
Lower Miss. R.	5. s. Miss. R., LA	30.469513	−91.196643	1993	24	126	0.59	0.79	0.026
Ohio R. drainage	6. Tippecanoe R., IN	41.109551	−86.568975	1994	24	94	0.61	0.69	0.011
Great Lakes									
L. Superior	7. Duluth, MN '95	46.743180	−92.124326	1989	24	103	0.46	0.59	0.034
	Duluth, MN '06				22	81	0.46	0.69	0.034
L. Michigan	8. Mackinac Straits, MI	45.815657	−84.793072	1989	24	84	0.52	0.66	0.023
L. Huron	9. Alpena, MI	45.068333	−83.435667	1990	24	74	0.56	0.64	0.013
L. Erie	10. Gibraltar Is., OH '02	41.657400	−82.821900	1998	24	94	0.51	0.76	0.047
	Gibraltar Is., OH '04				23	78	0.54	0.78	0.030
L. Ontario	11. Cape Vincent, NY	44.131871	−76.339059	1990	24	92	0.56	0.62	0.040
Erie Canal	12. Clyde, NY	43.080838	−76.871249	1990	18	75	0.44	0.57	0.023
Oneida Lake	13. Bridgeport, NY	43.183172	−75.979839	1991	24	116	0.52	0.75	0.031
St. Lawrence R.	14. Becancour, QC	46.240000	−72.230000	1989	27	98	0.55	0.71	0.024
Hudson R.	15. Catskill, NY '91	42.223580	−73.850380	1991	22	85	0.49	0.66	0.027
	Catskill, NY '03				18	113	0.49	0.66	0.038
Mediterranean S. drainage	16. Ebro R., ESP	41.252222	0.488889	2001	14	65	0.57	0.69	0.019
North S. drainage	17. L. Ijsselmeer, NLD	52.817147	5.273748	1827	23	71	0.54	0.67	0.021
Baltic S. drainage	18. Piasnica R., POL	54.831723	18.062399	1824	24	78	0.51	0.61	0.017
Black S. drainage	19. Danube R., HUN	46.833333	17.733333	1934	24	80	0.50	0.66	0.027
	20. Dnieper R., UKR	46.380000	32.340000	Native	30	151	0.62	0.80	0.023
Caspian S. drainage	21. Volga R., RUS	56.510000	35.340000	Native	26	107	0.49	0.77	0.036
(B) Quagga mussels									
Western expansion	A. L. Matthews, CA	33.843977	−117.441005	2007	10	39	0.34	0.50	0.032
	B. L. Mead, NV	36.057493	−114.749212	2007	24	56	0.40	0.70	0.043
Great Lakes									
L. Michigan	C. Grand Haven, MI	43.051010	−86.174650	~1997	24	95	0.46	0.85	0.046
L. Huron	D. Charity Is., MI	44.028982	−83.436818	~2000	24	110	0.49	0.83	0.041
L. Erie	E. Gibraltar Is., OH '02	41.657400	−82.821900	~1998	16	49	0.53	0.70	0.025
	Gibraltar Is., OH '07				25	90	0.57	0.79	0.045
L. Ontario	F. Olcott, NY	43.341584	−78.717157	~1990	22	69	0.45	0.72	0.038
	G. Cape Vincent, NY	44.131871	−76.339059	~1990	19	56	0.50	0.64	0.037
Oneida Lake	H. Bridgeport, NY	43.183172	−75.979839	~2008	20	88	0.52	0.77	0.033
St. Lawrence R.	I. Montreal, QC	45.300000	−73.350000	~1992	14	68	0.50	0.75	0.033
Black Sea	J. Bug R., UKR	46.712121	31.965884	Native	17	57	0.46	0.76	0.040
	K. Dnieper R., UKR	46.380000	32.340000	Native	30	75	0.41	0.72	0.043
Caspian Sea	L. Volga R., RUS	51.939930	47.305180	1992	24	90	0.51	0.81	0.036

Year discovered obtained from USGS (http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/) for North America, and DAISIE (http://www.europe-aliens.org/) for Europe

performed in our laboratory using 10 µl reaction volumes of 0.6 units *Taq*, 50 µM nucleotides, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris–HCl, 0.5 µM of each primer, and 30 ng of template; with a mineral oil overlay to maintain reaction volume. A thermal cycle of 2 min at 94°C for initial denaturation was followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 1 min at 52–58°C (primer-specific; Table 2), and extension at 72°C for 30 s; capped by a final extension at 72°C for 5 min. Amplification products were diluted 1:50, of which 1 µl was added to 13 µl of formamide and ABI (Applied Biosystems, Inc.; Foster City, California) Gene Scan 500 size standard, and loaded onto a 96-well plate for analysis on an ABI 3130xl Genetic Analyzer using GeneMapper v4.0. Output profiles were checked to confirm allelic size variants.

A portion of the mtDNA cyt *b* gene was amplified from randomly selected representative individuals to confirm species identity at sites using polymerase chain reactions (PCR) of 25 µl, including 1 unit *Taq* polymerase, 200 µM dNTPs, 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris–HCl, 0.5 µM of each primer (151F, 270R; Merritt et al. 1998), and 30 ng of template. Amplification on a MJR DYAD thermal-cycler (Bio-Rad Laboratories, Hercules, CA) consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min; concluded by a final 72°C extension for 3 min.

Sequencing of the mitochondrial (mt) cytochrome (cyt) *b* gene (336 bp) used the primers 151F and 270R, and was outsourced to the Cornell University Life Sciences Core Laboratories Center (http://cores.lifesciences.cornell.edu/brcinfo, Ithaca, NY). Sequences then were trimmed by us to remove primer sequences, aligned with CLUSTAL X v2.0 (Larkin et al. 2007), and adjusted using BIOEDIT v7.0 (Hall 1999, 2004). These sequences are deposited in

Table 2 Microsatellite primers used for amplification of (A) zebra mussel, (B) quagga mussel DNA, with source, annealing temperature (*T*A), the range of repeat numbers (R_N) and sizes (R_S; bp), number of alleles (N_A), average observed heterozygosity (H_O), and average F_{IS} and F_{ST} for each microsatellite locus

