

Molecular ecology of zebra mussel invasions

GEMMA E. MAY,* GREGORY W. GELEMBIUK,* VADIM E. PANOV,† MARINA I. ORLOVA† and CAROL EUNMI LEE*

*Wisconsin Institute of Rapid Evolution, Department of Zoology, 430 Lincoln Drive, University of Wisconsin-Madison, Madison, WI 53706, USA, †Zoological Institute of the Russian Academy of Sciences, Universitetskaya emb. 1, 199034 St Petersburg, Russia

Abstract

The invasion of the zebra mussel, *Dreissena polymorpha*, into North American waters has resulted in profound ecological disturbances and large monetary losses. This study examined the invasion history and patterns of genetic diversity among endemic and invading populations of zebra mussels using DNA sequences from the mitochondrial cytochrome oxidase I (COI) gene. Patterns of haplotype frequency indicate that all invasive populations of zebra mussels from North America and Europe originated from the Ponto-Caspian Sea region. The distribution of haplotypes was consistent with invasive populations arising from the Black Sea drainage, but could not exclude the possibility of an origin from the Caspian Sea drainage. Similar haplotype frequencies among North American populations of *D. polymorpha* suggest colonization by a single founding population. There was no evidence of invasive populations arising from tectonic lakes in Turkey, while lakes in Greece and Macedonia contained only *Dreissena stankovici*. Populations in Turkey might be members of a sibling species complex of *D. polymorpha*. Ponto-Caspian derived populations of *D. polymorpha* ($\theta = 0.0011$) and *Dreissena bugensis* (one haplotype) exhibited low levels of genetic diversity at the COI gene, perhaps as a result of repeated population bottlenecks. In contrast, geographically isolated tectonic lake populations exhibited relatively high levels of genetic diversity ($\theta = 0.0032$ to 0.0134). It is possible that the fluctuating environment of the Ponto-Caspian basin facilitated the colonizing habit of invasive populations of *D. polymorpha* and *D. bugensis*. Our findings were concordant with the general trend of destructive freshwater invaders in the Great Lakes arising from the Ponto-Caspian Sea basin.

Keywords: bivalve, colonization, introductions, invasive species, quagga mussels, range expansion

Received 30 March 2005; revision accepted 31 October 2005

Introduction

The introduction of exotic species into native ecosystems has been responsible for destruction of habitat, loss of species diversity, and the extinction of native species (Pimental *et al.* 2000). Aquatic ecosystems have been heavily impacted. For example, over 170 nonindigenous species are now established in the Laurentian Great Lakes (Grigorovich *et al.* 2003). The zebra mussel *Dreissena polymorpha* (Pallas, 1771) ranks among the most destructive exotic invaders in North American waters with an economic impact estimated in the billions of dollars (OTA 1993). Although many studies have focused on the ecological impact of zebra mussels, little is known about the geographical history of their invasions and patterns of genetic differentiation

among and within populations in their native and invaded ranges. In conjunction with data from other invaders, such knowledge might help predict future invasions and facilitate understanding of invasions in an evolutionary context, since potential sources might differ in characteristics critical for invasive success. Thus, the objectives of this study were to (i) reconstruct geographical pathways of invasions, and (ii) characterize patterns of genetic diversity within and among populations in the invaded and endemic ranges of zebra mussels, as well as those of other species in the genus *Dreissena*.

The endemic range of zebra mussels includes brackish and fresh waters of the northern regions of the Ponto-Caspian (Black, Caspian, and Azov) Sea drainages and brackish and freshwater tectonic lakes south of the Ponto-Caspian Seas (Kinzelbach 1986, 1992; Starobogatov 1994a). The endemic range of the subspecies *D. polymorpha polymorpha* includes estuaries and lower reaches of large

Correspondence: Carol Eunmi Lee, Fax: 608-265-6320; E-mail: carolle@wisc.edu

rivers draining into the northern regions of the Black and Caspian Seas (Starobogatov 1994a). In the 18th century, *D. p. polymorpha* began to expand beyond its endemic range following the construction of canal systems between Eurasian river basins (Jazdzewski 1980; Bij de Vaate *et al.* 2002). The establishment of waterways linking the Ponto-Caspian Seas with the Baltic and North Seas in the 19th century resulted in shipping traffic that facilitated rapid invasions of *D. p. polymorpha* throughout European waters (De Martonne 1927; Jazdzewski 1980; Bij de Vaate *et al.* 2002). Within North America, *D. p. polymorpha* was first reported in Lake St Clair, Ontario, in 1988, and was most likely introduced as veliger larvae in ballast water of transoceanic vessels (Hebert *et al.* 1989). These mussels have colonized vast reaches of interconnected waterways in Europe and North America and are continuing to expand their range (Pollux *et al.* 2003; Drake & Bossenbroek 2004; Orlova & Panov 2004).

Despite ample historical information, ultimate sources of zebra mussel invasions have remained elusive and subject to much speculation. Zebra mussels in North America have been hypothesized to originate from the Caspian/Aral Sea drainage (Ludyanskiy 1993), the Black Sea drainage (McMahon *et al.* 1994; Rosenberg & Ludyanskiy 1994; Spidle *et al.* 1994), or multiple European locations (Stepien *et al.* 2002). Another possibility raised by Strayer (1999) was that the invasions could have originated from ancient Eurasian freshwater lakes, such as those in Greece, Macedonia, and Turkey. A Ponto-Caspian origin has been viewed as most plausible, given that numerous species endemic to the Ponto-Caspian basin have invaded the Great Lakes since the mid-1980s (Lee & Bell 1999; Ricciardi & MacIsaac 2000; Reid & Orlova 2002). These invaders include a second dreissenid, the quagga mussel *Dreissena bugensis*, native to the estuaries of the northern Black Sea. Quagga mussels are currently displacing zebra mussels in parts of the Great Lakes (Mills *et al.* 1999; Jarvis *et al.* 2000).

