

Review of Genetic Studies of *Dreissena* spp.¹

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SYNOPSIS. Temperate fresh waters of eastern North America were invaded in the late 1980s by two bivalve species, the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*D. bugensis*). The point of origin of zebra mussels is unknown because they are widespread throughout western and eastern Europe, but the quagga mussel is confined to the Dnieper and Bug rivers in the Ukraine. Both species are highly variable in shell morphology. Zebra mussel color and pattern morphs defy classification, but at least one distinct pattern is found only in the Aral-Caspian region and North American populations. An extremely pale, laterally compressed quagga morph is found in eastern Lake Erie and southern Lake Ontario, but has not been noted in the Ukraine. Allozyme electrophoresis has revealed extraordinarily high levels of genetic variability in the zebra mussel. Average heterozygosities range from 27.0 to 43.5%, compared to 9.7 to 14.5% in the quagga mussel. Two surveys of North American and European populations indicated that little variability was lost when zebra mussels were transported to North America. Therefore, either the invader population was comprised of a large number of individuals, and/or more than one source population contributed to the invasion. No genetic differentiation among populations has occurred in North America in the nine years since the first invasion. Zebra mussels, like many bivalves, demonstrate heterozygote deficiencies at some loci. A weak correlation has been found between individual heterozygosity and shell length. The need for identification of enzymes, loci, and allelic mobilities to allow comparison of genetic data among studies is stressed, as is the need for vouchering specimens. Fruitful areas for future research include elucidation of dreissenid taxonomy and status of putative subspecies, monitoring for the potential invasion of the saline tolerant *D. rostriformis grimmi* into North American estuarine waters, and ecological and physiological comparison of extreme morphs such as the normal and profundal quagga mussels.

¹ From the Symposium *Biology, Ecology and Physiology of Zebra Mussels* presented at the Annual Meeting of the American Society of Zoologists, 4-8 January 1995, at St. Louis, Missouri.

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INTRODUCTION

Invasion of any organism into a new area demands a rapid assessment of the potential impacts of the organism on its new ecosystem. To assess such impacts, it is essential to first know the identity of the organism to at least the species level. Mistakes in identification, or assumptions about genetic uniformity of a population, can result in unfortunate consequences in pest control programs (Metcalf and Luckmann, 1975). The geographic origin of the invader must also be identified, so that predictions about its behavior in a new environment can be based on information about its native habitat.

When zebra mussels were first found in Lake St. Clair in 1988 (Hebert *et al.*, 1989), their identification as *Dreissena polymorpha* was straightforward. *D. polymorpha* had already invaded much of western Europe, and in fact had been predicted to invade North America (Johnson, 1921; Sinclair, 1964). The morphology, habitats, and behavior of zebra mussels were already well known in Europe, and were summarized for the benefit of North American investigators by Mackie *et al.* (1989). However, almost no genetic work has been done on the zebra mussel in Europe. An early examination of the species in North America using allozymes revealed a high level of variability which suggested that zebra mussels had the potential to adapt to a wide variety of ecological conditions, and to differentiate through founder effect into ecologically distinct populations (Hebert *et al.*, 1989).

The discovery of a second non-native dreissenid species in the Erie Canal in 1991, given the common name quagga mussel (May and Marsden, 1992), justified the concern that assumptions about genetic uniformity could be unwarranted. Genetic data, in combination with an extensive review of the taxonomic literature, ultimately resolved the identity of this species as *D. bugensis* (Rosenberg and Ludyanskiy, 1994; Spidle *et al.*, 1994). Little is known about *D. bugensis* in its native range, so much work needs to be done to determine how the range expansion and impacts of

this species will differ from those of the zebra mussel.

The purpose of this paper is to review genetic studies on both species including a summary of the current understanding of dreissenid taxonomy as it relates to the two species in North America. Compilation of these data reveals gaps in the literature which point the way for future work.

