NOTES

Absence of naturally occurring hybridization between the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*D. polymorpha*) in the lower Great Lakes

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Abstract: The coexistence of two dreissenids, the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*D. polymorpha*), in a new environment raises the possibility of natural hybridization and possible introgression. Animals of both species were collected in areas where they occur sympatrically (25–39% were quagga mussels) and screened at two protein-coding loci believed to differentiate between the two species. The occurrence of alleles diagnostic for both species in an individual would demonstrate hybridization between the species. No hybrid individuals were observed in a survey of 750 animals from four sites in Lake Ontario and one site in Lake Erie. Successful hybridization between these two genetically disparate species seems unlikely in the Great Lakes.

Résumé : La coexistence de deux espèces de dreissénidées, la Moule quagga (*Dreissena bugensis*) et la Moule zébrée (*D. polymorpha*), dans un nouveau milieu permet de croire à la possibilité d'hybridation naturelle et peut-être à l'introgression. Des animaux des deux espèces ont été recueillis dans des zones où les deux espèces cohabitent (25–39% de Moules quagga) et examinés à deux locus de codage des protéines, diagnostiques des deux espèces. La présence d'allèles diagnostiques des deux espèces chez un individu prouverait l'existence de l'hybridation. Aucun hybride n'a été rencontré chez les quelque 750 animaux recueillis en quatre points du lac Ontario et un site du lac Érie. Il semble donc que l'hybridation soit impossible entre ces deux espèces génétiquement très différentes.
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Introduction

The recent discovery of the quagga mussel (a morphologically cryptic relative of the zebra mussel, *Dreissena polymorpha*) in the Great Lakes raises a number of questions about this exotic mollusc: What is its identity? From where did it come? Will it have environmental limits different from those of the zebra mussel? Can it interbreed with the zebra mussel to produce offspring that would constitute a third unknown quantity in the Great Lakes? The quagga mussel has since been identified as *D. bugensis* (Rosenberg and Ludyanskiy 1994; Spidle et al. 1994). North American researchers have begun to infer ecological limits based on the range of the quagga mussel in the Old World (M.L. Ludyanskiy, Marine Biocontrol, P.O. Box 636, Sandwich, MA 02563 U.S.A., personal communication) and to perform laboratory experiments to measure differences in tolerance to temperature and salinity between these two species of *Dreissena* (Domm et al. 1993; A.P. Spidle, E.L. Mills, and B. May, unpublished data).

The zebra mussel has been successfully colonizing the Great Lakes for almost 10 years (Griffiths et al. 1991), and the quagga mussel has spread throughout much of Lake Erie and Lake Ontario (Dermott and Munawar 1993; Mills et al. 1993). The zebra mussel is capable of spawning throughout the year in water above 12°C (Stanczykowska 1977). The spawning periods of the *Dreissena* species will overlap unless the quagga mussel spawns exclusively in water at lower temperatures, allowing the broadcast gametes to mix in the water column and providing the opportunity for the development of hybrid individuals. Combining these congeneric species in a completely new range may provide

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Table 1. Sampling sites and sample sizes from the electrophoretic survey.

Site	Proportion of quagga mussels (%)[a]	Water body	No. of quagga mussels	No. of zebra mussels	Total
Cape Vincent	33	Lake Ontario	100	120	220
Thirty-Mile Point	39*	Lake Ontario	80	80	160
Olcott	36*	Lake Ontario	80	80	160
Rochester	25*	Lake Ontario	80	100	180
Dunkirk	33	Lake Erie	10	20	30
Total			350	400	750

NOTE: An asterisk indicates that the estimate of the proportion of quagga mussels is taken from Mills et al. (1993), who give details of the collection of the respective samples. The samples from Cape Vincent and Dunkirk, N.Y., were obtained separately and the proportions of quagga mussels collected at that time are given.

[a]Estimated proportion of quagga mussels in the population trawled.

habitat (unavailable in the Old World) where hybrids of these two species may be viable.