Broad sampling of the endemic range is a unique feature of this study. In order to reconstruct geographical pathways of invasions, we sampled invasive populations in North America and Europe, and endemic populations from the Ponto-Caspian basin, and from lakes in Greece, Macedonia, and Turkey. Previous attempts to identify sources of invasive zebra mussels using genetic markers have been limited by a lack of sampling from the endemic range (Marsden *et al.* 1995; Stepien *et al.* 1999, 2002; Astanei *et al.* 2005). Our analysis employed DNA sequences (606 bp) from the mitochondrial cytochrome oxidase I (COI) gene. We analysed patterns of COI haplotype frequencies from the invasive and endemic range of *D. polymorpha* in order to reconstruct geographical pathways of invasion. Our second objective, to characterize patterns of genetic diversity within and among populations, would reveal demographic characteristics of these populations, and may provide evi-

dence of population bottlenecks. Dreissenid populations inhabiting the Ponto-Caspian Seas and their estuaries would have been subjected to extreme fluctuations in sea level, salinity, and habitat boundaries. In contrast, populations inhabiting ancient lakes in Greece, Macedonia, and Turkey would have experienced relatively stable environments. We anticipated that the resulting demographic processes would have produced distinctive genetic signatures in COI haplotype composition, including differences in genetic diversity (also see Gelembiuk *et al.* in this issue).

Materials and methods

Population sampling

Our sampling included populations from both endemic and invaded ranges of the *Dreissena polymorpha* species complex (members of the subgenus *Dreissena sensu stricto*). By 'endemic' we are referring to its range during the Holocene. By this definition, the endemic range of the invasive subspecies *D. polymorpha polymorpha* resides primarily in the estuaries and lower reaches of large rivers in the northern portions of the Ponto-Caspian Seas, and not in the main basins of these seas (Starobogatov & Andreeva 1994). Specifically, the Black Sea proper had not contained *D. polymorpha* or any other dreissenid since the last seawater incursion from the Mediterranean c. 7000–10 000 years ago (Babak 1983; Reid & Orlova 2002). The near-coastal regions of the Caspian Sea proper contain *D. polymorpha andrusovi* and possibly other sibling species of uncertain systematic status (Rosenberg & Ludyanskiy 1994). In the Aral Sea, *D. polymorpha obtusicarinata* and *D. caspia pallasii* once occurred, but are now extinct due to increases in salinity, while *D. polymorpha aralensis* currently occurs in rivers and lakes adjacent to this sea (Starobogatov & Andreeva 1994).

Populations of *D. p. polymorpha* were collected between 2000 and 2003 from a wide geographical area that included the North American Great Lakes, Western Europe, and the Black and Caspian Sea drainages (Fig. 1; Table 1). *D. polymorpha* contains many morphotypes, or sibling species (Kinzelbach 1992; Schütt 1993; Rosenberg & Ludyanskiy 1994), for which systematic relationships are unclear. Morphologically distinct samples of *Dreissena* corresponding to the described geographical range of *D. polymorpha anatolica* and *D. polymorpha gallandi* (Locard 1893) were collected in 2003 from several lakes in Turkey (Fig. 1; sites 22–28). These two sets of specimens will be referred to throughout this paper using the provisional *D. polymorpha gallandi* and *D. polymorpha anatolica* subspecific designations. Another sample of *Dreissena* collected in 2003 from the Seyhan Dam in Turkey (Fig. 1; site 27) morphologically resembled *Dreissena caputlacus* (Schütt 1993), and the type locality of this species is nearby. These specimens will be referred to using the provisional *D. caputlacus* designation.

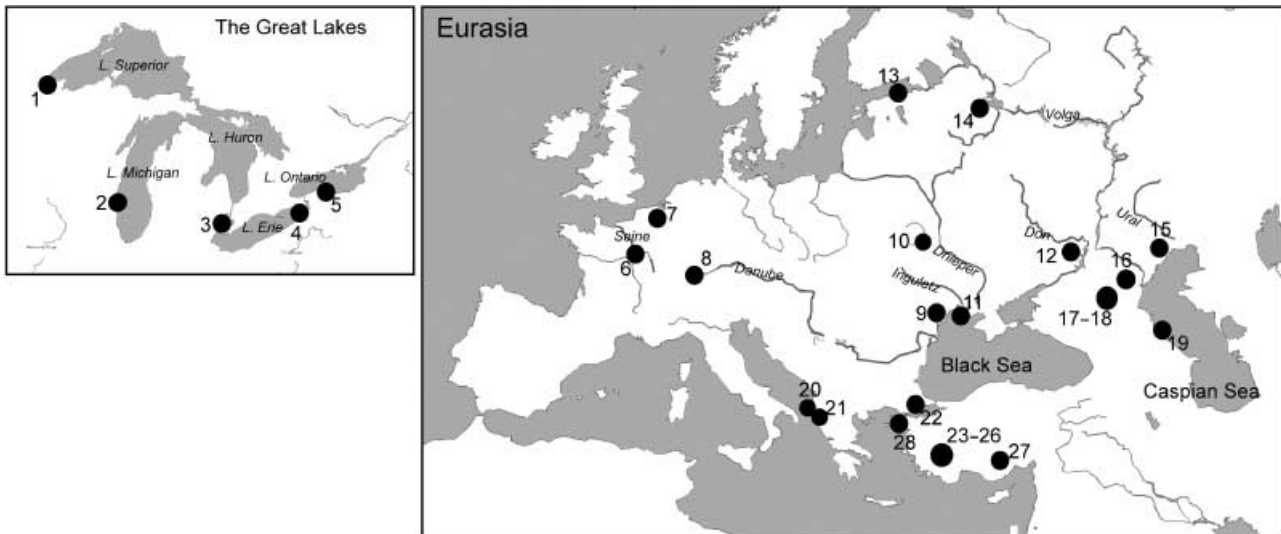


Fig. 1 Sampling locations of populations of the zebra mussel, *Dreissena polymorpha*, and species of *Dreissena*. Populations are numbered from 1 to 28 (listed in Table 1), with North American populations shown in the map on the left, and Eurasian populations shown in the map on the right. Lakes Ohrid and Prespa (sites 20 and 21) are in closer proximity than shown.

