

Population genetics of the zebra mussel, *Dreissena polymorpha* (Pallas): local allozyme differentiation within midwestern lakes and streams

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Abstract: Several aquatic invertebrates with free-swimming larvae have paradoxically demonstrated fine-scale genetic heterogeneity. In this study, we tested for genetic heterogeneity in an exotic freshwater bivalve, the zebra mussel, *Dreissena polymorpha* (Pallas), which like many marine molluscs has a free-swimming larval stage. Zebra mussels were collected from 22 sites in the Great Lakes and from a small inland lake complex in southwestern Michigan and scored for 13 allozyme loci. Sites were sampled in a hierarchical fashion to assess the spatial scale of genetic variation. Zebra mussel populations exhibited significant genetic heterogeneity on a local scale within lakes, even though populations remained homogenous on a larger regional scale between lakes or lake complexes. The allozyme loci that exhibited heterogeneity differed from lake to lake. Populations also displayed significant heterozygote deficiencies from Hardy–Weinberg expectations for a majority of loci, implying population subdivision and (or) inbreeding on a fine scale. Our results suggest that local genetic differentiation for zebra mussels is both spatially and temporally fluid and is the product of stochastic processes, such as spawning asynchrony and uneven mixing of larval cohorts, rather than natural selection.

Résumé : On a paradoxalement observé une hétérogénéité génétique fine chez plusieurs invertébrés aquatiques à larves nageuses. Dans la présente étude, nous avons examiné s'il y a hétérogénéité génétique chez le bivalve exotique d'eau douce, la moule zébrée, *Dreissena polymorpha* (Pallas), qui, comme de nombreux mollusques marins, passe par un stade de larve nageuse. On a recueilli des moules zébrées à 22 sites des Grands Lacs et dans un petit complexe lacustre enclavé du sud-ouest du Michigan pour en examiner 13 loci d'allozymes. On a échantillonné les sites hiérarchiquement pour évaluer l'échelle spatiale de la variation génétique. Les populations de moules zébrées ont montré une hétérogénéité génétique significative à l'échelle locale à l'intérieur des lacs, mais demeuraient homogènes à l'échelle régionale entre lacs ou complexes lacustres. Les loci d'allozymes qui étaient hétérogènes n'étaient pas les mêmes d'un lac à l'autre. On a aussi observé chez les populations des réductions cas importantes de l'hétérozygotie par rapport aux prévisions de la loi de Hardy–Weinberg pour une majorité de loci, ce qui laisse croire en une subdivision des populations et (ou) à une endogamie à petite échelle. Nos résultats montrent que la différenciation génétique locale des moules zébrées est spatialement et temporellement fluide, et qu'elle est le produit de processus stochastiques, associés à l'asynchronie des pontes ou au mélange inégal des cohortes de larves, plutôt que celui de la sélection naturelle.

[Traduit par la Rédaction]

Introduction

An aquatic species with large population numbers and a free-swimming larval stage should be genetically homogenous across great distances within connected bodies of water (Palumbi 1995). Genetic shifts that occur in local populations, whether due to genetic drift or natural selection, should be quickly erased by gene flow from surrounding populations. Yet this does not always appear to be the case, especially in marine molluscs (Johnson and Black 1984;

Watts et al. 1990; Hedgecock 1994; Edmands et al. 1996; David et al. 1997; reviewed in Palumbi 1995 and Mitton 1997).

Sources of genetic heterogeneity fall into two general categories, those primarily due to deterministic processes (i.e., natural selection) and those due to stochastic processes (i.e., genetic drift). In the deterministic category, genetic variation (usually for a single locus) is related to a particular environmental variable. An example of this type of population structure is the cline for leucine aminopeptidase (LAP) among populations of the blue mussel, *Mytilus edulis* (Koehn et al. 1980). Hilbish and Koehn (1985a, 1985b) have demonstrated that selection against certain LAP genotypes is sufficient to maintain allele frequency differences between estuarine and marine populations of *M. edulis*, despite the exchange of larvae between the two habitats. In the stochastic category, population structure is characterized by an unstable, chaotic pattern of genetic variation across populations (Johnson and Black 1984; Watts et al. 1990; Johnson et al.