Locus	Source	T_A (°C)	R_N	R_S (bp)	N_A	H_O	F_{IS}	F_{ST}
(A) Zebra mussel								
Dpol A6	Naish and Boulding (2001)	58	14–53	280–397	29	0.833	0.028	0.089
Dpol B8	Naish and Boulding (2001)	54	4–90	122–380	58	0.704	0.053	0.232
Dpol B9	Naish and Boulding (2001)	58	6–75	195–402	46	0.873	0.031	0.054
*Dpo*04	Feldheim et al. (in review)	50	5–52	225–366	13	0.615	0.085	0.050
*Dpo*101	Feldheim et al. (in review)	52	2–43	186–339	35	0.811	0.012	0.050
*Dpo*171	Feldheim et al. (in review)	50	1–74	125–271	41	0.853	0.052	0.070
*Dpo*173	Feldheim et al. (in review)	52	12–60	96–288	38	0.708	0.015	0.134
*Dpo*221	Feldheim et al. (in review)	52	3–46	66–195	34	0.776	0.036	0.122
*Dpo*260	Feldheim et al. (in review)	50	1–46	112–292	35	0.716	0.042	0.114
*Dpo*272	Feldheim et al. (in review)	50	1–37	142–286	13	0.382	0.010	0.106
*Dpo*281	Feldheim et al. (in review)	52	1–79	124–280	44	0.617	0.033	0.307
(B) Quagga mussel								
*Dbug*1	Wilson et al. (1999)	56	4–55	121–325	36	0.787	0.060	0.125
*Dbug*4	Wilson et al. (1999)	56	1–66	158–353	31	0.758	0.069	0.038
*Dbug*5	Wilson et al. (1999)	62	4–68	214–388	40	0.908	0.047	0.035
*Dbu*74	Feldheim et al. (in review)	50	5–77	153–297	34	0.772	0.050	0.149
*Dbu*75	Feldheim et al. (in review)	50	4–19	152–212	13	0.747	0.020	0.043
*Dbu*92	Feldheim et al. (in review)	50	2–34	170–298	13	0.541	0.028	0.080
*Dbu*93	Feldheim et al. (in review)	52	2–23	94–178	19	0.593	0.051	0.323
*Dbu*110	Feldheim et al. (in review)	52	1–25	151–199	17	0.764	0.022	0.034
*Dbu*141	Feldheim et al. (in review)	50	5–41	234–378	25	0.846	0.023	0.070

GenBank as accession numbers GQ988724-32 for *D. polymorpha* and GQ988733-45 for *D. rostriformis bugensis*.

Genetic analyses

ARLEQUIN (v3.11; http://cmpg.unibe.ch/software/arlequin3; Excoffier et al. 2005) was used to assign individuals to the correct mtDNA cyt *b* haplotype; and allelic frequencies, number of private alleles, conformance of μsats to Hardy–Weinberg (HW) equilibrium expectations, and linkage disequilibrium were evaluated in GENEPOP v4.0 (http://kimura.univ-montp2.fr/%7Erousset/Genepop.htm; Rousset 2008). HW deviations were tested for heterozygosity deficiency or excess, and for the presence of null alleles using MICRO-CHECKER v2.23 (http://www.microchecker.hull.ac.uk; van Oosterhout et al. 2004, 2006). Levels of significance for all tests were adjusted using Bonferroni correction (Sokal and Rohlf 1995).

Microsatellite genetic composition of the samples was analyzed to identify true populations (i.e., those distinguished by significantly divergent gene pools) using the *F*-statistic analog θ_{ST} (Weir and Cockerham 1984) and contingency tests (Raymond and Rousset 1995; Goudet et al. 1996). Relationships among recently diverged samples, such as those tested here, have been shown to be better resolved in models using contingency tests (see Balloux and Lugon-Moulin 2002), which are independent of HW equilibrium assumptions, non-parametric, and unaffected by sample size (Raymond and Rousset 1995; Goudet et al. 1996). Our use of the *F*-statistic analog facilitated direct comparisons with other studies (see Brown and Stepien 2008, 2009). Probability values of both tests were adjusted using sequential Bonferroni corrections (Rice 1989). In order to further analyze the relationships among population sites, pairwise genetic distances were calculated based on the microsatellite data using Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) and neighbor-joining trees (Saitou and Nei 1987) were constructed in PHYLIP v.3.68 (http://www.phylip.com/; Felsenstein 1989). The trees were rooted to their respective close relatives *D. stankovici* and *D. rostriformis grimmi* (see Stepien et al. 2003, 2005 for phylogeny) using microsatellite data from Feldheim et al. (in review). Three-dimensional factorial correspondence analysis (3D-FCA; Benzecri 1973) was used to examine population divisions in GENETIX v.4.05 (http://www.genetix.univ-montp2.fr/genetix/genetix.htm; Belkhir et al. 2004) via a visual representation of the relationships among populations without *a priori* assumptions about populations, evaluating variation within and among sites.

Populations that experience a genetic bottleneck usually exhibit a decrease in the number of alleles at polymorphic microsatellite loci faster than their respective heterozygosity declines (Luikart et al. 1998a). In order to detect such a signal, a test for heterozygosity excess was carried out using the program BOTTLENECK 1.2.02 (http://www.ensam.inra.fr/URLB/bottleneck/bottleneck.html; Cornuet and Luikart 1996; Piry et al. 1999). This test compared the observed gene diversity (Nei 1987) to the expected equilibrium gene diversity, which is calculated from the observed number of alleles assuming a constant-size (i.e., equilibrium) population (Luikart et al. 1998b). Wilcoxon tests using a stepwise mutation model were used to establish whether the number of loci showing heterozygosity excess was significantly greater than that expected in populations at equilibrium, with distribution of expected gene diversity at equilibrium estimated from 10,000 simulations (Cornuet and Luikart 1996).

The Bayesian model-based methods of Rannala and Mountain (1997) in STRUCTURE v 2.3.1 (http://pritch.bsd.uchicago.edu/structure.html; Pritchard et al. 2000; Pritchard and Wen 2004) and GENECLASS2 (http://www.ensam.inra.fr/URLB/GeneClass2/Installation.htm; Piry et al. 2004) use the individual as the unit, assigning it to the most likely population group(s) regardless of geographic origin. STRUCTURE was used to assign individuals to population groups, ranging from $K = 1$ (a single population group, i.e., the null hypothesis of panmixia) to $K = N$ (the total N of sampling groups), with the relative frequency of individual membership per group totaling 1.00. Ten independent runs for each K were used with burn-ins of 100,000 replicates and 1,000,000 replicates. We complemented this analysis with assignment tests using GENECLASS2, which assigned each individual a probability of membership among each sampled location using simulated population sizes of 10,000 individuals per sampling site and a 0.01 rejection level (Cornuet et al. 1999).

Results

We recovered 386 zebra mussel alleles from 11 loci and 228 quagga mussel alleles using nine loci. All populations for both species conformed to Hardy–Weinberg equilibrium expectations, all loci were unlinked, and there was no evidence of null alleles.

Our results support that both dreissenid introductions to North America originated from multiple Eurasian sources. For the zebra mussel, the 3D-FCA (Fig. 2A) and the neighbor-joining tree (Fig. 3A) analyses indicate at least 2 different introductions, with Lake Erie samples from both years being distinct from other North American samples. The majority of the North American samples group with introduced locations in northern Europe and the Volga River, but the Lake Erie samples do not group with any locations we tested here.

The 3D-FCA (Fig. 2B) and the neighbor-joining tree (Fig. 3B) for quagga mussels are largely congruent, highlighting distinct introduction events—one that contributed the majority of the diversity found in North American samples, and a second that influenced diversity in Lake Erie (evident in samples from both years). Quagga mussels that were introduced to the North American Great Lakes appear to trace to the general area of the Southern Bug and Dnieper River estuaries, which are geographically proximate estuaries ($\sim$60 km).