Our study also included additional subgenera of *Dreissena* (*Carinodreissena* and *Pontodreissena*) following the classification of Starobogatov (1994b) and Rosenberg & Ludyanskiy (1994). *Dreissena stankovici*, belonging to the subgenus *Carinodreissena*, were originally referred to as *D. polymorpha* (Stanković 1960), but are now recognized as a distinct species endemic to Lake Ohrid in Macedonia and Albania (L'vova & Starobogatov 1982; Starobogatov 1994b). We collected *D. stankovici* samples from Lakes Ohrid and Prespa in the summer of 2001. Species and subspecies of *Pontodreissena*, *Dreissena bugensis*, *Dreissena rostriformis distincta*, and *Dreissena rostriformis compressa* (Logvinenko & Starobogatov 1968; Starobogatov 1977), were collected from the Great Lakes, Volga River, and the Caspian Sea between 2000 and 2001 (Table 1). The subgenus *Pontodreissena* inhabits a broad range of habitats, from brackish seas to freshwater rivers and reservoirs. For example, *D. bugensis* occurs in fresh water with salinities up to 1 PSU (Neveeskaya 1965), while *D. rostriformis* inhabits the middle and southern Caspian Seas at salinities of 7–12 PSU (Rosenberg & Ludyanskiy 1994). *D. rostriformis* is divided into several subspecies, including *D. r. distincta*, and *D. r. compressa* (Logvinenko & Starobogatov 1968; Starobogatov 1994b), which often overlap morphologically, but are distributed at different depths (Rosenberg & Ludyanskiy 1994; Starobogatov & Andreeva 1994).

DNA Extraction and Cytochrome Oxidase I DNA sequencing

Genomic DNA was extracted from muscle tissues of ethanol-preserved samples using a methylene chloride/isoamyl

alcohol (MCIA) DNA extraction protocol (Maureen Krause, personal communication, 2000) as follows: muscle tissue was excised from individuals and 20 mg of tissue was dissolved in 800 μ L of extraction buffer (1 \times SSC, 1.1% SDS, 0.4 mg Proteinase K) and incubated at 55 $^{\circ}$ C for 16–20 h. Samples were mixed with 260 μ L of 5 M NaCl and 88 μ L of MCIA (24:1 MCIA), incubated on ice for 1–2 h, and centrifuged for 5 min at 2000 g, then 10 min at 6000 g. The aqueous phase was re-extracted with 880 μ L of MCIA using a 5-min centrifugation at 6000 g. DNA was precipitated with two volumes of 100% EtOH and dissolved in 50 μ L of Tris-EDTA pH 8.5. Polymerase chain reaction (PCR) primers COIL 1490 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994) and COI-CB (5'-GAATCTCCTAACCTGTWGGATCAA-3') (Claxton *et al.* 1998) were used to amplify a 710-bp fragment of COI. A temperature profile of 33 cycles of 94 $^{\circ}$ C (30 s), 59 $^{\circ}$ C (60 s), and 72 $^{\circ}$ C (45 s) was used for PCR amplification. PCR product was run on 1.5% agarose gels, excised, and purified using a QIAquick extraction kit (QIAGEN). PCR product was cycle-sequenced using ABI BigDye version 2 and sequenced using an ABI PRISM 377XL automated sequencer (Applied Biosystems). For all species, complementary strands of most individuals were sequenced to confirm accuracy. A total of 606 nucleotide positions of the COI gene were analysed for 280 individuals of *D. p. polymorpha*, 21 individuals of *D. bugensis*, 3 individuals of *D. rostriformis*, and 25 individuals of *D. p. gallandi*, 603 nucleotide positions for 81 individuals of *D. p. anatolica*, 599 nucleotide positions for 27 individuals of *D. caputlacus*, and 591 nucleotide positions for 18 individuals of *D. stankovici*.

Table 1 Sampled populations of *Dreissena*

Site	Country (city)	Body of water	Subgenus	Species	Sample size
1	USA	Lake Superior	<i>Dreissena s. str.</i> *	<i>D. p. polymorpha</i>	19
2	USA	Lake Michigan	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	19
3	USA	Lake St. Clair	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	19
4	USA	Lake Erie	<i>Pontodreissena</i>	<i>D. bugensis</i>	3
5	USA	Lake Ontario	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	17
			<i>Pontodreissena</i>	<i>D. bugensis</i>	3
6	France (Vernon)	Seine River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	14
7	The Netherlands (Amsterdam)	IJsselmeer Lake	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	18
8	Germany (Kelheim)	Danube River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	18
9	Ukraine (Zaichevskoie)	Ingulets River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	17
10	Ukraine (Kiev)	Dnieper River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	13
11	Ukraine (Kherson)	Dnieper River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	19
12	Russia (Volgograd)	Volga River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	19
13	Russia (St Petersburg)	Gulf of Finland	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	20
14	Russia (Rybinsk)	Volga River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	14
			<i>Pontodreissena</i>	<i>D. bugensis</i>	2
15	Kazhakstan (Atyrau)	Ural River	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	20
16	Russia (Astrakhan)	Volga River Delta	<i>Pontodreissena</i>	<i>D. bugensis</i>	14
			<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	6
17	Russia (Liman)	Caspian Sea canal	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	4
			<i>Pontodreissena</i>	<i>D. bugensis</i>	1
18	Russia (Lagan)	Caspian Sea canal	<i>Dreissena s. str.</i>	<i>D. p. polymorpha</i>	24
19	Azerbaijan	Mid Caspian Sea	<i>Pontodreissena</i>	<i>D. rostriformis</i>	3
20	Macedonia	Lake Ohrid	<i>Carinodreissena</i>	<i>D. stankovici</i>	8
21	Greece	Lake Prespa	<i>Carinodreissena</i>	<i>D. stankovici</i>	10
22	Turkey (Istanbul)	Lake Büyükçekmece	<i>Dreissena s. str.</i>	<i>D. p. gallandit</i>	20
23	Turkey	Lake Beyşehir	<i>Dreissena s. str.</i>	<i>D. p. anatolica†</i>	15
24	Turkey	Lake Kovada	<i>Dreissena s. str.</i>	<i>D. p. anatolica†</i>	19
25	Turkey	Lake Eğirdir	<i>Dreissena s. str.</i>	<i>D. p. anatolica†</i>	20
26	Turkey	Karacaören Dam	<i>Dreissena s. str.</i>	<i>D. p. anatolica†</i>	15
27	Turkey	Seyhan Dam	<i>Dreissena s. str.</i>	<i>D. p. anatolica†</i>	12
			<i>Dreissena s. str.</i>	<i>D. caputlacust</i>	27
28	Turkey	Uluabat Lake	<i>Dreissena s. str.</i>	<i>D. p. gallandit</i>	5