Taxonomy

In 1991, a genetic survey of zebra mussels in North America using allozyme electrophoresis detected a second species of dreissenid mussel (May and Marsden 1992). *D. polymorpha* and the new species, dubbed the quagga mussel, were initially separated by Nei's genetic distance of 1.22, later revised to 1.69 (Spidle *et al.*, 1994); congeneric species are generally separated by genetic distances between 0.16 and 1.39 (Avice and Smith 1977). A literature search for the identity of the quagga mussel revealed the taxonomic disarray of the dreissenid lineages. Confusion and disagreement among dreissenid taxonomists has been in part engendered by the fact that much of relevant literature is in Russian, and has not been translated. The identification of the North American quagga mussel as *D. bugensis* is based on three lines of evidence. First, quagga mussels are genetically identical with tissue collected from *D. bugensis* in the Dnieper River (Spidle *et al.*, 1994). Second, quagga mussels are more brackish water intolerant than zebra mussels (Spidle 1994), although there is considerable adaptive variation in their response to low salinity levels (Mills *et al.*, 1996). Third, quagga mussels are morphologically comparable to illustrations and archived shells of *D. bugensis*, and shells of *D. bugensis* collected in the Dnieper River (Rosenberg and Ludyanskiy, 1994; Spidle *et al.*, 1994). No prior common name for *D. bugensis* has been found, so the common name quagga has been submitted to the American Fisheries Society nomenclature committee by G. Rosenberg.

Resolution of the identity of the quagga mussel was facilitated by an extensive review of the Russian literature and examination of original collections of dreissenid

mussels in Russian institutions by Rosenberg and Ludyanskiy (1994). Seven species of *Dreissena* were originally recognized by Andrusov (1897), including *D. polymorpha*, *D. rostriformis*, and *D. bugensis*. The next major review of the taxa (Zhadin, 1952) agreed substantially with Andrusov's work, but omitted *D. rostriformis* as a species. Western workers have tended to reduce the number of species to one (*D. polymorpha*; Marelli and Gray, 1985) or two (*D. polymorpha* and *D. rostriformis*; Nuttall, 1990). The most recent Russian taxonomies recognize four living species in the Caspian Sea, *D. polymorpha*, *D. elata*, *D. caspia*, and *D. rostriformis* (Mordukhai-Boltovskoi, 1960; Logvinenko, 1965), with the recent restoration of *D. bugensis* as a full species (Starobogatov, in press, cited by Rosenberg and Ludyanskiy, 1994). Of these species, *D. rostriformis* and *D. bugensis* belong in the sub-genus *Pontodreissena*. At no point have *D. rostriformis* and *D. bugensis* been synonymous, as suggested in a note by Marelli (1994). *D. r. grimmi* and *D. bugensis* are the two living descendants of the extinct *D. r. rostriformis*. The two taxa differ chiefly in their ranges and their salinity tolerances. *D. r. grimmi* is confined to the middle and upper Caspian while *D. bugensis* is found in the Dnieper and Bug rivers which drain into the Black Sea in the Ukraine. *D. r. grimmi* cannot reproduce in salinities below 6-7 ppt, whereas these salinity levels are lethal to *D. bugensis* (see also Mills *et al.*, 1996). Thus, Rosenberg and Ludyanskiy (1994) concluded that there are six extant species of *Dreissena* based on morphological evidence: *D. polymorpha* (with three subspecies), *D. andrusovi*, *D. caspia* (with two subspecies), *D. (Carinodreissena) stankovici*, *D. (Pontodreissena) rostriformis*, and *D. (P.) bugensis*. No molecular genetic data exist to substantiate any subspecies designations.

Elucidation of the taxonomic status of all extant dreissenid species is important, and can clearly be facilitated using genetic data. The primary obstacle to this work is the collection and exportation of specimens from geographically or politically inaccessible areas of eastern Europe. North American investigators should remain alerted to

the possibility that additional species or sub-species with different environmental tolerances could still appear on the continent if we do not establish and maintain strict quarantine precautions.