Invasive population structure

All introduced zebra mussel populations appear significantly different from each other in pairwise tests (Tables 3A, 4A), showing a high degree of genetic heterogeneity across their ranges, with the exception of a single comparison between the Kansas locations. These two locations do not significantly differ from each other, but diverge from all other populations (Table 3A). Similarly, the only population sites with low self-assignment in the GENECLASS test were the Kansas samples, which had high assignments to each other (Table 5A). Bayesian assignment analysis reveals 5 major groups within the zebra mussel data set (Fig. 4B). One (colored blue in Fig. 4A) links most of the introduced Eurasian populations (sites 16–19) with the Volga River Russia samples (site 21). A second group (colored pink) differentiates the Lake Erie (site 10, '02, '04) population from all others. The third group (colored tan) links the Dnieper River (site 20) with samples from the Mississippi River drainage (sites 4–6), Oneida Lake (site 13) and the Hudson River (site 15, 2003 only). The fourth group (colored green) links samples from the upper Great Lakes (sites 7–9) and the earlier Hudson River sampling date (site 15, 1991). The final group (colored brown) assigns zebra mussel locations in the western United States (site 1–3) to a group that includes the Lake Ontario (site 11) and St. Lawrence River (site 14) samples, suggesting that the eastern Great Lakes may have served as the source for this westward expansion. This grouping also has strong support in the 3D-FCA (Fig. 2A) and the neighbor-joining tree (Fig. 3A).

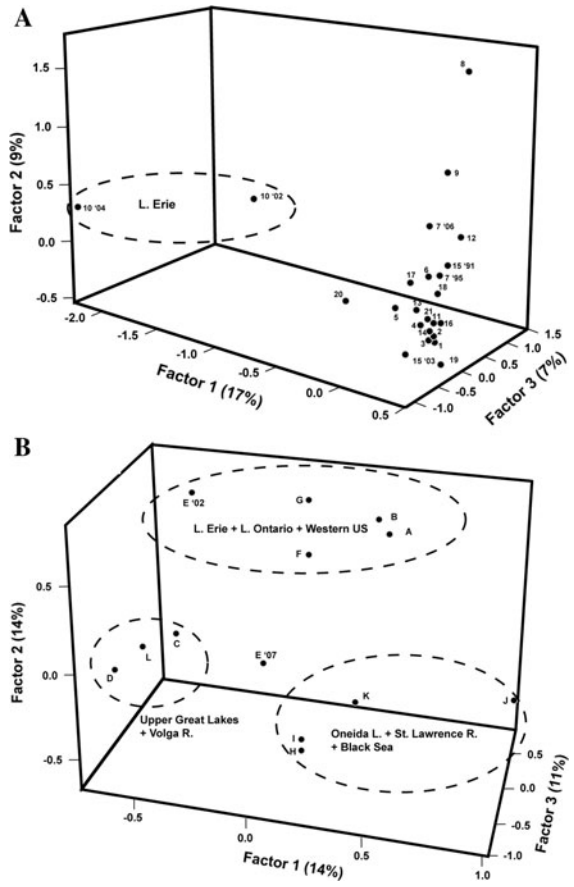

Fig. 2 Three-dimensional factorial correspondence analysis (GENETIX v.4.05; Belkhir et al. 2004) for **A** zebra mussels and **B** quagga mussels. **A** Zebra mussels in L. Erie were distinct from all other samples. **B** Quagga mussel samples separate into 3 distinct groups, with western North American samples clustering with those from L. Ontario. Years are given for samples from the same location, but from different years

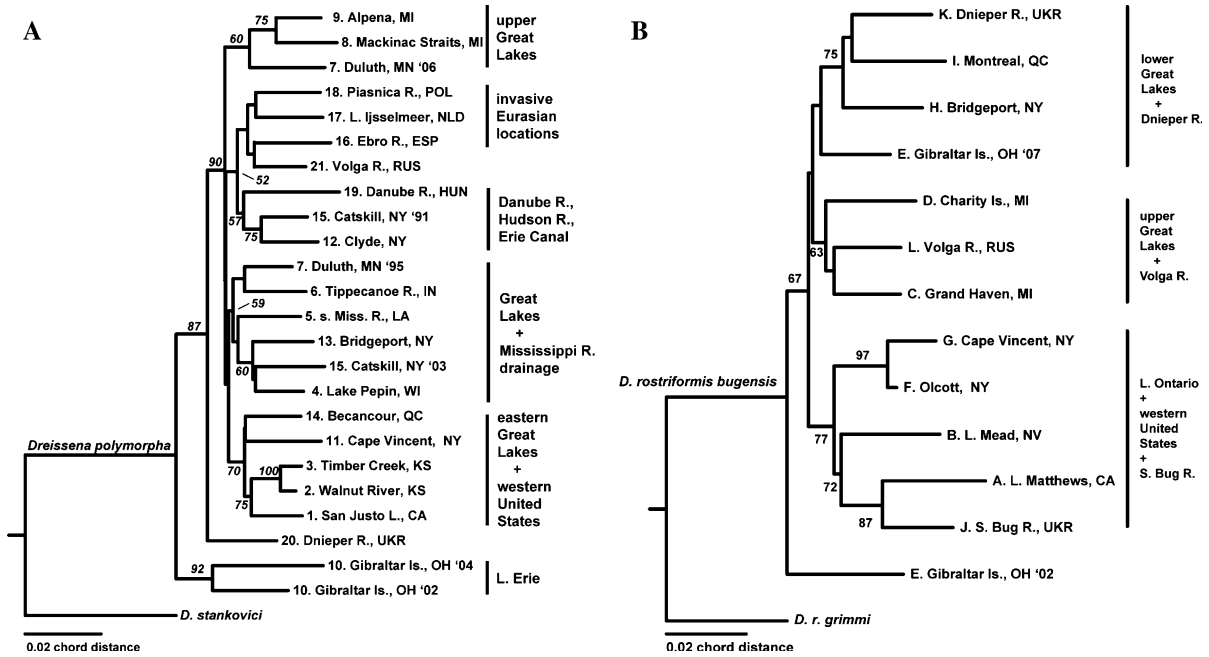

Fig. 3 Genetic distance trees among **A** zebra mussel and **B** quagga mussel population sites constructed in PHYLIP v3.6 (Felsenstein 1989) with Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) based on microsatellite data. Bootstrap percentage values are from 1000 pseudoreplicates. Branch lengths are proportional to genetic divergences. Years are given for samples from the same location, but from different years

All quagga mussel populations significantly differ from one another in the pairwise tests (Table 3B). Their AMOVA groupings also are significant (Table 4A), and the populations show high self-assignment (Table 5B). The 3D-FCA (Fig. 2B), neighbor-joining tree (Fig. 3B), and Bayesian assignment test (Fig. 4B) are congruent in recovering 3 population groups. The first grouping links quagga mussel populations from the upper Great Lakes (sites C–D) with the invasive Volga River sample (site L). The second group comprises samples from Oneida Lake (H), the St. Lawrence River (I), the Dnieper River (site K), and some individuals from Lake Erie (E) in 2007. Quagga mussel sampling locations in the western United States (sites A, B) group with the Lake Ontario (F, G) samples in the 3D-FCA (Fig. 2B), the neighbor-joining tree (Fig. 3B), and the Bayesian assignment analysis (Fig. 4B); these suggests that Lake Ontario was a likely source for the quagga mussels in the Colorado River and southern California—similar to the pattern shown in zebra mussels.