**sensu stricto*.

†systematic status uncertain.

Analysis of molecular covariance

In order to test the hierarchical distribution of molecular covariance for the *D. polymorpha* species complex at different geographical scales, an analysis of molecular covariance (AMOVA) (Excoffier *et al.* 1992) was performed using the software package ARLEQUIN version 2.001 (Schneider *et al.* 2000). The analysis was performed at four hierarchical scales: (i) among endemic regions [South-central Turkey (sites 23–27), Northwestern Turkey (sites 22 and 28), Black Sea (sites 9 and 11), and Caspian Sea drainages (sites 12, 15–18)], (ii) among and within endemic populations nested within regions, (iii) among nonendemic regions (North American sites 1, 2, 3, 5 and Eurasian sites 6, 7, 8, 10, 13, 14), and (iv) among and within nonendemic (invading) populations nested within regions (with the two nonendemic continental regions analysed both jointly and separately).

Analysis among endemic regions tests the degree to which molecular variation is partitioned among these regions, defined a priori according to biogeographical and morphological criteria (see below). Substantial differentiation would be expected if the endemic regions had long independent evolutionary histories. Analysis between the two nonendemic continental regions would reveal whether North America and nonendemic Eurasia show genetic differentiation. Such differentiation might occur if these invasions arose from different sources or if severe founder effects occurred during colonization of one of these regions. Analysis among and within populations nested within regions would reveal the degree of population differentiation, providing a statistic analogous to Wright's F_{ST} . Such structure within regions would arise from genetic drift and mutation given limited migration between populations. High levels of population differentiation might be expected,

for example, if site colonizations were relatively recent and bottlenecked.

A further test of invasion history was conducted by examining the variance structure of invading populations grouped with putative source populations. Specifically, invading populations were grouped with either the Black Sea or the Caspian Sea drainage in an AMOVA incorporating all endemic and invading populations. The grouping that combined the invading populations with their ultimate source would be expected to explain a larger share of the covariance.

The four endemic regions designated for the AMOVA, Caspian Sea drainage (*D. p. polymorpha*), Black Sea drainage (*D. p. polymorpha*), Northwestern Turkey (*D. p. gallandi*), and South-central Turkey (*D. p. anatolica*), were based on (i) biogeographical/phylogeographical partitions observed in other taxa, and (ii) morphology and location of population samples when compared with those of recognized subspecies of *D. polymorpha*. Specifically, Caspian and Black Sea drainages, which together constitute the native range of *D. p. polymorpha*, were treated as separate units, given differences in endemic species composition and a semi-independent history in the geological and fossil record (Reid & Orlova 2002). Such a division is also supported by the phylogeographical break observed across several species of amphipods and cladocerans (Cristescu *et al.* 2003, 2004). Specimens from Turkey were collected from sites falling within two distinct zoogeographical provinces, the Euro-Siberian and Mediterranean regions (Davis 1965). Turkey is furthermore a conglomerate of five tectonic blocks, each having separate geological and biogeographical histories (Hrbek & Meyer 2003). Lakes Büyükkçekmece (site 22) and Ulubat (site 28) are located on the Pontides block located within the Euro-Siberian region, in an area adjoining the Sea of Marmara. This area in Northwestern Turkey corresponds to the known endemic range of the subspecies *D. p. gallandi*, and shell morphology of specimens collected from these two sites appears to match the taxonomic description for this subspecies (Locard 1893; Schütt 1993). Lakes Eğirdir (site 25), Kovada (site 24), and Beyşehir (site 23), and the Karacaören (site 26) and Seyhan (site 27) reservoirs are all located within the Mediterranean region in the Menderes-Taurus (sites 23–26) and East Taurus (site 27) blocks. This area in South-central Turkey largely corresponds to the known endemic range of the subspecies *D. p. anatolica*, and shell morphology of specimens collected from these five sites also appear to match its taxonomic description (Locard 1893; Schütt 1993).

In all AMOVAs, the relative partitioning of molecular covariance was estimated using pairwise differences between the *D. polymorpha* complex COI haplotypes. Significance of covariance components was tested by permutation under the null hypothesis of no population structure.