Shell morphology

The zebra mussel earned its specific name "polymorpha" from the obvious, extreme variation in shell color and pattern. Closer examination reveals additional variation in shell shape, particularly in the ratio of shell height to width and relative lengths of the anterior and posterior portions of the shell (Fig. 1). A preliminary study of shape variation in North American and European populations did not reveal consistent differences among populations (JEM, unpublished data). Biochino (1989, 1990) reduced the color morphs to five basic patterns (Fig. 2), and then categorized the zig-zag patterns (CC) on the basis of stripe width and location and type of zig-zag interruptions in each stripe. Smirnova *et al.* (1993) added a sixth color morph (MM) to this classification (Fig. 2). These classifications are an oversimplification of the complexity of zebra mussel patterns, both because radical color morphs are missing from the classification (Fig. 3c, g, l), and because patterns can apparently change drastically from one growing season to the next (Fig. 3j, k). Intergrades between Biochino's patterns are also often seen (Fig. 3f). The double-letter nomenclature used by Biochino is misleading, as it implies that they are known homozygous traits. Biochino's classification prompted Ludyanskiy (1993) to note that the single longitudinal stripe or 'DD' pattern is observed in the Aral-Caspian area and throughout North America (albeit at low frequencies), but has not been observed by western European dreissenid biologists. This finding may suggest that at least one source population that contributed to the North American invasion likely came from the Aral-Caspian area. Smirnova *et al.* (1993), using additional distribution data of the same color morphs, concluded that the Lake Erie population was unlikely to have been founded by mussels from the Ponto-Caspian, middle-Russian, or Baltic areas. However, they did not examine morph fre-



FIG. 1. Example of extreme variation in shell shape in zebra mussels collected in the North American Great Lakes. The two upper shells are the same individual and the two lower shells are the same individual.

quencies from the Aral-Caspian area or northeastern European Russia. Inheritance patterns of the various color morphs have not been examined to determine the effect of environmental influences on the expression of shell colors and patterns.

Quagga mussels do not show pattern variation as dramatic as that shown by zebra mussels. However, Dermott and Munawar (1993) and others have noted a distinctive quagga morph which is laterally compressed and is pale or completely white. This morph was initially found in the profundal zone of eastern Lake Erie, but it appears to extend throughout southern Lake Ontario. Intergrades between normal and profundal morphs have also been noted (APS, personal observation). Allozyme comparison with North American and Ukrainian *D. bugensis* confirmed that this profundal morph is genetically similar to the more familiar quagga morphs. To our knowledge, this morph has not been detected in Europe. Physiological comparison of the two morphs should provide an interesting area for future research.

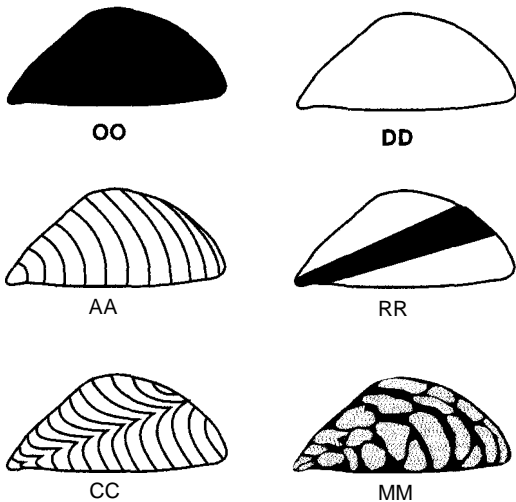


FIG. 2. Zebra mussel color morphs classified by Biochino (1989) and Smirnova *et al.* (1993).

Karyotype

Zebra mussels have a karyotype with $2N = 32$ composed of 20 small metacentric and 12 acrocentric chromosomes (Grishanin, 1990). No karyological studies have been done on *D. bugensis*.