Given the substantial genetic distance between these species, successful natural hybridization seems unlikely (Nei's genetic identity, $I = 0.20$; Spidle et al. 1994). However, two pairs of oyster species have been observed to fertilize in both directions despite seemingly large genetic distances (*Crassostrea rivularis* × *C. gigas*, $I = 0.49$, Buroker et al. 1979*b*; *C. virginica* × *C. rhizophorae*, $I = 0.51$, Hedgecock and Ozaki 1984). In contrast, two species that are more similar have been found to be capable of interbreeding in only one direction (*C. gigas* × *C. sikamea*, $I = 0.64-0.85$, Buroker et al. 1979*a*; also see the review in Gaffney and Allen 1993).

Zebra mussels have been demonstrated to emit gametes in the laboratory upon stimulation with serotonin (Ram and Nichols 1993). This technique has been used to make single-pair interspecific matings in both directions in the laboratory (Nichols and Black 1993). These putative hybrid offspring have not been successfully reared to the settling stage, however.

Two diagnostic protein-coding loci differentiate between zebra and quagga mussels and can be used to identify hybrids (the nomenclature for protein-coding loci follows Shaklee et al. 1990). Inorganic pyrophosphatase, EC 3.6.1.1 (*PP**), is fixed for alternate alleles in each species (*PP**100 in the zebra mussel and *PP**110 in the quagga mussel; May and Marsden 1992; Spidle et al. 1994). The second locus is phosphoglucomutase, EC 5.4.2.2 (*PGM1**). The quagga mussel has been observed to be fixed for the *PGM1**107 allele (May and Marsden 1992; Spidle et al. 1994). In an extensive survey of genetic variation in *D. polymorpha* in Eurasia and North America, five *PGM1** alleles were found in the zebra mussel (two common alleles, *PGM1**100, 90%, and *PGM1**111, 9%, and three rare alleles, *PGM1**88, 96, and 107, all less than 0.4%; the allele frequencies from Marsden et al. 1995). The occurrence of the *PGM1**107 allele in the zebra mussel introduces the possibility that some introgression may have occurred, with the quagga allele entering the gene pool of the zebra mussel, if the alleles are in fact identical by descent in each species.

Six individual zebra mussels, all heterozygous, have previously been observed to possess the *PGM1**107 allele (over 2000 mussels were sampled, most from areas where the quagga mussel does not occur; Marsden et al. 1995). Two of these zebra mussels did occur in areas where the quagga mussel is currently sympatric (Colchester, Ont., on Lake Erie and Cape Vincent, N.Y., on Lake Ontario). The Colchester sample was collected in June 1990 and none of the 43 mussels collected possessed the morphological or electrophoretic phenotype of the quagga mussel (Marsden et al. 1995). The quagga mussel appears to have colonized the Colchester area, beginning in the summer of 1992 (Dermott and Munawar 1993). The other four zebra mussels with the *PGM1**107 allele did not occur in the known range of the quagga mussel (Zhadin 1952; Dermott and Munawar 1993; Mills et al. 1993): two were from Lake Michigan (Sheboygan, Wis., and Holland, Mich.), one was from the Hudson River drainage (Catskill, N.Y.), and the last was from the Wtoctawek Reservoir in Poland.

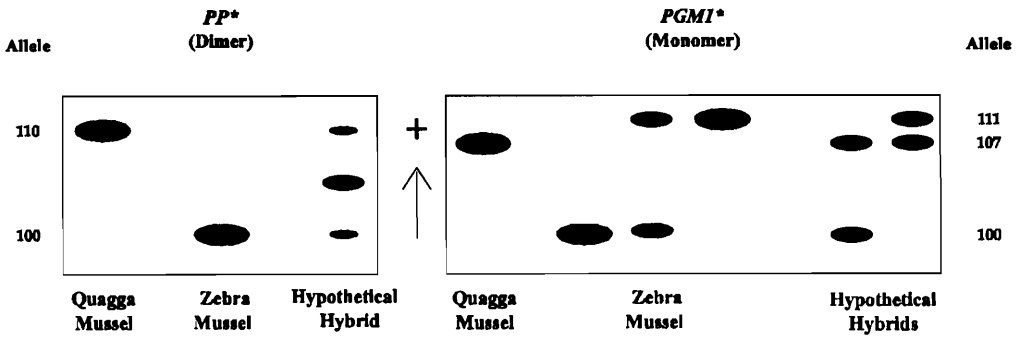
The occurrence of the *PGM1**107 allele in Poland suggests that not all copies of this allele in the zebra mussel are identical by descent, since there is no evidence that the zebra mussels in North America could have been introduced from the Wtoctawek Reservoir (genetic data suggest that this population is quite distant from all surveyed North American populations of *D. polymorpha;* Nei's $D = 0.07$; Marsden et al. 1995). *PGM1**, therefore, may still be a locus that is diagnostic between the zebra and quagga mussels. Sympatric populations of *D. bugensis* and *D. polymorpha* that both exhibit heterozygosity for the *PGM1**107 allele would provide solid evidence of hybridization or introgression.