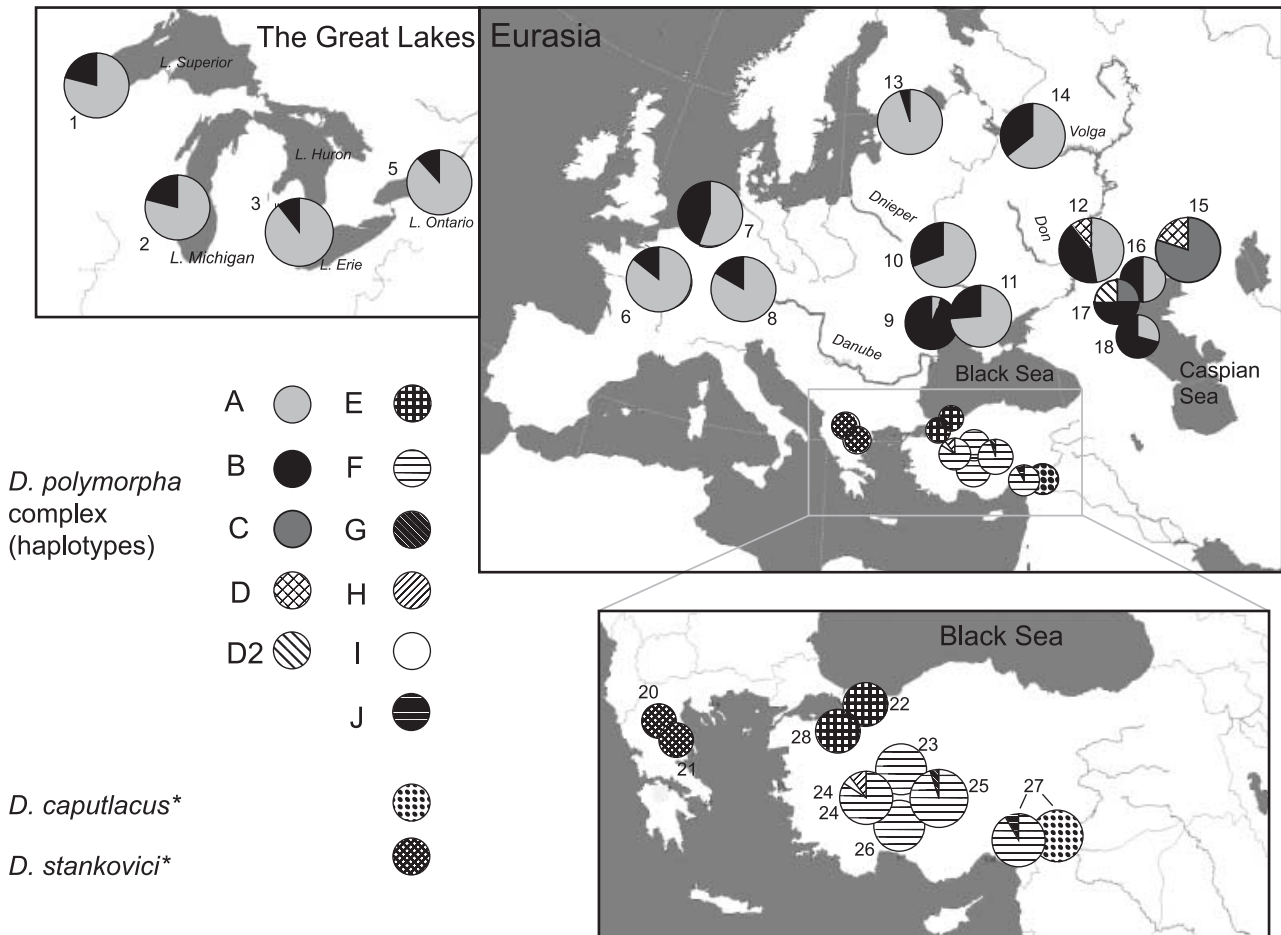
Analysis of population parameters

Population genetic parameters were estimated and coalescent analyses were conducted to extract information on genetic diversity, population size, and demographic history from the DNA sequence data. The software package DNASP version 4.0 (Rozas & Rozas 1999) was used to conduct preliminary analysis, estimating nucleotide diversity (π) and the population parameter θ ($\theta = N_e\mu$ for mtDNA, where μ = mutation rate and N_e = effective population size provided $N_e = 2N_f$; calculated from segregating sites) (Watterson 1975).

Results

Phylogeography of endemic and invading populations of *Dreissena polymorpha*

Of the haplotype diversity found in the endemic range (COI haplotypes A–J), only two haplotypes (A and B) were found in the invaded regions (Fig. 2). Haplotypes A and B were found in the Black Sea drainage, the Volga River, areas proximal to the Volga Delta, and every invasive population in North America and Eurasia. Our results are most compatible with the Black Sea drainage as the source of invasive populations, because the Caspian Sea drainage contained three unique haplotypes C, D, and D2. However, the Caspian Sea drainage could not be excluded as a potential source, as it also contained haplotypes A and B. None of the haplotypes found in tectonic lakes in Greece, Macedonia, and Turkey (sites 20–28) were found outside the endemic range. Turkish populations (sites 22–28) contained unique haplotypes E–J that were not shared elsewhere (Fig. 2) and the 'zebra mussel populations' in Lakes Prespa and Ohrid (sites 20 and 21) (Strayer 1999) proved to be a distinct species, *Dreissena stankovici* (see Phylogenetic Reconstruction in Gelembiuk *et al.* in this issue). We performed an analysis of molecular covariance (AMOVA) at multiple geographical scales (Table 2). The AMOVA revealed that much of the genetic covariance was distributed among the four endemic ranges [Northwestern Turkey (sites 22, 28), South-central Turkey (sites 23–27), Black Sea drainage (sites 9, 11), Caspian Sea drainage (sites 12, 15–18)] selected a priori based on geological and morphological systematic boundaries. The percentage of covariance among endemic regions was high, 54.89% ($P \leq 0.000$). In contrast, the covariance component distributed between nonendemic populations in Eurasia (sites 6–8, 10, 13–14) vs. those in North America (sites 1–3, 5) was low and nonsignificant (0.26%, $P \leq 0.389$), indicating a lack of subdivision between these two invaded continental regions. Further AMOVA of invading populations indicated no measurable covariance component among North American populations (0.0%, $P \leq 0.731$), and a slightly higher covariance component distributed among



*Individual haplotypes are not shown.