Received November 5, 1998. Accepted October 7, 1999.
J14881

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1993; Kordos and Burton 1993). This pattern is suggestive of genetic drift caused by spatial and temporal variation in juvenile recruitment (Watts et al. 1990; Johnson et al. 1993; Kordos and Burton 1993).

The zebra mussel, *Dreissena polymorpha* (Pallas), was introduced into North American freshwater ecosystems (originally the Great Lakes) during the mid-1980s via ballast discharge from European vessels (Hebert et al. 1989). Zebra mussels have a free-swimming planktonic larval stage that develops into a sessile, benthic adult, an unusual trait for freshwater molluscs but common in marine molluscs. This exotic mussel therefore provides a model to test whether the same population genetics that characterize many marine invertebrates also exist for a freshwater benthic organism.

Many previous studies have focused on the ecological and economic impacts of zebra mussels on North American aquatic habitats, but less is known about the population genetics of this species. *Dreissena polymorpha* populations in the United States and Canada have been found to contain extensive allozyme polymorphism, characteristic of species with large effective population sizes (Hebert et al. 1989; Garton and Haag 1991; Boileau and Hebert 1993; Haag and Garton 1995; Marsden et al. 1995). Nevertheless, significant geographic variation in allele frequency has been reported among populations within Lakes St. Clair, Erie, Michigan, and Ontario (Boileau and Hebert 1993; Marsden et al. 1995). The cause for this variation (i.e., deterministic or stochastic) is unclear. Russian researchers have claimed that allele frequencies for three allozyme loci (esterase-D, LAP, and malic enzyme) in zebra mussel populations surrounding the Chernobyl power plants were correlated with water temperature (Fetisov et al. 1992). It is therefore possible that water temperature (or an environmental factor correlated with water temperature) could explain the genetic heterogeneity present among North American *D. polymorpha* populations.

Here, we report on an extensive genetic survey of *D. polymorpha* populations collected from the Laurentian Great Lakes and from a small inland lake complex in southwestern Michigan (Eagle Lake complex). Our objective was to characterize the spatial scale (e.g., between complexes of lakes, among lakes, within lakes) over which genetic differentiation occurs for zebra mussels. We also tested for the possible effects of water temperature on allozyme variation in Milwaukee Harbor, Lake Michigan, Wis., to determine whether any differentiation observed could be due to natural selection.

Materials and methods

Collection sites and methods

Zebra mussels were collected from two lake complexes: the Laurentian Great Lakes and the Eagle Lake complex (an inland lake complex located in the St. Joseph River basin in southwestern Michigan; see Fig. 1 and accompanying legend for information on collection sites and sample sizes). For the Laurentian Great Lakes, we collected zebra mussels from three sites each in Lakes Erie, Michigan, and Ontario during the summer of 1995 (Fig. 1A). Within the Eagle Lake complex, we collected zebra mussels from three sites each in Eagle Lake (surface area = 153 ha), Christiana Lake (surface area = 72 ha), and Christiana Creek during the summer of 1994 (Fig. 1C). A "site" refers to a 10-m² collecting area within a lake. To measure the amount of variation present on a

microgeographic scale, one 10-m² site within Eagle Lake (EL1) (Fig. 1C) was divided into three subsites to examine microgeographic differentiation.

Our sampling design allowed for a four-level hierarchical analysis of population structure: (i) between complexes of lakes (i.e., the Great Lakes versus the Eagle Lake complex), (ii) among lakes within a complex (e.g., Lake Erie versus Lake Michigan versus Lake Ontario), (iii) among sites within lakes (e.g., site CL1 versus site CL2 versus site CL3 within Christiana Lake), and (iv) among microgeographic subpopulations within one site (i.e., EL1).

To test for the effects of temperature on North American zebra mussel populations, we collected adult zebra mussels from four sites in Lake Michigan near Milwaukee (Fig. 1B). Two sites (MH1 and MH2) were located in the industrial inner harbor of the city, while the remaining two sites (MH3 and MH4) were located in the outer harbor, separated from Lake Michigan by a breakwall. The outer harbor receives a greater influx of lake water, while the inner harbor is warmed by industrial outputs. As a result, temperatures differ between the two sets of sites (A.S. Brooks, University of Wisconsin-Milwaukee, personal communication). Water temperatures on the day of collection (23 May 1996) were 17.0 and 16.3°C for sites MH1 and MH2, respectively, and 11.0 and 8.9°C for sites MH3 and MH4, respectively.