Natural hybrids would be expected to demonstrate a combination of alleles from both species at both loci. Interspecific fertilization would be expected to occur in areas where gametes of both species have the opportunity to combine during external fertilization. This note reports the results of a survey designed to find hybrid adults in natural populations by using genetic differences diagnostic for each of the two North American species of *Dreissena*.

Methods

Quagga and zebra mussels were collected by bottom trawling at depths of 25–30 m at four sites along the southern coast of Lake Ontario and one site in southeastern Lake Erie (Table 1) where the quagga mussel is known to occur in large numbers (Dermott and Munawar 1993; Mills et al. 1993). Cape Vincent was sampled in the fall of 1991 and the remain-

Fig. 1. Predicted electrophoretic phenotypes of a *D. bugensis* × *D. polymorpha* hybrid based on the quaternary structures of the proteins encoded by two loci diagnostic for each species. The diagnostic alleles observed for each species are shown for comparison (zebra mussel: *PP**100, *PGM1**100, and *PGM1**111; quagga mussel: *PP**110 and *PGM1**107). The arrow indicates the anodal direction.



ing sites were sampled in the fall of 1992. The animals were packed in dry ice and shipped overnight to the Genome Variation Analysis Facility in Ithaca, N.Y., where they were stored at −70°C until use.

Mussels possessing a shell shape that appeared intermediate between the typical zebra and typical quagga mussel shapes were selected for electrophoresis. The samples were prepared for electrophoresis following Spidle et al. (1994). Shells of mussels used were sent as voucher specimens to the American Museum of Natural History (accession numbers are not yet available). Horizontal starch gel electrophoresis, histochemical visualization, and banding pattern interpretation were carried out according to May (1992). Each animal was scored for *PP** and *PGM1**. The quaternary structures of the proteins encoded by both loci were used to predict the electrophoretic phenotype of a hypothetical hybrid individual (Fig. 1).

Results and discussion

No hybrid individuals were observed among the 350 quagga mussels and 400 zebra mussels scored at the diagnostic *PP** locus. All quagga mussels surveyed possessed the diagnostic *PGM1**107 allele, while the zebra mussels surveyed possessed only the two common alleles *PGM1**100 and *PGM1**111. None of the zebra mussels surveyed possessed the *PGM1**107 allele which had previously been found in populations of *D. polymorpha* (Marsden et al. 1995). Cape Vincent is the only site where the *PGM1**107 allele was found in zebra mussels collected in the presence of quagga mussels (Marsden et al. 1995). Extensive resampling of that area in this study (Table 1) revealed no further instances of the *PGM1**107 allele in *D. polymorpha*, however. The zebra mussels surveyed for this report also lacked the other two rare *PGM1** alleles, *PGM1**88 and *PGM1**96, previously reported in the zebra mussel (J.E. Marsden, unpublished data). All mussels surveyed exhibited the electrophoretic phenotypes expected for either *D. polymorpha* or *D. bugensis* (Fig. 1).

If natural fertilization occurs between *D. bugensis* and *D. polymorpha*, it occurs at an extremely low rate and (or) any hybrid veligers do not successfully settle and mature, strongly suggesting that successful interspecific hybridization does not occur between these species. The laboratory production of hybrids by Nichols and Black (1993) would provide more convincing evidence for natural hybridization if larvae could be raised to maturity. In that case, it would still be necessary to demonstrate that the offspring are not products of contamination (cf. Allen et al. 1993) or gynogenesis, where the sperm triggers development of the egg without contributing genetic material from the father (demonstrated in Guo et al. 1993 and Guo and Gaffney 1993). To exclude these two possibilities, any putative hybrids between quagga and zebra mussels must be shown to contain gene products contributed by both parent species (cf. Allen and Gaffney 1993).

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