Fig. 2 Distribution and frequency of COI haplotypes of the *Dreissena polymorpha* complex and related species in North America (left map), Eurasia (right map), and Turkey and Greece (bottom map). Populations from North America include Lakes (1) Superior, (2) Michigan, (3) St Clair, and (5) Ontario. Populations from Eurasia include (6) Seine River, (7) IJsselmeer Lake, (8) Danube River, (9) Ingulets River, (10) Kiev Reservoir, Dnieper River, (11) Kherson, Dnieper River, (12) Volgograd, Volga River, (13) Gulf of Finland, (14) Rybinsk Reservoir, Volga River, (15) Ural River, (16) Astrakhan, Volga River, (17) Liman, Caspian Sea canal, and (18) Lagan, Caspian Sea canal. Populations from Turkey and Greece include (20) Lake Ohrid, (21) Lake Prespa, (22) Lake Büyükçekmece, (23) Lake Beyşehir, (24) Lake Kovada, (25) Lake Eğirdir, (26) Karacaören Dam, (27) Seyhan Dam, and Uluabat Lake (28).

invading Eurasian populations (7.38%, $P \leq 0.054$). These results suggest a higher degree of similarity among North American populations than among nonendemic Eurasian populations.

The covariance component distributed between Black Sea drainage populations vs. all nonendemic populations pooled with Caspian Sea drainage populations was low and nonsignificant (3.06%, $P \leq 0.246$). In contrast, the covariance component distributed between Caspian Sea drainage populations and nonendemic populations pooled with Black Sea drainage populations was much higher and significant (19.84%, $P \leq 0.008$). Thus, significant subdivision exists between the Caspian Sea drainage and all other populations of *D. p. polymorpha*, while there is greater genetic proximity between the invasive populations and

those in the Black Sea drainage. As a whole, these AMOVA results are consistent with the observation that populations from Turkey and the Caspian Sea drainage possess haplotypes and haplotype frequencies that are distinct from those in Europe, North America, and the Black Sea drainage (Fig. 2).

Intraspecific genetic polymorphism

Genetic diversity was low in *D. polymorpha*, *D. bugensis* and *D. rostriformis* (Table 3). In addition, COI sequences from *D. rostriformis distincta* and *D. rostriformis compressa* were identical, even though morphological characters from field samples could distinguish them. In contrast, levels of polymorphism were much higher for *D. caputlacus* and

Table 2 Results from an analysis of molecular covariance (AMOVA; Excoffier *et al.* 1992) among haplotypes at multiple geographical scales: among endemic regions [between south-central Turkey (sites 23–27), northwestern Turkey (sites 22, 28), and Black (sites 9, 11) and Caspian Sea (sites 12, 15–18) drainages], among and within endemic populations, among nonendemic regions (North American sites 1, 2, 3, 5 and Eurasian sites 6, 7, 8, 10, 13, 14), and among and within nonendemic populations. The last column shows the probability of obtaining an estimated covariance component equal to or larger than the observed value under the null hypothesis of an absence of structure at that level (tested by permutation)

Source of variation	DF	SS	Covariance components	% Variation	Fixation indices	Probability
(1) a) Among BSD, CSD, S Turkey, NW Turkey	3	43.92	0.2588	54.89	$F_{CT} = 0.549$	$P \leq 0.000$
b) Among populations	10	13.57	0.0853	18.09	$F_{ST} = 0.730$	$P \leq 0.000$
c) Within populations	201	25.61	0.1274	27.03	$F_{SC} = 0.401$	$P \leq 0.000$
(2) a) Between nonendemic Eurasia and NA	1	0.24	-0.0004*	-0.26	$F_{CT} = -0.003$	$P \leq 0.389$
b) Among populations	8	2.14	0.0064	3.91	$F_{ST} = 0.037$	$P \leq 0.112$
c) Within populations	161	25.46	0.1582	96.35	$F_{SC} = 0.039$	$P \leq 0.103$
(3) a) Between CSD vs. nonendemic populations plus BSD	1	8.12	0.0614	19.84	$F_{CT} = 0.198$	$P \leq 0.008$
b) Among populations	15	19.91	0.0709	22.91	$F_{ST} = 0.428$	$P \leq 0.000$
c) Within populations	263	46.58	0.1777	57.25	$F_{SC} = 0.286$	$P \leq 0.000$
(4) a) Between BSD vs. nonendemic populations plus CSD	1	2.41	0.0086	3.06	$F_{CT} = 0.031$	$P \leq 0.246$
b) Among populations	15	25.62	0.0941	33.64	$F_{ST} = 0.367$	$P \leq 0.000$
c) Within populations	263	46.58	0.1771	63.31	$F_{SC} = 0.347$	$P \leq 0.000$
(5) a) Among non endemic Eurasia	5	1.95	0.0137	7.38	$F_{ST} = 0.074$	$P \leq 0.054$
b) Within non endemic Eurasia	91	15.59	0.1713	92.62		
(6) a) Among NA populations	3	0.18	-0.0043*	-3.15	$F_{ST} = -0.032$	$P \leq 0.731$
b) Within NA populations	70	9.87	0.1410	103.15		

Abbreviations: Black Sea Drainage, BSD; Caspian Sea Drainage, CSD; North America, NA.

*A negative variance component was calculated. A slight negative parameter estimate can result if the parameter value is zero.