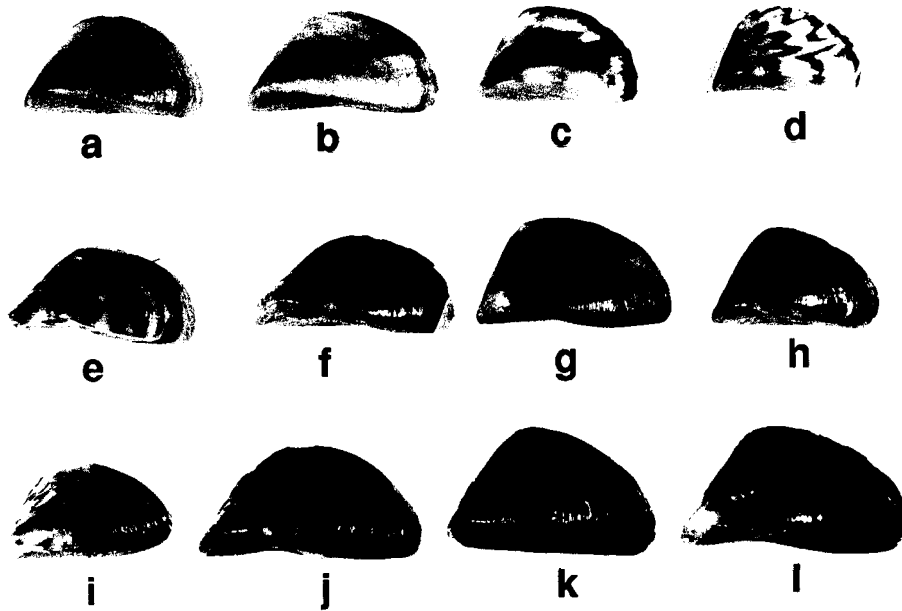


FIG. 3. Variability in shell pattern among zebra mussels collected in the North American Great Lakes. The "a" shell corresponds to DD in Figure 2, b = RR, d = CC, e = AA, h = OO, and g is similar to MM. The "c" shell is similar to "b" but has pigmentation inside the shell which is visible externally.

TABLE 1. Summary of genetic data on zebra and quagga mussels.¹

| # Enzyme(s) screened | # Enzymes used | # Poly-morphic loci | # Mono-morphic loci | Average # alleles among polymorphic loci | Estimated overall heterozygosity | Source |
|----------------------------|----------------|---------------------|---------------------|--|----------------------------------|------------------------------|
| Zebra mussel | | | | | | |
| 18 | 16 | 17 | 6 | 3.1 | 31.6 | Hebert <i>et al.</i> , 1989 |
| 9 | 9 | 11 | 0 | 3.32 | 43.5 | Boileau and Hebert, 1993 |
| 5 | 5 | 7 | 0 | 3.4 | 30.7 | Garton and Haag, 1991 |
| 27 | 14 | 16 | 1 | 3.92 | 27-43 | Marsden <i>et al.</i> , 1995 |
| 11 | 10 | 11 | 0 | 4.0 | 29.1-34.1 | Spidle <i>et al.</i> , 1994 |
| 55 | 11 | 11 | 1 | 3.5 | 31.1-40.2 | May and Marsden, 1992 |
| Quagga mussel | | | | | | |
| North American populations | | | | | | |
| 55 | 11 | 5 | 7 | 2.4 | 9.7 | May and Marsden, 1992 |
| 12 | 11 | 5 | 6 | 3.3 | 10.1 | Spidle <i>et al.</i> , 1994 |
| European populations | | | | | | |
| 12 | 11 | 3 | 8 | 3.1 | 10.6-14.5 | Spidle <i>et al.</i> , 1994 |

¹ All data are from North American populations only unless otherwise noted.

² Includes data from European populations.

) Monomorphic loci were defined as those having a single allele with 20.95 frequency (Hartl and Clark 1989).

TABLE 2. Number of alleles resolved and population sample sizes used by different investigators at allozyme loci in zebra and quagga mussels.¹