Genetic diversity levels

Individual zebra mussel sampling locations have 65–151 total alleles (Table 1). Loci *Dpo*04 and *Dpo*272 have the fewest alleles (13), whereas locus *Dpol*B8 has the most (58; Table 2A). The introduced population site in Spain (map location 16) possesses the fewest alleles, and the native Dnieper River (site 20) population has the greatest number (Fig. 1A). Great Lakes samples average 90 alleles, whereas native Eurasian populations average 113, which is a significant difference (*t*-test $t = 2.168$, $df = 13$, $P = 0.0492$). Samples from long established locations in the Mississippi River Basin (sites 4–6) average 105 alleles and the recent western expansion locations (1–3) average 106 alleles. Both are significantly higher than the average for the Great Lakes locations ($P = 0.042$ and $P = 0.002$, respectively). Zebra mussels show a decrease in average number of alleles per location from Eurasia to the Great Lakes, but subsequent expansion areas have higher numbers of alleles. However, these consist mostly of low

Table 3 Pairwise tests for (A) zebra mussel and (B) quagga mussel locations

	1	2	3	4	5	6	7 '95	7 '06	8	9	10 '02	10 '04	11
(A) Zebra mussel													
1. San Justo L., CA	~	**	**	**	**	**	**	**	**	**	**	**	**
2. Walnut River, KS	0.050**	~	NS	**	**	**	**	**	**	**	**	**	**
3. Timber Creek, KS	0.049**	0.006	~	**	**	**	**	**	**	**	**	**	**
4. Lake Pepin, WI	0.082**	0.054**	0.058**	~	**	**	**	**	**	**	**	**	**
5. s. Miss. R., LA	0.077**	0.080**	0.079**	0.054**	~	**	**	**	**	**	**	**	**
6. Tippecanoe R., IN	0.116**	0.107**	0.110**	0.100**	0.072**	~	**	**	**	**	**	**	**
7. Duluth, MN '95	0.110**	0.094**	0.097**	0.083**	0.091**	0.052**	~	**	**	**	**	**	**
Duluth, MN '06	0.088**	0.125**	0.107**	0.130**	0.099**	0.156**	0.154**	~	**	**	**	**	**
8. Mackinac Straits, MI	0.143**	0.175**	0.162**	0.158**	0.148**	0.125**	0.091**	0.116**	~	**	**	**	**
9. Alpena, MI	0.162**	0.151**	0.155**	0.127**	0.118**	0.069**	0.064**	0.153**	0.065**	~	**	**	**
10. Gibraltar Is., OH '02	0.170**	0.164**	0.161**	0.163**	0.122**	0.153**	0.177**	0.191**	0.221**	0.214**	~	**	**
Gibraltar Is., OH '04	0.175**	0.184**	0.184**	0.161**	0.115**	0.172**	0.192**	0.182**	0.217**	0.211**	0.091**	~	**
11. Cape Vincent, NY	0.091**	0.048**	0.047**	0.088**	0.130**	0.161**	0.132**	0.170**	0.216**	0.184**	0.210**	0.225**	~
12. Clyde, NY	0.189**	0.191**	0.182**	0.186**	0.183**	0.157**	0.119**	0.195**	0.155**	0.133**	0.241**	0.263**	0.226**
13. Bridgeport, NY	0.101**	0.081**	0.076**	0.045**	0.057**	0.062**	0.071**	0.110**	0.125**	0.107**	0.161**	0.156**	0.136**
14. Becancour, QC	0.054**	0.029**	0.037**	0.065**	0.075**	0.099**	0.090**	0.109**	0.160**	0.146**	0.167**	0.176**	0.058**
15. Catskill, NY '91	0.153**	0.157**	0.149**	0.125**	0.125**	0.088**	0.098**	0.164**	0.125**	0.102**	0.209**	0.210**	0.192**
Catskill, NY '03	0.078**	0.060**	0.054**	0.022**	0.055**	0.107**	0.106**	0.118**	0.164**	0.164**	0.144**	0.136**	0.101**
16. Ebro R., ESP	0.134**	0.134**	0.120**	0.099**	0.087**	0.141**	0.164**	0.171**	0.212**	0.175**	0.175**	0.170**	0.180**
17. L. Ijsselmeer, NLD	0.131**	0.134**	0.142**	0.119**	0.081**	0.137**	0.161**	0.171**	0.211**	0.154**	0.172**	0.166**	0.196**
18. Piasnica R., POL	0.128**	0.144**	0.140**	0.134**	0.116**	0.151**	0.195**	0.152**	0.217**	0.206**	0.205**	0.210**	0.209**
19. Danube R., HUN	0.141**	0.131**	0.120**	0.134**	0.118**	0.167**	0.155**	0.169**	0.206**	0.180**	0.188**	0.205**	0.171**
20. Dnieper R., UKR	0.091**	0.085**	0.087**	0.073**	0.036**	0.067**	0.090**	0.097**	0.137**	0.118**	0.120**	0.112**	0.123**
21. Volga R., RUS	0.083**	0.091**	0.079**	0.067**	0.067**	0.124**	0.111**	0.120**	0.159**	0.134**	0.150**	0.147**	0.114**

	12	13	14	15 '91	15 '03	16	17	18	19	20	21
(A) Zebra mussel											
1. San Justo L., CA	**	**	**	**	**	**	**	**	**	**	**
2. Walnut River, KS	**	**	**	**	**	**	**	**	**	**	**
3. Timber Creek, KS	**	**	**	**	**	**	**	**	**	**	**
4. Lake Pepin, WI	**	**	**	**	**	**	**	**	**	**	**
5. s. Miss. R., LA	**	**	**	**	**	**	**	**	**	**	**