Table 3 DNA polymorphism and inferred population parameters for species of *Dreissena*

Species	No. of individuals	No. of haplotypes	No. of polymorphic sites	$\pi \pm SE$	$\theta \pm SE^*$
<i>D. p. polymorpha</i>	280	5	4	0.0011 \pm 0.000	0.0011 \pm 0.0000
<i>D. p. anatolica</i>	81	5	5	0.0017 \pm 0.000	0.0003 \pm 0.0000
<i>D. p. gallandi</i>	25	1	0		
<i>D. caputlacus</i>	27	8	19	0.0059 \pm 0.000	0.0085 \pm 0.0006
<i>D. stankovici</i>	18	14	27	0.0075 \pm 0.001	0.0134 \pm 0.0052
<i>D. bugensis</i>	21	1	0		
<i>D. rostriformis</i>	3	1	0		

*estimated from segregating sites (Watterson 1975).

D. stankovici (Table 3). Analysis of DNA polymorphisms revealed no deletion or insertion of bases for all DNA sequences across all species.

Nucleotide diversity π and the population parameter θ (estimated from segregating sites; Watterson 1975) were low in subspecies of *D. polymorpha* but much higher in *D. stankovici* and *D. caputlacus* (Table 3). This difference was particularly apparent at the level of individual populations. For individual populations of *D. polymorpha*, the median values of θ and π were low, both 0.00049 (θ range: 0, 0.0028; π range: 0, 0.0026). In contrast, these values were much higher for the two populations of *D. stankovici*

($\theta = 0.0067, 0.0108$; $\pi = 0.0041, 0.0079$), and the sole population of *D. caputlacus* ($\theta = 0.0084$; $\pi = 0.0058$). There was no measurable nucleotide diversity in sequences of *D. bugensis* or *D. rostriformis*.

Discussion

Pathways of anthropogenic invasions

Patterns of COI haplotype diversity suggest that all invasive populations of zebra mussels in Europe and North America were derived from the Ponto-Caspian Sea basin.

The North American and nonendemic European populations contained two COI haplotypes (A and B), which were shared with populations in the Black Sea drainage. While this pattern of haplotype distribution was most concordant with an origin from the Black Sea drainage for the North American and European populations, the Caspian Sea drainage could not be excluded as a potential source (see below). The analysis of molecular covariance (AMOVA, Table 2) was most consistent with the Black Sea drainage as the ultimate source of invasive populations, given that the covariance component distributed between Black Sea drainage populations vs. all invasive populations grouped with Caspian Sea drainage populations was low and nonsignificant (3.06%, $P \leq 0.246$) (Table 2). In contrast, the covariance component distributed between Caspian Sea drainage populations vs. all invasive populations grouped with Black Sea drainage populations was considerably higher and significant (19.84%, $P \leq 0.008$). This pattern of genetic covariance indicates that populations from the Caspian Sea drainage were genetically more distinct from all other populations than the populations from the Black Sea drainage. A Black Sea drainage origin was consistent with hypotheses based on temperature tolerance (McMahon *et al.* 1994) and shipping routes (J. Carlton, personal communication, in Rosenberg & Ludyanskiy 1994). In addition, such an origin is consistent with invasion pathways of several other species that have spread extensively in Europe and North America, such as the amphipod *Echinogammarus ischnus* (Cristescu *et al.* 2004), the cladoceran *Cercopagis pengoi* (Cristescu *et al.* 2001, 2004), and the quagga mussel *Dreissena bugensis* (Spidle *et al.* 1994).

The COI haplotype data provided no evidence of invasive populations arising from lakes in Turkey, Greece, and Macedonia. Only *Dreissena polymorpha* haplotypes E to J and *Dreissena caputlacus* were found in Turkish lakes, while Greek and Macedonian lakes contained only *Dreissena stankovici*.

The Caspian Sea could not be excluded as a potential source of invasive populations because it contained haplotypes A and B, in addition to haplotypes C, D, and D2. However, haplotypes A and B might not be native to the Caspian Sea drainage. The presence of these haplotypes could be the result of an invasion by Black Sea populations after the construction of the Volga-Don canal in 1952. It is also possible that Baltic Sea populations, possibly originating from the Black Sea drainage, could have colonized the Caspian Sea drainage by flowing southward down the Volga River. Patterns observed across other taxa are consistent with such a hypothesis. Bivalves native to the Black Sea basin such as *D. bugensis* and *Monodacna colourata* have heavily colonized the southern Volga River (Orlova, personal observation). Furthermore, recently established populations of the cladocerans *Cornigerius* and *Cercopagis* in reservoirs along the Volga River possessed mitochondrial

DNA haplotypes that grouped with the Black Sea populations, and not with those from the Caspian Sea, deviating from the phylogeographical break observed between Black and Caspian Sea drainages (Cristescu *et al.* 2003). Nevertheless, the possibility that *D. p. polymorpha* haplotypes A and B are native to the Caspian Sea drainage could not be excluded. Even though the Black and Caspian Seas have semi-independent histories dating back to the Miocene, they were fairly recently interconnected about 9000 years ago (Reid & Orlova 2002). The final point of contact between these seas corresponds to the low-lying Manych Depression/northeastern Caspian region, where haplotypes A and B occur in the Caspian Sea drainages.