Zebra mussels from the Great Lakes and the Eagle Lake complex were collected from hard substrates at depths of 0.5–1.5 m. Collections were made at random within a 10 × 10 m area. Individuals were transported to the laboratory on ice (nearby sites) or were immediately frozen on dry ice (distant sites). Samples were stored at -80°C prior to electrophoresis. Zebra mussels from Milwaukee Harbor were scraped from harbor walls at all four sites and immediately frozen on dry ice.

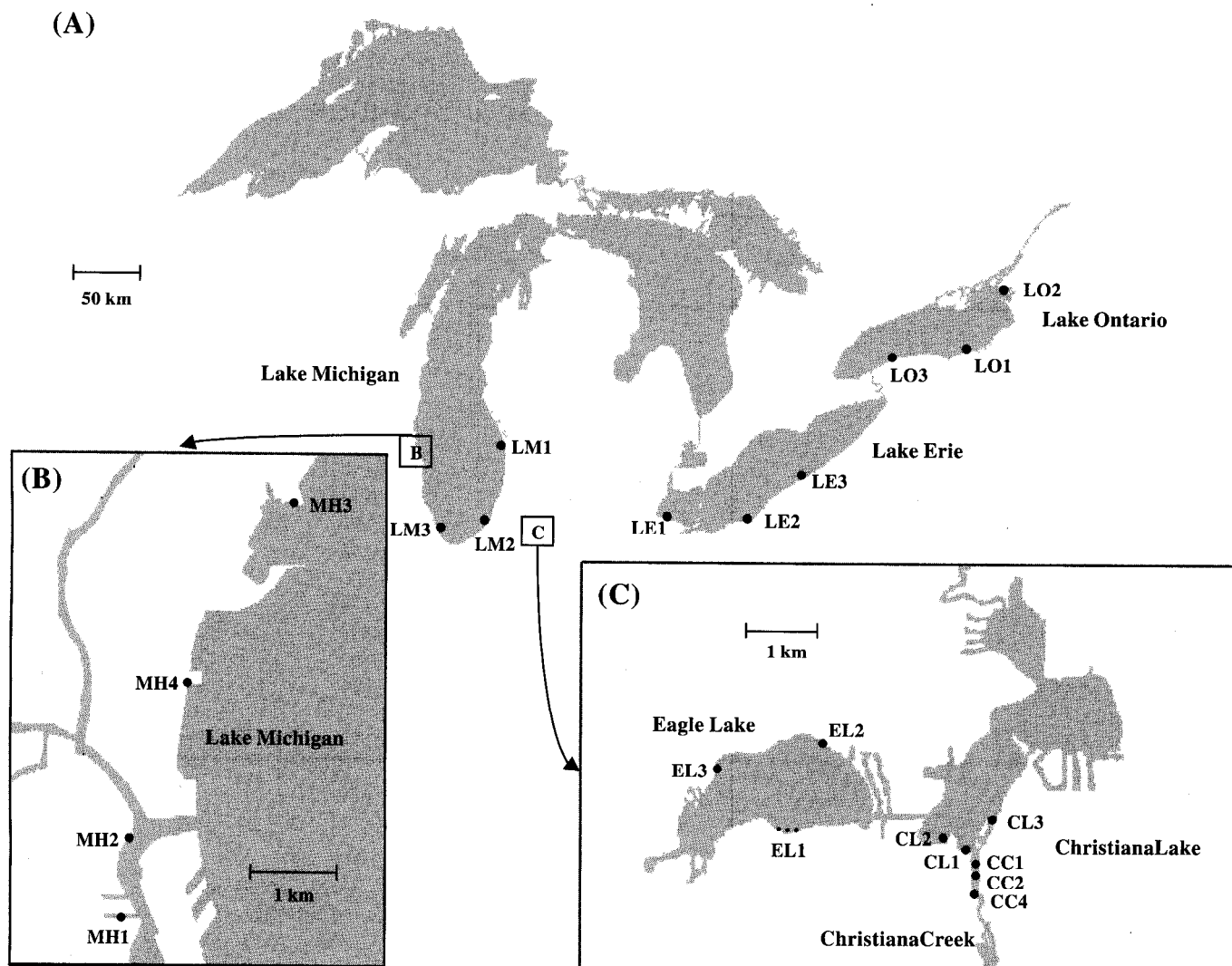
Genetic techniques

Soft tissue was removed from zebra mussel shells and a portion was homogenized in 15 µL of grinding buffer for allozyme analysis. Zebra mussels from the Eagle Lake complex and the Great Lakes were scored for a total of nine enzyme systems using standard starch gel electrophoresis (Murphy et al. 1990). We resolved a total of 13 different loci for these systems (Table 1). Individuals from the four Milwaukee Harbor sites were scored for a subset of these loci: ACO, IDHP, MDH1, ODH, and GPI. Five of the allozymes, ACO, IDHP, MDH1, ODH, and GPI, were resolved using a Tris-citrate electrode buffer system (pH = 6.3). The remaining loci, EST1, EST2, MEP, MDH2, and the peptidases, were resolved using a Tris-citrate electrode buffer system (pH = 8.0). Stain recipes were those of Murphy et al. (1990).

Data analysis

Populations were tested for allele frequency variation using *G* heterogeneity tests. Rare alleles were pooled as necessary to ensure that average expected frequency within cells was >6 (Zar 1999). Tablewide significance was determined using a sequential Bonferroni procedure (Holm 1979; Rice 1989). The *F* statistics (F_{IT} , F_{IS} , and F_{ST}) and expected and observed heterozygosities (H_e and H_o) were calculated using the Genetic Data Analysis system of Lewis and Zaykin (1996) based on the methods of Weir and Cockerham (1984). The *F* statistics were derived using a hierarchical design (Weir 1996) in which the total reduction in heterozygosity F_{IT} was partitioned into three components: (i) the portion due to local inbreeding within sites, F_{IS} , (ii) the portion due to differentiation among sampling sites within lakes, $F_{(ST)S}$, and (iii) the portion due to between-lake differentiation, $F_{(ST)P}$. We confined this analysis to the 18 study sites for which zebra mussels were scored for all 13 allozymes. Ninety-five percent confidence intervals were calculated for the *F* statistics by bootstrapping across loci (number of replications = 10 000; Weir 1996). Values for aver-

Fig. 1. (A) Collection sites and sample sizes (n , the number of mussels genetically scored) in the Great Lakes. Lake Michigan samples were from Douglas, Mich. (LM1, $n = 50$), New Buffalo, Mich. (LM2, $n = 49$), and Chicago, Ill. (LM3, $n = 50$). Lake Erie samples were from Maumee, Ohio (LE1, $n = 77$), Huron, Ohio (LE2, $n = 50$), and Headlands Beach, Ohio (LE3, $n = 48$). Lake Ontario samples were from Sodus Point, N.Y. (LO1, $n = 50$), Cape Vincent, N.Y. (LO2, $n = 50$), and 30 Mile Point, N.Y. (LO3, $n = 49$). (B) Collection sites in Milwaukee Harbor, Lake Michigan, Wis. Sites MH1 ($n = 50$) and MH2 ($n = 50$) were located within the warm industrial inner harbor of the city, and MH3 ($n = 50$) and MH4 ($n = 50$) were located in the colder, outer harbor. (C) Collection sites within the Eagle Lake Complex. Zebra mussels were collected from three sites each in Eagle Lake (EL1, $n = 432$; EL2, $n = 142$; EL3, $n = 142$), Christiana Lake (CL1, $n = 127$; CL2, $n = 128$; CL3, $n = 124$), and Christiana Creek (CC1, $n = 128$; CC2, $n = 142$; CC4, $n = 138$). One site in Eagle Lake (EL1) was subdivided into three microgeographic sites.



age heterozygosity (H) and average number of alleles per locus (A) are reported as mean \pm SE.