Table 3 continued

	12	13	14	15 '91	15 '03	16	17	18	19	20	21
6. Tippecanoe R., IN	**	**	**	**	**	**	**	**	**	**	**
7. Duluth, MN '95	**	**	**	**	**	**	**	**	**	**	**
Duluth, MN '06	**	**	**	**	**	**	**	**	**	**	**
8. Mackinac Straits, MI	**	**	**	**	**	**	**	**	**	**	**
9. Alpena, MI	**	**	**	**	**	**	**	**	**	**	**
10. Gibraltar Is., OH '02	**	**	**	**	**	**	**	**	**	**	**
Gibraltar Is., OH '04	**	**	**	**	**	**	**	**	**	**	**
11. Cape Vincent, NY	**	**	**	**	**	**	**	**	**	**	**
12. Clyde, NY	~	**	**	**	**	**	**	**	**	**	**
13. Bridgeport, NY	0.165**	~	**	**	**	**	**	**	**	**	**
14. Becancour, QC	0.180**	0.079**	~	**	**	**	**	**	**	**	**
15. Catskill, NY '91	0.051**	0.108**	0.144**	~	**	**	**	**	**	**	**
Catskill, NY '03	0.204**	0.046**	0.061**	0.140**	~	**	**	**	**	**	**
16. Ebro R., ESP	0.165**	0.101**	0.130**	0.085**	0.093**	~	**	**	**	**	**
17. L. Ijsselmeer, NLD	0.168**	0.130**	0.132**	0.116**	0.135**	0.076**	~	**	**	**	**
18. Piasnica R., POL	0.186**	0.146**	0.137**	0.140**	0.145**	0.107**	0.090**	~	**	**	**
19. Danube R., HUN	0.137**	0.143**	0.141**	0.122**	0.130**	0.114**	0.117**	0.141**	~	**	**
20. Dnieper R., UKR	0.158**	0.051**	0.075**	0.121**	0.066**	0.093**	0.078**	0.121**	0.109**	~	**
21. Volga R., RUS	0.139**	0.100**	0.088**	0.115**	0.073**	0.050**	0.068**	0.095**	0.085**	0.084**	~

	A	B	C	D	E '02	E '07	F	G	H	I	J	K	L
(B) Quagga mussel													
A. L. Matthews, CA	~	**	**	**	**	**	**	**	**	**	**	**	**
B. L. Mead, NV	0.267**	~	**	**	**	**	**	**	**	**	**	**	**
C. Grand Haven, MI	0.211**	0.094**	~	**	**	**	**	**	**	**	**	**	**
D. Charity Is., MI	0.215**	0.094**	0.008	~	**	**	**	**	**	**	**	**	**
E. Gibraltar Is., OH '02	0.293**	0.172**	0.104**	0.125**	~	**	**	**	**	**	**	**	**
Gibraltar Is., OH '07	0.173**	0.094**	0.038**	0.027**	0.124**	~	**	**	**	**	**	**	**
F. Olcott, NY	0.212**	0.113**	0.057**	0.080**	0.064**	0.089**	~	*	**	**	**	**	**
G. Cape Vincent, NY	0.297**	0.176**	0.122**	0.150**	0.093**	0.158**	0.016	~	**	**	**	**	**
H. Bridgeport, NY	0.180**	0.151**	0.055**	0.052**	0.164**	0.056**	0.108**	0.188**	~	**	**	**	**
I. Montreal, QC	0.223**	0.152**	0.069**	0.071**	0.188**	0.069**	0.124**	0.204**	0.034**	~	**	**	**
J. Bug R., UKR	0.118**	0.122**	0.092**	0.099**	0.153**	0.077**	0.087**	0.143**	0.068**	0.099**	~	**	**

Table 3 continued

	A	B	C	D	E '02	E '07	F	G	H	I	J	K	L
K. Dnieper R., UKR	0.245**	0.142**	0.090**	0.088**	0.185**	0.083**	0.131**	0.193**	0.062**	0.056**	0.115**	~	**
L. Volga R., RUS	0.214**	0.125**	0.029**	0.034**	0.097**	0.051**	0.061**	0.129**	0.080**	0.089**	0.116**	0.104**	~

Below diagonal = F_{ST}, above diagonal = contingency test

* $P < 0.05$, ** sig. after Bonferroni correction (Rice 1989), *NS* not significant

frequency alleles (<0.01%) that also are found in various Great Lakes locations. Great Lakes zebra mussel samples have average observed heterozygosity (H_O) values that are slightly lower than those found in the native Eurasian range (0.67 ± 0.08 vs. 0.74 ± 0.09), which is significant ($t = 2.700$, $df = 13$, $P = 0.018$), although the heterozygosity distribution does not suggest a genetic bottleneck for the North American introduction ($P = 0.735$) in the BOTTLENECK analysis. Zebra mussel populations outside the Great Lakes have somewhat lower H_O (0.67 ± 0.08) than those within the Great Lakes (0.73 ± 0.04), which is not significant ($t = -1.789$, $df = 16$, $P = 0.093$). However, testing for genetic bottlenecks indicates that the three western expansion locations experienced bottlenecks ($P = 0.0001$ in each). In contrast, this analysis approach suggests that populations from Lake Superior ($p = 0.011$) and Lake Ontario ($p = 0.005$) underwent continuous population expansions.

Quagga mussel samples have 39–110 total alleles (Table 1), with loci *Dbu*75 and *Dbu*92 having the fewest alleles (13) and locus *Dbug*05 having the most (40; Table 2B). The introduced site in California (site A) has the fewest number of alleles, and the introduced population in Lake Huron (site D) possesses the most. Great Lakes sites average 78 alleles and native locations average 74; whose means significantly differ ($t = 4.763$, $df = 9$, $P = 0.001$). The recently established location in California and Nevada averages 48 alleles, which is significantly lower than the Great Lakes locations ($t = 4.014$, $df = 8$, $P = 0.004$). Great Lakes populations of quagga mussels have an average H_O that appears slightly lower than in their native Eurasian range (0.76 ± 0.08 vs. 0.74 ± 0.05), but is not significant ($t = -0.709$, $df = 9$, $P = 0.496$). Quagga mussel samples outside the Great Lakes have lower H_O than those found within (0.76 ± 0.08 vs. 0.60 ± 0.18), which is significant ($t = 4.007$, $df = 8$, $P = 0.039$). Great Lakes populations of quagga mussels have a greater than average number of alleles, but do not differ in observed heterozygosity from native Eurasian populations, with their heterozygosity distribution refuting a genetic bottleneck ($P = 0.338$). However, recent western expansions of the quagga mussel have lower average numbers of alleles and lower observed heterozygosities, supporting a founder effect. Moreover, heterozygosity excesses detected by the

Table 4 Analysis Of MOlecular VAriance (AMOVA; Excoffier et al. 1992) results showing partitioning of genetic variation within and among (A) zebra mussel and (B) quagga mussel sampling sites using microsatellites (μsat)

Division of variation partitioning tested	Measure of variation partitioning	
(A) Zebra mussel		
Among 5 primary population groups: (1–3, 11, 14); (12, 15–19, 21); (4–7 '95, 13, 15 '03); (10, 20); (7 '06, 8, 9)	Proportion of variation	0.06
	Φ_{CT}	0.06**
Among sampling sites within the groups	Proportion of variation	0.08
	Φ_{SC}	0.08**
Within sampling sites	Proportion of variation	0.86
	Φ_{ST}	0.14**
(B) Quagga mussel		
Among 3 primary population groups: (A, B, F, G, J); (C, D, H, I, L); (E, K)	Proportion of variation	0.02
	Φ_{CT}	0.05**
Among sampling sites within the groups	Proportion of variation	0.10
	Φ_{SC}	0.10**
Within sampling sites	Proportion of variation	0.89
	Φ_{ST}	0.11**

** Φ-statistics (*F*-statistic analogues) significant at $P < 0.0001$. A. Comparisons among five primary population groups: (1–3, 11, 14); (12, 15–19, 21); (4–7 '95, 13, 15 '03); (10, 20); (7 '06, 8, 9). B. Comparisons among three primary quagga mussels population groups: (A, B, F, G, J); (C, D, H, I, L); (E, K)

BOTTLENECK analysis for Lake Matthews CA ($P = 0.018$) and Lake Mead NV ($P = 0.040$) samples support a genetic bottleneck in the western expansion of the quagga mussel. The reduction appears greatest in the California sample, which traces to an origin from Lake Mead via the Colorado River aqueduct. Two samples show signs of population expansion detectable in the BOTTLENECK analysis: Lake Huron ($P = 0.003$) and western Lake Ontario ($P = 0.024$); these suggest rapid, continuous population growth following introduction.