Similar haplotype frequencies among North American populations of *D. polymorpha* (Fig. 2) tend to support the hypothesis of Marsden *et al.* (1995) of a single founding population for North America, rather than the claim of multiple independent European origins (with these origins differing among the North American Great Lakes), based on random amplified polymorphic DNAs (RAPDs) (Stepien *et al.* 2002). Results from Marsden *et al.* (1995) and Stepien *et al.* (2002) are incongruent with each other despite being based on specimens from the same locations (A. P. Spidle, personal communication, 2000). In addition, a recent study by Astanei *et al.* (2005), using five microsatellite loci, found no significant differentiation between their two Great Lakes samples (eastern Lake Ontario and Lake St Clair; $F_{ST} = -0.012$; 92% bootstrap support for monophyly), in contrast to the relatively high differentiation between lakes claimed by Stepien *et al.* (2002) (e.g. eastern Lake Ontario and western Lake Erie near Lake St Clair; $F_{ST} = 0.055 \pm 0.004$). Our data conform poorly to the claim of multiple European origins given (i) similar haplotype frequencies among North American populations (Fig. 2), with no measurable covariance component among them (Table 2), and (ii) variable haplotype frequencies in Europe (Fig. 2; Table 2). If different North American populations were colonized independently from distinct European sources, haplotype frequencies among populations in North America would likely have been more disparate. Results from an ongoing study using amplified fragment length polymorphisms (AFLPs) (Gelembiuk *et al.* in prep.) are concordant with Marsden *et al.*'s (1995) observation, based on allozymes, of reciprocal monophyly between Europe and North America. It is likely that as zebra mussel populations moved westward in the Great Lakes, genetic drift acted upon existing variation resulting in the observed slight cline in haplotype frequencies (Fig. 2).

Our study was primarily designed to examine the ultimate, endemic-range sources of zebra mussel invasions. Additional data will be required to determine whether populations in the endemic range served directly as the proximal source for the North American invasion, or whether the proximal source lies elsewhere in the European

invaded range (as hypothesized by Stepien *et al.* 1999; Astanei *et al.* 2005). Further sampling is also needed for additional key locations in the native range, including the Azov Sea and the delta of the Danube.

Lack of haplotype polymorphism and evidence for population bottlenecks

The low levels of haplotype variation in populations of *D. p. polymorpha*, *D. p. gallandi*, *D. p. anatolica*, *D. bugensis*, and *D. rostriformis* were striking (Fig. 2; Table 3). Compared to the levels of haplotype polymorphism we found for *D. p. polymorpha* (4 polymorphic sites), Stepien *et al.* (1999) found no intraspecific DNA polymorphism in either *D. p. polymorpha* or *D. bugensis* using mitochondrial 16S ribosomal RNA sequences. For *D. p. polymorpha*, the lack of polymorphism in their study might have resulted from relatively slow mutation rate of 16S rRNA and from the limited geographical scope (no Caspian Sea samples). Low levels of genetic variance at COI would not necessarily correspond to low quantitative genetic variance (e.g. in physiological or morphological traits), since several studies show that genetic variance at neutral markers and quantitative traits are not strongly correlated (Butlin & Tregenza 1998; Reed & Frankham 2001).

Such low levels of polymorphism are inconsistent with a history of constant large effective population size and suggest historical bottlenecks. Furthermore, at least in the cases of *D. stankovici* from Lake Prespa, *D. p. polymorpha*, and *D. p. anatolica*, a bottleneck is implied by the presence of one very common central haplotype, with additional haplotypes only one or a few mutational steps removed in a star phylogeny (Fig. 1 in Gelembiuk *et al.* in this issue). Such a pattern, as opposed to the presence of multiple haplogroups, suggests that only one lineage survived the population constrictions. Signals of population growth, observed across all taxa containing polymorphism suitable for coalescent analysis, may be due to a common pattern of postbottleneck expansion (Table 1 in Gelembiuk *et al.* in this issue). Interestingly, a pattern of populations characterized by mutually exclusive subsets of haplotypes separated by few mutational steps was observed across several taxa (Fig. 2; Fig. 1 in Gelembiuk *et al.* in this issue), suggesting that shared haplotypes were lost in each region due to founder effects.

Low levels of haplotype diversity in *D. p. polymorpha* and *D. bugensis* might reflect repeated bottlenecks due to the colonizing habit of these two species. Models of metapopulation structure predict that high rates of extinction/turnover relative to migration should result in low global and intrapopulation genetic diversity, with significant differentiation between populations (Austerlitz *et al.* 2000; Pannell 2003), a pattern observed in *D. p. polymorpha* (Fig. 2; Table 2). Dreissenids that have been subjected to fluctuat-

ing habitats or rapid range expansions might have suffered greater loss of genetic diversity relative to those inhabiting comparatively stable ancient tectonic lakes, such as Lake Ohrid. High variance in reproductive success is common among marine organisms, and might be partly responsible for some of the low effective population sizes seen here (Hedgcock 1994; Li & Hedgcock 1998). Further analysis and discussion of these patterns and other aspects of historical demography can be found in the accompanying paper (Gelembiuk *et al.* in this issue).

Acknowledgements

Research funds were provided by grants from the Wisconsin Sea Grant Institute, R/LR-87-PD and R/LR-88, and NSF DEB-0130543 to C. E. Lee. The UW Anna Birge Memorial Fund for Undergraduate Research provided funds for Michael Gerner to collect *Dreissena polymorpha* from Russia, and *D. stankovici* from Lakes Prespa and Ohrid. Director Alexander Alimov sponsored M. Gerner as a visiting scholar at the Zoological Institute of the Russian Academy of Sciences to facilitate his field collections. Greg Gelembiuk was supported by Sea Grant R/LR-87-PD, an NSF Graduate Research Fellowship, and the UW Anna Birge Memorial Fund. Gemma May was supported by Sea Grant R/LR-88, the UW Carl A. Bunde Fund, and the UW Anna Birge Memorial Fund. Vadim Panov was supported by the European Commission 6th Framework Programme Integrated Project ALARM (contract No GOCE-CT-2003-506675). The following people assisted with sample collections: Ebru Unal, Yuriy Slynko, Alexander Gorbunov, F. de Valence, Dawn Dittman, Helena Belyaeva, Shafia Powell, T. Naehar, F. Shakirova, Christoph Shubart, Fabian Skibinski, J. de Leeuw, Konstantin, and Galina. Dianna Padilla provided initial contacts with scientists in Russia. Emily Ruell aided in DNA sequencing. Louis Bernatchez, Stanley Dodson, Jenny Boughman, David Baum, Sara Hotchkiss, and Lee Laboratory members provided useful suggestions and editorial comments.

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