| Enzyme | E.C. number | Locus abbreviation | Zebra mussels | | | | | | | |
|---|-------------|----------------------------|--------------------------------|--------------------------|--------------------------|-----------------------------|--------------------------------|---------------------------------|-----------------------------|--------------------------------|
| | | | Hebert <i>et al.</i> , 1989 | Garton and Haag, 1991 | May and Marsden, 1992 | Boileau and Hebert, 1993 | Spidle <i>et al.</i> , 1994 | Marsden <i>et al.</i> , 1995 | May and Marsden, 1992 | Spidle <i>et al.</i> , 1994 |
| Aldehyde oxidase | 1.2.3.1 | <i>AO*</i> | unresolved | — | — | — | — | — | — | — |
| Arginine kinase ² | 2.7.3.3 | <i>AK-1*</i> | 1 | — | — | — | — | — | — | — |
| | | <i>AK-2*</i> | 1 | — | — | — | — | — | — | — |
| Aspartate aminotransferase (= glutamate oxaloacetate transaminase <i>GOT*</i>) | 2.6.1.1 | <i>AAT*</i> | 3 | — | — | — | — | — | — | — |
| Diaphorase | 1.6.2.2 | <i>DIA*</i> | — | — | 6 | — | 6 | 6 | 1 | 2 |
| Esterase | 3.1.1.1 | <i>EST-1*</i> | 1 | 5 | 3 | — | — | 2 | 1 | — |
| | | <i>EST-2*</i> | 4 | — | — | — | — | — | — | — |
| | | <i>EST-3*</i> | 1 | — | — | — | — | — | — | — |
| Formaldehyde dehydrogenase | 1.2.1.1 | <i>FDH*</i> | — | — | — | — | 2 | 2 | 2 | 2 |
| Fumarase | 4.2.1.2 | <i>FH*</i> | 3 | — | 2 | 3 | — | 3 | — | — |
| Glucose-6-phosphate isomerase (= phospho-glucose isomerase <i>PGI*</i>) | 5.3.1.9 | <i>GPI-1*</i> | 4 | 6 | 8 | 5 | 9 | 9 | 3 | 6 |
| Glyceraldehyde-3-phosphate dehydrogenase | 1.2.1.12 | <i>GAPDH*</i> ² | 2 | — | 3 | — | 3 | 3 | — | 1 |
| Isocitrate dehydrogenase | 1.1.1.42 | <i>IDH*</i> | 2 | — | 2 | 2 ⁴ | 3 | 3 ⁴ | 2 | 2 |
| L-lactate dehydrogenase | 1.1.1.27 | <i>LDH*</i> | 2 | — | — | 2 ⁴ | — | — | — | — |
| Leucine aminopeptidase | 3.4.11.1 | <i>LAP*</i> | 5 | — | — | — | — | — | — | — |
| Malate dehydrogenase | 1.1.1.37 | <i>MDH-1*</i> | 2 | 2 | 3 | 2 | 3 | 3 | 1 | 1 |
| | | <i>MDH-2*</i> | 2 | 3 | 2 | — | 2 | 2 ⁴ | 1 | 1 |
| Malic enzyme | 1.1.1.40 | <i>ME*</i> | 2 | 3 | — | — | — | — | — | — |
| Mannose-6-phosphate isomerase | 5.3.1.8 | <i>MPI*</i> | 5 | — | — | 5 | — | — | — | — |
| Peptidase with leucyl-alanine (≡ peptidase with leucyl-glycine ⁵) | 3.4.11-13 | <i>PEP-LA-1*</i> | 4 | — | — | 3 | — | — | — | — |
| | | <i>PEP-LA-2*</i> | 4 | — | — | 3 | — | — | — | — |
| | | <i>PEP-LA-3*</i> | 2 | — | — | 3 | — | — | — | — |
| Peptidase with phenyl-alanyl-proline | 3.4.11-13 | <i>PEP-PAP*</i> | — | — | — | 3 ³ | — | 2 | — | — |
| Peptidase (unspecified) | 3.4.11-13 | <i>PEP-1*</i> | — | 3 | — | — | — | — | — | — |
| | | <i>PEP-2*</i> | — | 2 | — | — | — | — | — | — |
| Phosphoglucomutase | 5.4.2.2 | <i>PGM-1*</i> | unresolved | — | 3 | — | 5 | 5 | 1 | 2 |
| | | <i>PGM-2*</i> | — | — | unresolved | — | — | 5 | — | — |
| Phosphogluconate dehydrogenase (= PGD, 6PGDH) | 1.1.1.44 | <i>PGDH*</i> | 3 ⁶ | — | 4 | 3 | 4 | 4 | 2 | 4 |
| Inorganic pyrophosphatase | 3.6.1.1 | <i>PP*</i> | — | — | 1 | — | 2 | 1 ⁴ | 1 | 1 |
| Triosphosphate isomerase | 5.3.1.1 | <i>TPI-1*</i> | 4 ⁶ | — | 5 | — | 5 | 5 | 3 | 5 |
| N | | | 51-64 | 120 | 19-45 | 18-48 | 40-243 | 30-80 | 21 | 10-22 |