Temporal genetic change

Temporal comparisons among three zebra mussel sampling locations reveal significant differences among years using both F_{ST} and contingency tests (Table 3A), with strong self-assignment using GENECLASS (Table 5A). Thus all samples are most similar to their original population compositions, despite some temporal changes. Temporal comparisons of zebra mussel samples from Lake Erie (−16 alleles; 17%; $\theta_{ST} = 0.091$, $P < 0.0001$) and Lake Superior (−22 alleles; 21%; $\theta_{ST} = 0.154$, $P < 0.0001$) show a net loss of alleles (Table 1), whereas the Hudson River population reveals a net gain of alleles (+28 alleles; 33%; $\theta_{ST} = 0.140$, $P < 0.0001$). According to the BOTTLENECK analysis, the Lake Superior population lost the signal of rapid population expansion between 1995 and 2006. None of the other time pair comparisons show demographic signals in the BOTTLENECK analysis, suggesting that those changes did not result from changes in the size of the reproducing population.

For quagga mussels, only the Lake Erie location was tested for temporal change, and the two sampling years show a significant difference, with a large gain in alleles (+41 alleles; 84%; $\theta_{ST} = 0.124$, $P < 0.0001$). This renders the 2007 Lake Erie samples more similar to those from the St. Lawrence River and from the Black Sea, possibly due to population mixing caused by additional introductions into Lake Erie (Figs. 2B, 3B).

Discussion

Multiple founding sources contributed to significant genetic structure among dreissenid mussel populations of both species across their North American invasive ranges, likely reflecting some maintenance of native population differences due to differential colonization and expansion. In addition, the relatively small founder effects shown in the invasive dreissenid populations highlight the ease of introducing

Table 5 Assignment test using GENECLASS2 (Piry et al. 2004) (A) zebra mussels, (B) quagga mussels

	1	2	3	4	5	6	7 '95	7 '06	8	9	10 '02	10 '04	11	12	13	14	15 '91	15 '03	16	17	18	19	20	21	% Correct
(A) Zebra mussel																									
1. San Justo L., CA	**44**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	92
2. Walnut River, KS	2	**7**	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
3. Timber Creek, KS	1	15	**6**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	25
4. Lake Pepin, WI	0	0	0	**11**	2	0	2	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	46
5. s. Miss. R., LA	0	0	0	0	**13**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	54
6. Tippecanoe R., IN	0	0	0	0	0	**19**	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	79
7. Duluth, MN '95	0	0	0	0	1	1	**13**	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	54
Duluth, MN '06	1	0	1	0	0	0	0	**15**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	68
8. Mackinac Straits, MI	0	1	0	0	0	1	0	1	**16**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	67
9. Alpena, MI	0	0	0	0	0	0	1	0	0	**19**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	79
10. Gibraltar Is., OH '02	0	0	0	0	0	0	0	0	0	0	**17**	0	0	0	0	0	0	0	0	0	0	0	3	0	71
Gibraltar Is., OH '04	0	0	0	0	0	0	0	0	0	0	0	**17**	0	0	0	0	0	0	0	0	0	0	0	0	74
11. Cape Vincent, NY	1	0	0	0	0	0	1	0	0	0	0	0	**14**	0	0	1	0	1	0	0	0	0	1	0	58
12. Clyde, NY	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	0	0	3	0	0	0	0	1	1	0	50
13. Bridgeport, NY	0	0	0	1	1	0	0	0	0	0	0	0	0	0	**14**	0	0	1	0	0	0	0	1	0	58
14. Becancour, QC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**24**	0	1	0	0	0	0	0	0	89
15. Catskill, NY '91	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**16**	0	1	0	0	0	0	0	73
Catskill, NY '03	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**5**	0	0	0	0	2	0	28
16. Ebro R., ESP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	0	0	0	0	1	71
17. L. Ijsselmeer, NLD	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**16**	0	0	0	3	70
18. Piasnica R., POL	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	**14**	0	1	3	58
19. Danube R., HUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**21**	0	0	88
20. Dnieper R., UKR	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**19**	0	63
21. Volga R., RUS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	**18**	69

	A	B	C	D	E '02	E '07	F	G	H	I	J	K	L	% Correct
(B) Quagga mussel														
A. L. Matthews, CA	**2**	0	0	0	0	0	1	0	1	0	4	0	0	20
B. L. Mead, NV	0	**18**	1	3	0	1	0	0	0	0	0	0	0	75
C. Grand Haven, MI	0	0	**9**	6	0	0	0	0	1	0	0	0	1	38
D. Charity Is., MI	0	0	2	**11**	0	0	0	0	0	0	0	0	2	46
E. Gibraltar Is., OH '02	0	0	0	0	**11**	2	0	0	0	0	0	0	0	69
Gibraltar Is., OH '07	0	0	1	2	0	**13**	0	0	1	0	0	0	0	52
F. Olcott, NY	0	0	2	1	0	0	**9**	7	0	2	0	0	0	41
G. Cape Vincent, NY	0	0	2	0	0	0	9	**7**	0	0	0	0	0	37
H. Bridgeport, NY	0	0	0	3	0	0	0	0	**10**	1	0	0	0	50

Table 5 continued

	A	B	C	D	E '02	E '07	F	G	H	I	J	K	L	% Correct
I. Montreal, QC	0	0	0	1	0	1	0	0	3	**2**	0	0	0	14
J. Bug R., UKR	2	2	1	0	0	0	0	0	1	0	**9**	1	0	53
K. Dnieper R., UKR	0	0	0	0	0	0	0	0	0	1	0	**17**	0	57
L. Volga R., RUS	0	0	0	2	0	0	0	0	0	0	0	0	**15**	**63**

Self-assignment is in bold down diagonal, percent correctly assigned is on the right. Most individuals assigned to their population of origin, and those that did not usually assigned to a nearby location

these organisms in large numbers—especially during their veliger larval stages. In both species, samples from Lake Erie appear genetically distinct, suggesting the likelihood of separate introductions from Eurasia. This appears especially likely given that both Mills et al. (1996) and Carlton (2008) found evidence of Lake Erie samples of both species (including shells on beaches, reports from divers on natural gas wellheads in northern Lake Erie, on water treatment filters in Ontario, and on the hull of a fishing vessel) preceding the formal discovery of dreissenid mussels in the Great Lakes. Boileau and Hebert (1993) also found that the Lake Erie population of the zebra mussel appeared genetically distinct from other Great Lakes and European populations using allozymes (which may have been influenced by the "cryptic" presence of the quagga mussel). Like Boileau and Hebert (1993), the Lake Erie zebra mussels we sampled do not show strong connections with any of the analyzed European locations in the present study. However, Stepien et al. (2002) found that Lake Erie samples were most similar to those from Poland and the Netherlands using randomly amplified nuclear polymorphic DNA. Our samples from those regions grouped with the other Great Lakes populations, and not with Lake Erie. Additional sampling and analyses from possible source regions thus is warranted.