Results

High levels of allozyme polymorphism existed within all zebra mussel populations. The average heterozygosity (H) for the 13 loci resolved in the study was 0.36 ± 0.003 in the Eagle Lake complex and 0.38 ± 0.005 in the Great Lakes (Table 2). The average number of alleles per locus (A) was 3.3 ± 0.04 in the Eagle Lake complex and 3.1 ± 0.06 in the Great Lakes (Table 2). One locus, GPI, was extremely polymorphic, possessing 10 different electrophoretic alleles across all populations (Table 1). Over 69% of all individuals scored for this locus in the Eagle Lake complex were hetero-

zygotes, while 76% of Great Lakes individuals were heterozygotes (Table 2). Nonetheless, there was a general deficiency of heterozygotes from Hardy-Weinberg genotypic expectations at all sites (Table 2), suggesting population subdivision and local inbreeding within lakes. Values of F_{IS} were significantly > 0 for seven loci (ACO, EST2, ODH, PEPD, PEPGEN1, PEPGEN3, and GPI) (Table 2). Only MDH2 displayed a significant excess of heterozygotes (Table 2).

Allele frequencies were similar across lakes (K.M. Lewis et al., unpublished data, available upon request). Values of $F_{(ST)P}$ were not significantly > 0 between the Great Lakes and the Eagle Lake complex, among the Great Lakes, or among lakes and the stream of the Eagle Lake complex (Table 3). There was a slight but significant reduction in hetero-

Table 1. List of enzyme systems resolved giving the abbreviations, enzyme commission numbers (EC code), metabolic function, and number of alleles resolved for each enzyme.

| Enzyme locus | Abbreviation | EC code | Metabolic function | No. alleles |
|--|--------------|----------|-----------------------|-------------|
| Aconitase | ACO | 4.2.1.3 | Citric acid cycle | 3 |
| Esterase (two isozymes) | EST1 | 3.1.1.1 | Carboxylic ester | 3 |
| | EST2 | | Hydrolysis | 2 |
| Isocitrate dehydrogenase | IDHP | 1.1.1.42 | Citric acid cycle | 5 |
| Malate dehydrogenase (two isozymes) | MDH1 | 1.1.1.37 | Citric acid cycle | 3 |
| | MDH2 | | | 3 |
| Malic enzyme | MEP | 1.1.1.40 | Fatty acid oxidation | 3 |
| Octopine dehydrogenase | ODH | 1.5.1.11 | Anaerobic respiration | 3 |
| Peptidase-D (Phe-Pro) | PEPD | 3.4.13.9 | Peptide hydrolysis | 4 |
| Peptidase general (Leu-Ala) (three isozymes) | PEPGEN1 | 3.4.11 | Peptide hydrolysis | 4 |
| | PEPGEN2 | | | 3 |
| | PEPGEN3 | | | 4 |
| Phosphoglucose isomerase | GPI | 5.3.1.9 | Glycolysis | 10 |

Table 2. Average heterozygosity ($H \pm SE$) and average number of alleles ($A \pm SE$) per locus for the Great Lakes and Eagle Lake complex.

| Locus | Great Lakes | | Eagle Lake complex | | F_{IS} |
|---------|-------------|----------|--------------------|----------|---------------|
| | H | A | H | A | |
| ACO | 0.34±0.011 | 3.0±0.00 | 0.36±0.011 | 3.0±0.00 | 0.055±0.023* |
| EST1 | 0.28±0.024 | 2.1±0.17 | 0.24±0.008 | 2.3±0.11 | 0.005±0.025 |
| EST2 | 0.43±0.031 | 2.0±0.00 | 0.38±0.011 | 2.0±0.00 | 0.144±0.024* |
| IDHP | 0.37±0.026 | 2.7±0.29 | 0.44±0.016 | 3.0±0.24 | 0.032±0.017 |
| MDH1 | 0.46±0.023 | 2.3±0.17 | 0.48±0.020 | 2.7±0.17 | 0.001±0.031 |
| MDH2 | 0.30±0.020 | 3.0±0.00 | 0.24±0.011 | 3.0±0.00 | -0.046±0.020* |
| MEP | 0.02±0.005 | 1.8±0.18 | 0.04±0.007 | 2.6±0.15 | 0.022±0.040 |
| ODH | 0.21±0.018 | 3.0±0.00 | 0.21±0.012 | 3.0±0.00 | 0.212±0.031* |
| PEPD | 0.61±0.019 | 3.6±0.15 | 0.52±0.022 | 4.3±0.18 | 0.051±0.025* |
| PEPGEN1 | 0.34±0.024 | 2.9±0.24 | 0.27±0.011 | 2.8±0.20 | 0.070±0.026* |
| PEPGEN2 | 0.42±0.019 | 3.0±0.00 | 0.39±0.006 | 3.0±0.00 | 0.028±0.023 |
| PEPGEN3 | 0.37±0.023 | 3.9±0.11 | 0.41±0.018 | 4.1±0.11 | 0.198±0.030* |
| GPI | 0.76±0.023 | 6.9±0.40 | 0.69±0.016 | 7.2±0.26 | 0.049±0.014* |
| Overall | 0.38±0.005 | 3.1±0.06 | 0.36±0.003 | 3.3±0.04 | 0.067±0.019* |