Allozyme variation

Several studies using allozyme electrophoresis indicate that *D. polymorpha* has extraordinarily high levels of genetic variability; individual heterozygosities ranging from 27 to 43.5% rank zebra mussels among the highest reported heterozygosity values (Table 1; Nevo *et al.*, 1984; Hebert *et al.*, 1989; Boileau and Hebert, 1993; Marsden *et al.* 1995). Quagga mussels have about one third the average heterozygosity of zebra mussels, ranging from 9.7 to 14.5% (May and Marsden, 1992; Spidle *et al.*, 1994). Average heterozygosities for both species are similar in Europe and in North America (Spidle *et al.*, 1994; Marsden *et al.* 1995). The number of alleles resolved per locus varies from 1 to 10, and differs considerably among investigators (Table 2; a tenth allele was noted at the *GPI** locus by APS). Genetic variability has also been noted among some individual zebra and quagga mussels at the cytochrome b gene in the mitochondrial genome (C. Stepien, Case-Western Reserve University, personal communication).

The high levels of genetic diversity in both zebra and quagga mussels suggest that the founder populations were comprised of a large number of individuals, and that no genetic bottleneck occurred when they were transported to North America (Hebert *et al.*, 1989; Marsden *et al.*, 1995). This finding is generally true of invasive molluscs with veliger larvae, though the invasion of the Asian clam *Corbicula* into North America is an exception to this rule. *Corbicula fluminea* arrived on the west coast in 1924 and spread across the continent within 50 years (McMahon, 1983), yet genetic studies indicate that this species is monomorphic at most allozyme loci which have been re-

solved (Smith *et al.*, 1972; Hillis and Patton, 1982; McLeod, 1986). High genetic variability is not necessarily a predictor of the potential of an organism to be a successful invader (Barrett and Richardson, 1986; but see Nevo, 1978).

The rapid spread of zebra mussels to disjunct habitats across North America suggests that there is potential for population differentiation to occur via founder effect or through micro-evolution as they encounter new habitats. An extensive genetic survey of 21 North American populations of zebra mussels did not find any evidence that such population differentiation has occurred (Marsden *et al.*, 1995). Upon reflection, this result should not be surprising. The veliger life stage ensures that a high level of inter-population mixing is likely to occur, and that downstream populations will consistently be supplemented with larvae from upstream populations. However, Boileau and Hebert (1993) examined seven North American populations and reached the opposite conclusion, *i.e.*, that little intermixing of individuals between populations has occurred since their initial establishment. The large frequency differences between populations which they observed is in marked contrast to the results of Marsden *et al.* (1995); one possible explanation for this contrast, until further studies are done, is that their samples may have included quagga mussels (see discussion below).

The original introductions of mussels from Europe did not involve any significant loss of genetic variability (Marsden *et al.* 1995), and may have occurred more than once, suggested by the apparently separate introductions of zebra and quagga mussels (May and Marsden, 1992). Intracontinental ballast water and other boat traffic transfers

¹ Designations for subcellular localization and multilocus isozyme identification are not given as these items were not identified in most studies. When only one locus was resolved for a given enzyme, we arbitrarily gave it the designation 1. E. C. numbers were not supplied in several papers, and so the numbers given here correspond to the enzyme name given in each paper.

² Listed as arginine phosphofructokinase (APK) by Hebert *et al.* (1989).

³ Mismatch between locus name and abbreviation; locus name taken to be correct.

⁴ Additional allele found in European populations.

⁵ Frick, 1983.

⁶ Also listed as monomorphic by Hebert *et al.* (1989).

are equally likely to contain large numbers of individuals and may occur repetitively between the same areas. Such repetitive transfer is not conducive to spatial differentiation among populations. On the other hand, it will be interesting to observe whether evolutionary change of North American populations occurs relative to their counterparts in Europe now that stricter controls on ballast water have been imposed. Alternatively, the neutral or nearly neutral nature of most allozyme variation would not necessarily reflect changes that occur at other loci in the genome that are susceptible to selective pressures. Therefore, major divergence may occur over time due to selective environmental pressures which are not revealed by examination of allozyme data.