Previously invaded locations often serve as hubs for further spread of an invader (Bossenbroek et al. 2009; Floerl et al. 2009; Kelly et al. 2009), which appears likely for the zebra mussel. These genotypes may be pre-adapted for invasive success. For example, the bloody red shrimp *Hemimysis anomala* is another Ponto-Caspian invader whose previously invaded Northern European locations served as sources for their establishment in the North American Great Lakes (Audzijonyte et al. 2008). Likewise, the Chinese mitten crab has been introduced to the Great Lakes from several European ports, but has failed to establish (Tepolt et al. 2007). Similarly, the Eurasian ruffe *Gymnocephalus cernuus* invaded the Great Lakes from the Elbe River via the Baltic Sea region (Stepien et al. 2005). The Great Lakes populations then served as such a hub for dreissenid expansion into the rest of North America (this study), and are predicted to serve as hubs for future further expansion into other parts of the world (Kelly et al. 2009).

In contrast to zebra mussels, North American quagga mussels appear to have arrived directly from

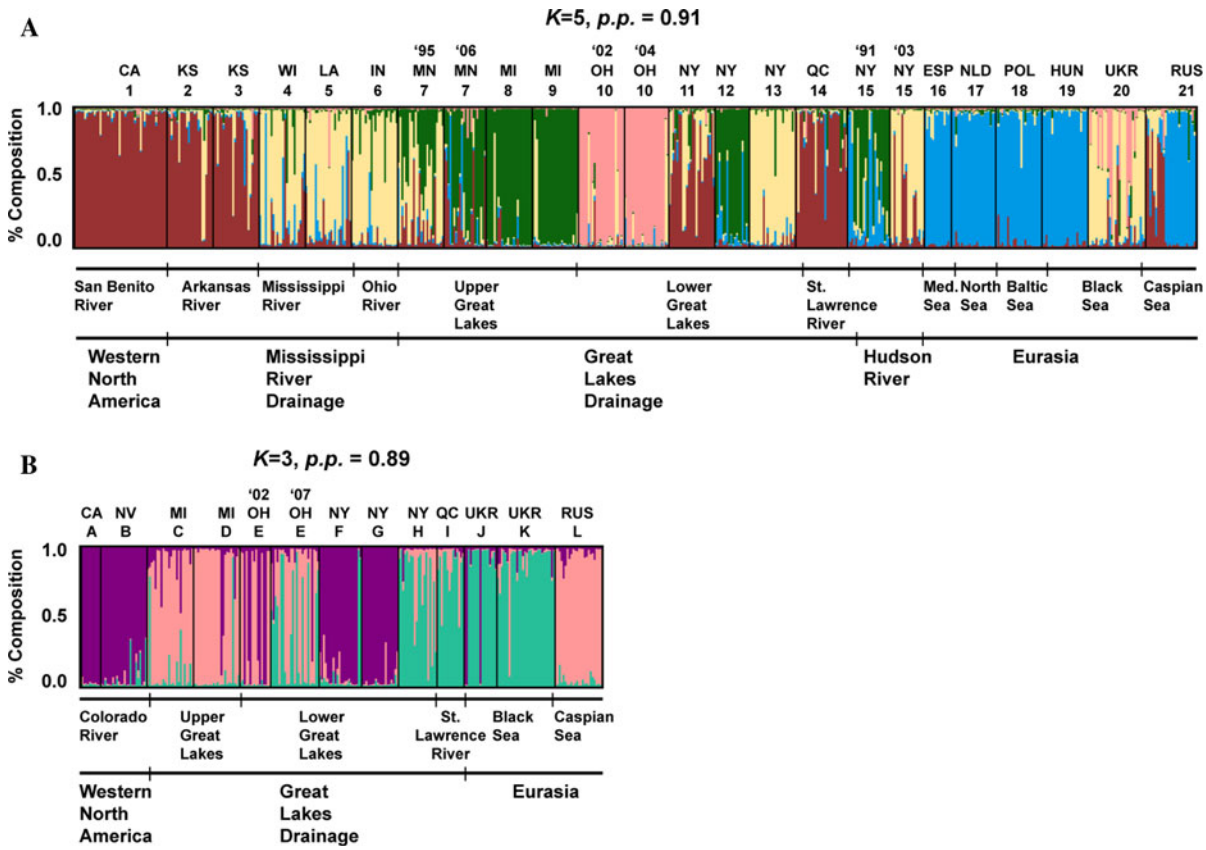

Fig. 4 Bayesian STRUCTURE analysis (Pritchard et al. 2000; Pritchard and Wen 2004) for **A** zebra mussels and **B** quagga mussels. Analysis discerned 5 groups (posterior probability = 0.91) for zebra mussels and 3 groups (posterior probability = 0.89) for quagga mussels. Both species show evidence of multiple introductions. Recent populations established in western North America link to the eastern portions of the Great Lakes. Years are given for samples from the same location, but from different years

their native Ponto-Caspian region, with their invasive populations tracing to the Southern Bug and Dnieper Rivers. This is congruent with results from Stepien et al. (2002), who analyzed nuclear RAPD markers to discern that quagga mussels from western Lake Erie were distinct from other North American quagga mussels, with all tracing back to a Dnieper River source (that study preceded our collections from the Southern Bug River that we analyzed here). Similarly, the North American round goby *Neogobius melanostomus* invasion traced back to the Dnieper River (Brown and Stepien 2009). The Dnieper River watershed thus served as a common source population for invasive species that became successfully established in the Great Lakes. The geographic difference in founding sources discerned between the two dreissenid species' suggests that zebra and quagga mussels each arrived in the Great Lakes in unrelated introduction events—the zebra mussel originating on ships from the Baltic Sea and northern Europe, and the quagga mussel on ships from the Black Sea. These correspond to two of the main invasion pathways out of the Ponto-Caspian identified previously (Ricciardi and MacIsaac 2000; Grigorovich et al. 2002).