Note: Also given are F_{IS} values (± 1 SD) estimated for loci across sites by the method of Weir and Cockerham (1984). Standard deviations for F_{IS} were determined by jackknifing over populations. Negative values reflect an excess of heterozygotes and positive values a deficiency compared with Hardy-Weinberg expectations. * $P < 0.05$ that $F_{IS} \neq 0$, as determined by jackknifing over populations.

zygosity in the Eagle Lake complex relative to the Great Lakes (Wilcoxon signed rank test, $Z = 1.994$, $P = 0.045$) (Table 2).

Despite the lack of broad-scale geographic variation between lake complexes and among lakes, significant genetic differentiation existed on both local and microgeographic scales within lakes. Thirteen out of 89 single-locus G tests displayed significant allele frequency heterogeneity among sites within lakes (Table 4). Three of these tests were significant on a tablewide basis (PEPD (Eagle Lake), PEPGEN3 (Christiana Creek), and GPI (Christiana Lake)). The 13 significant tests were distributed across seven different loci, and at least one locus was significant in each lake surveyed, except for Lake Michigan (Table 4). The F statistics indicated significant variation among all sites considered together ($F_{(STS)} = 0.0024$), sites within each Great Lake ($F_{(STS)} = 0.0063$), and sites within the Eagle Lake complex ($F_{(STS)} = 0.0027$) (Table 3). Genetic differentiation was as great among sites within Eagle Lake, Christiana Lake, or

Christiana Creek ($F_{(STS)} = 0.0027$) as across the entire Great Lakes ($F_{(STS)} = 0.0024$). Finally, four loci displayed significant allele frequency differences among the three microgeographic subpopulations collected within a 10-m² area in Eagle Lake (Table 4).

Local genetic variation was not correlated with water temperature among the Milwaukee Harbor sites (K.M. Lewis et al., unpublished data). The warm, inner Milwaukee harbor populations did not differ significantly in allele frequencies from the colder, outer harbor populations for any locus. No significant spatial heterogeneity was found among these four sites.

Discussion

The pattern of allozyme differentiation for the freshwater mussel *D. polymorpha* is similar to that observed for many marine invertebrates (e.g., Johnson and Black 1984; Watts et al. 1990; Johnson et al. 1993; Kordos and Burton 1993;

Table 3. *F* statistics for the Great Lakes and Eagle Lake complex calculated according to the method of Weir and Cockerham (1984).

| Sites compared | F_{IT} | F_{IS} | $F_{(ST)P}$ | $F_{(ST)S}$ |
|---------------------------------|----------|----------|-------------|-------------|
| All sites ^a | 0.0719* | 0.0674* | 0.0014 | 0.0024* |
| Great Lakes ^b | 0.0472* | 0.0412 | 0.0025 | 0.0063* |
| Eagle Lake complex ^c | 0.0779* | 0.0754* | 0.0006 | 0.0027* |

Note: * $P < 0.05$ as determined by bootstrap analysis of 10 000 replicates.

^aAnalysis for the Great Lakes and Eagle Lake complex combined; $F_{(ST)P}$ quantifies the level of genetic differentiation between the Great Lakes and Eagle Lake complex, while $F_{(ST)S}$ is a measure of differentiation within each complex of lakes.

^bAnalysis for the Great Lakes sites; $F_{(ST)P}$ quantifies the level of genetic differentiation among the Great Lakes, while $F_{(ST)S}$ is a measure of differentiation among sites within lakes.