A question which has drawn particular attention in genetic studies of North American zebra mussels is whether the source population in Europe could be identified using genetic surveys. The source of the quagga mussel, in contrast, is not in question, because its European distribution is limited to the Dnieper and Bug River drainages. Identification of the source population(s) of zebra mussels would only be possible if (1) there were very few source populations, (2) the source population(s) were genetically distinct from other potential sources, and (3) the founder population was sufficiently large to represent the majority of unique alleles or genotypes from the source(s). So far, genetic surveys of European populations do not implicate any single or small group of populations which could be the donor populations (Marsden *et al.*, 1995). However, populations of zebra mussels from eastern Europe and Russia, which may have contributed to the North American invasion (e.g., Ludyanskiy, 1993; Spidle *et al.*, 1994) have not been sampled adequately.

Genetic studies of bivalve molluscs have frequently noted an unusual prevalence of heterozygote deficiency, or Wahlund effect, and correlations between individual heterozygosity and growth rates (Berger, 1983; Zouros and Foltz, 1984). Both characteristics have also been observed in zebra mussels. Boileau and Hebert (1993) did not find

consistent heterozygote deficiencies among loci from different populations, but Marsden *et al.* (1995) found a predominance of heterozygote deficiencies at the *PGM-2** and *DIA** loci. Marsden *et al.* suggest that, given the relatively large number of alleles at each of these loci (5 and 6, respectively), the presence of a null allele is possible and would explain the deficiency.

Garton and Haag (1991) found a significant, though small, relationship between individual heterozygosity and size; multiple locus heterozygosity at seven loci explain 7% of the total variation in shell length. They suggest that growth and/or survival may be greater in more heterozygous individuals. However, weight-specific oxygen consumption was not significantly related to single or multiple locus heterozygosity. In contrast, Boileau and Hebert (1993) examined early post-settlement mussels and did not find any correlation between heterozygosity and early growth. Resolution of these disparate findings awaits replication of the studies.

Haag and Garton (1995) observed a significant difference in genotype frequencies between wild-caught larval and parental populations at a *PGI** locus; this difference was attributable to changes in the frequency of a single allele. Controlled laboratory parent-offspring studies are required to verify this implication of massive selection in the first two weeks of life. Fetisov *et al.* (1990) found a directed shift in allelic frequencies at *EST-D**, *LAP-1**, *LAP-2**, and *ME** during an eight-to-ten year exposure to elevated temperatures in the cooling reservoir of a power plant. They concluded that the shift was due to selection rather than genetic drift or inbreeding. Unfortunately, this study is available only as an English abstract, and other studies on this phenomenon have not yet been translated into English (Shevchenko and Fetisov, 1989; Zapkuvieni and Nakrevich, 1989). This work has not yet been supported by experimental results. No other work has been published on relationships between zebra mussel genotypes and phenotypic effects.

The majority of published studies on dreissenid genetics have neglected to identify allelic mobilities, so that allelic fre-

quencies cannot be compared among studies (see discussion of Boileau and Hebert's (1993) data in Marsden *et al.* (1995)). Multiple loci for a particular enzyme are also frequently not identified by relative mobility, and locus nomenclature is inconsistent. For example, the PP used by Boileau and Hebert (1993), identified as phenylalanyl-proline peptidase without an IUBNC number, is not the diagnostic PP locus (inorganic pyrophosphatase 3.6.1.1) used by May and Marsden (1992) to separate zebra and quagga mussels. Inability to compare allelic frequencies makes it impossible to rule out the possibility that investigators have inadvertently combined zebra and quagga mussels in their samples. This possibility would explain the order of magnitude differences in genetic distances found among geographically overlapping populations between the study by Boileau and Hebert (1993) and Marsden *et al.* (1995). Mixing of quagga and zebra mussels could provide an alternate explanation for the differences in genotype frequencies between adults and larvae, noted by Haag and Garton (1995), because the life stage that they would least be able to identify to species would be the larvae. Data from Spidle *et al.* (1994) indicate that zebra and quagga mussels share six of the nine alleles found at the *GPI** (= *PGI**) locus used by Haag and Garton (1995) to ensure the absence of quagga mussels in their study. The common *GPI** allele in quagga mussels (*GPI*83*), which is extremely rare in zebra mussels (<0.01), could only be resolved with the Ridgway buffer (Ridgway *et al.*, 1970; APS, unpublished data); clearly, citation of buffers used for such diagnostic loci would be useful in published work. Use of a standard for allozyme nomenclature such as the one widely used in fisheries biology (Shaklee *et al.*, 1990) also should be strongly encouraged in dreissenid studies; this nomenclature has already been used by May and Marsden (1992), Spidle *et al.* (1994), Marsden *et al.* (1995), and in this paper. Of equal importance is the vouchering of specimens. If shells from Boileau and Hebert's study had been saved, the issue of potential contamination with quagga mussels could be readily resolved. It is interesting to note that the