The observed heterozygosities we found for sampling locations of the North American quagga mussel (average $H_O = 0.73$) appears higher than the diversity Therriault et al. (2005) discerned for invasive quagga mussel locations along the Volga River in Europe (average $H_O = 0.50$; although the latter used fewer microsatellite markers than we did—6 compared to our 9). The number of alleles per population is similar between the two studies when populations

are adjusted for sample size, suggesting that introductions in North America and the Volga River each had large founding populations.

Neither species experienced a genetic bottleneck when it was introduced to North America suggesting that a large number of propagules were introduced into the Great Lakes, which likely contributed to their successful establishment. Likewise, the brown mussel *Perna perna* introduction into the Gulf of Mexico did not show a bottleneck and successfully expanded its range following ballast water introduction from multiple sources in South America and southern Africa (Holland 2001). Similarly, the round goby did not undergo a genetic bottleneck during its introduction into the Great Lakes (Stepien and Tumeo 2006; Brown and Stepien 2009). All of these invasion examples thus were founded by large numbers of successful colonists. The high volumes of ship ballast tanks facilitate massive introductions (Kelly et al. 2009), providing early life history stages of species introduced by this vector a good chance of establishment (Bax et al. 2003; Drake and Lodge 2004; Holeck et al. 2004).

We also detected fine scale patterns of divergences among North American populations of both dreissenid mussel species. Their distinct population genetic structures likely resulted from long distance "jump" dispersal patterns from divergent sources via ballast water or recreational boating, followed by local spread. Many of these populations have high genetic variation, likely due to large founding numbers that may have been supplemented by continued migration events via commercial and recreational boating traffic. The genetic bottlenecks shown in the western populations of both species suggest that they likely were founded by relatively small numbers of individuals, with little subsequent gene flow. This fits the model of low-frequency transport via recreational boats predicted for dreissenid expansions into the western regions of North America (Johnson et al. 2001; Bossenbroek et al. 2007). In contrast, our samples from the edges of the Great Lake show evidence for having experienced periods of rapid growth, likely fueled by large introductions via commercial shipping. We discern significantly different genetic compositions of zebra mussel populations between the northern and southern Mississippi River sites, corroborating the gradient observed from allozyme data by Elderkin and Klerks (2001) and from nuclear AFLPs (amplified frequency length polymorphisms) by Elderkin et al. (2004). This genetic structure is attributed to boat-mediated introductions and the difficulty for free-spawning mussels to maintain consistent recruitment in lotic systems (Elderkin and Klerks 2001; Elderkin et al. 2004). Stepien et al. (2002) also found a marked genetic difference between the southern Mississippi River samples of the zebra mussel versus those from the Great Lakes. That neutral genetic difference may be accompanied by adaptive variation of genotypes in the south versus the north, which merits further analysis of quantitative adaptive traits and their heritability.

The significant temporal change in genetic structure of the tested populations suggests some allelic turnover at those locations, due to contribution by some recruits from other areas. However, the colonies retain their original population genetic structure, thus exhibiting genetic "resilience". Haag and Garton (1995) found significant genetic differences among zebra mussel larvae and adults from western Lake Erie (the same location as our Lake Erie samples), indicating possible selection. Additionally, the temporal differences observed in our zebra mussel samples from the Hudson River likely are linked to the cycling population dynamics observed in that system, where large year classes dominate recruitment on a 2–4 year cycle, with population size fluctuating over an order of magnitude (Strayer and Malcolm 2006). We detected evidence for rapid population expansion in our 1995 Lake Superior samples, but by 2006 the population had stabilized, and no such signal remained. These signals are ephemeral (Cornuet and Luikart 1996) and would be predicted to fade after a decade, as found here. Although we did not test for temporal sampling regimes in other populations, additional evidence indicates that 10 years is sufficient for zebra mussel populations to return to equilibrium after demographic events. When Boileau and Hebert (1993) examined the newly established Oneida Lake zebra mussel population, it had much lower heterozygosity than their other North American samples. A decade later, our Oneida Lake sample has a heterozygosity level that is very similar to those characterizing Great Lakes sites, suggesting that its initial bottleneck faded with time and was augmented by new recruitment. Similarly, the invasion of New Zealand by

Drosophila pseudoobscura revealed a founder effect, followed by a return to equilibrium values within two decades, although that invasive population—unlike dreissenids—retained low genetic diversity (Reiland et al. 2002).

The 100th Meridian Initiative

Western expansion populations of both dreissenid species did not originate from the closest geographic sources—which would have been either the Mississippi River basin or the southwestern Great Lakes. Instead, they appear to trace to population origins in the eastern Great Lakes. This represents a possible mixed success for the 100th Meridian Initiative (Bossenbroek et al. 2009), with our study results suggesting that few individuals colonized from the Mississippi River drainage or the western Great Lakes, where 100th Meridian control resources were concentrated (Mangin 2001). Instead, it appears that the successful colonists originated from areas much further east—in Lake Ontario and the St. Lawrence River. This sort of long-distance dispersal event is hard to predict and observe (Buchan and Padilla 1999; Leung et al. 2006), but can be detected using genetic methods, as shown in our study.

Under the 100th Meridian Initiative, more easterly population areas may not have received the program's intensive education programs that focused on the western frontier of the zebra mussel's distribution, rather than towards its core distribution in the Great Lakes. The effectiveness of those education programs is supported by the apparent lack of spread from targeted regions, as shown by our findings and by the slowing spread of infestations to inland lakes in areas with intense education campaigns (Johnson et al. 2006; J. Bossenbroek, personal communication, 2009). Thus, education efforts carried out to prevent the spread of dreissenid mussels likely were effective (Johnson et al. 2001) in those target areas, but should have been more comprehensive.

Conclusions

The dreissenid mussel introductions to North America show some similarities between the two species and with other Great Lakes invaders, as well as some key differences. Notably, both quagga and zebra mussels were introduced from multiple source locations in Europe. North American populations of zebra mussels originated from multiple previously-invaded locations within northern Europe and the Baltic Sea regions. In contrast, quagga mussels colonized directly from native populations originating from estuaries of the Southern Bug and Dnieper Rivers. The genetic differences of these historic colonization patterns led to population structure observed in the Great Lakes for both species, which are genetically heterogeneous across their ranges and show considerable population genetic divergence across North America. Genetic diversity levels in both species reflect no overall founder effects in comparisons of the Great Lakes populations to likely Eurasian source locations, indicating very high numbers of introduced individuals. Zebra mussel populations outside of and within the Great Lakes have similar levels of genetic diversity, whereas quagga mussel populations show reduced genetic diversity in newly established western populations. Once established, the genetic composition of given populations genetically changed over time, but retained their original genetic signatures. External recruitment and long distance gene flow contributed to population genetic diversity, which increased over time. Since the genetic signatures of these populations remain distinctive, showing closest assignment with themselves, the genotypes that became established first retained "genetic population resilience" over time, leading to differentiation among sites. These population factors thus significantly shaped the genetic identity of the dreissenid mussel invasions and provided the raw material governing their genetic adaptations.

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