first quagga mussel collected in North America was actually noticed and commented upon, due to its "odd" shell shape, in 1990 (Ted Schaner, Ontario Ministry of Natural Resources, personal communication), over a year before the genetic study revealed its identity as a separate species.

CONCLUSIONS

Genetic studies using protein electrophoresis indicate that zebra mussels have extremely high levels of genetic variability; average heterozygosities in zebra mussels range from 27.0 to 43.5%, compared to 9.7-14.5% in quagga mussels. This variability is also reflected in morphological variation; a wider range of color patterns and shell shapes is seen in zebra than quagga mussels, though a distinct, pale, laterally compressed quagga mussel has been noted in Lake Erie. Color patterns may provide clues to the source(s) of zebra mussels in North America. Apparently little genetic variability was lost when zebra mussels were transported to North America, and no genetic differentiation has occurred in North American populations since the invasion. Either the invader population was comprised of a large number of individuals, and/or more than one source population contributed to the invasion. Zebra mussels, like many bivalves, demonstrate heterozygote deficiencies at some loci. Studies on the relationship between heterozygosity and growth in zebra mussels have produced conflicting results. Review of genetic studies of dreissenid mussels emphasizes the need for vouchering specimens, and the need for identification of enzymes, loci, and allelic mobilities to allow comparison of genetic data among studies.

The high levels of genetic and morphological variability seen in zebra and quagga mussels offer a wealth of opportunities for studies of their taxonomy, inheritance of color patterns, temporal and spatial microevolution in response to unique habitats, correlation of genetic variability with ecological lability, and correlations of genotypes with phenotypic traits. However, surprisingly little work has been done in these areas. This review points out several areas

for further research, including the following:

- Examination of populations of zebra mussels from eastern Europe as possible source populations for the North American zebra mussels.
- Comparison of the habitat and range expansion of quagga mussels *versus* zebra mussels, with particular reference to the differences in their overall genetic variability. Such comparisons will add to our understanding of how genetic variability affects the ability of a species to colonize new habitats.
- Further examination of genetic variability of *D. bugensis* in its native range. This examination would include a search for the profundal morph in Europe.
- Comparison of the ecology and physiology of the profundal and "normal" morphs of the quagga mussel, which are genetically very similar but differ widely in their morphology and color patterns. Rearing of the profundal and normal morphs in identical habitats would help to resolve the genetic component of this phenotypic variation.
- Monitoring for the potential invasion of *D. rostriformis* into saline waters of North America.
- Genetic examination of other dreissenid species, including *Mytilopsis*, to elucidate the taxonomy of this group and verify the existence of sub-species.

Further work is also needed to resolve some of the differences in work conducted to date, such as the differences in genetic distances among sympatric groups of populations (Boileau and Hebert 1993; Marsden *et al.*, 1995). The work of Garton and Haag (Garton and Haag, 1991; Haag and Garton 1995) and Fetisov *et al.* (1990) provide interesting suggestions of genotype-dependent phenotypic traits which should be pursued further. Finally, our lack of awareness of two morphologically similar species that co-occur in nature should once again alert us to the need for detailed genetic studies of the organisms upon which we do research. All studies on larval samples, adult distributions, and physiology of mussels in the Great Lakes that do not dis-

criminate between quagga and zebra mussels must remain suspect.

ACKNOWLEDGMENTS

We thank the conference organizers, Robert McMahon and Jeff Ram, for their invitation to participate in this conference, and Martha Kneuer for preparing Figure 2. The authors' work was funded by the U.S. Environmental Protection agency under Cooperative Agreement Number CR8 18947-01. Tammy Keniry collected the shell shape and color pattern data on zebra mussels.

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