^cAnalysis of the Eagle Lake complex; $F_{(ST)P}$ quantifies the level of genetic differentiation among Eagle Lake, Christiana Lake, and Christiana Creek, while $F_{(ST)S}$ is a measure of differentiation among sites within each lake and the creek.

Hedgecock 1994; Edmands et al. 1996). We found limited allele frequency variation for zebra mussels at a broad geographic scale among regions or lakes, consistent with organisms having a great dispersal capacity. However, significant genetic differentiation was observed locally within lakes. Furthermore, these differences persisted even on a microgeographic scale within a 10-m² area at one site. Allele frequency differences within lakes were not large in magnitude. Significant frequency differences for the common allele at a locus averaged 0.104 (range 0.043–0.160) among sites within lakes and 0.0639 (range 0.0419–0.0906) among the microgeographic sites at EL1 (K.M. Lewis et al., unpublished data). Consequently, the corresponding F_{ST} values were not particularly high (e.g., 0.0027 for the Eagle Lake complex and 0.0063 within the Great Lakes). Nevertheless, this genetic variation is significant, which is surprising given the presumed high potential for population mixing of *D. polymorpha* as veligers.

The small but significant reduction in heterozygosity observed between zebra mussel populations of the Great Lakes and the Eagle Lake complex may indicate that a population bottleneck occurred during the original colonization of the Eagle Lake complex. Zebra mussels were presumably introduced into the Eagle Lake complex from the Great Lakes by recreational boaters ca. 1991, perhaps as a single event (Horvath et al. 1996). However, there was little genetic differentiation between the two sets of lakes, and virtually all alleles were present in both sets of lakes. Consequently, there is no strong evidence for a founder effect during the colonization of the Eagle Lake complex.

One possible explanation for the pattern of genetic differentiation among *D. polymorpha* populations within lakes is natural selection. Strong selection tied to differences in environmental conditions among sampling sites could counteract gene flow, even if settling larvae were genetically homogeneous. Our findings, however, do not support the selection hypothesis. First, allozyme frequencies did not differ between populations at different water temperatures in our study. Contrary to reports from Chernobyl, Russia (Fetisov et al. 1992), we found no evidence for thermal selection on zebra mussels in Milwaukee Harbor. Second, although significant local heterogeneity was found for a number of loci, the set

Table 4. Loci displaying significant allele frequency variation among sites within lakes as determined with *G* heterogeneity tests.

| Locus | Location | <i>G</i> | df | <i>P</i> |
|---------|---------------------|----------|----|----------|
| IDHP | Lake Erie | 6.78 | 2 | 0.0348 |
| IDHP | Christiana Lake | 6.70 | 2 | 0.0367 |
| MDH1 | Lake Ontario | 6.64 | 2 | 0.0347 |
| MDH2 | Lake Erie | 8.40 | 2 | 0.0105 |
| MDH2 | Christiana Creek | 8.34 | 2 | 0.0147 |
| MDH2 | Eagle Lake | 8.81 | 4 | 0.0341 |
| ODH | Lake Erie | 9.64 | 2 | 0.0134 |
| ODH | Eagle Lake subsites | 14.00 | 4 | 0.0070 |
| PEPD | Eagle Lake | 22.26 | 4 | 0.0001* |
| PEPD | Eagle Lake subsites | 10.40 | 4 | 0.0287 |
| PEPGEN1 | Eagle Lake subsites | 5.81 | 2 | 0.0498 |
| PEPGEN3 | Lake Erie | 12.26 | 4 | 0.0230 |
| PEPGEN3 | Christiana Creek | 33.95 | 6 | 0.0001* |
| PEPGEN3 | Eagle Lake | 19.69 | 6 | 0.0026 |
| PEPGEN3 | Eagle Lake subsites | 13.03 | 6 | 0.0492 |
| GPI | Christiana Creek | 16.13 | 6 | 0.0146 |
| GPI | Christiana Lake | 30.51 | 6 | 0.0002* |

Note: * $P < 0.05$ on a tablewide basis as determined with the sequential Bonferroni procedure.

of loci showing variation differed among lakes. It seems unlikely that multiple allozyme loci would be under such diverse selection pressures in adjacent and interconnected bodies of water such as the Eagle Lake complex.

A more plausible explanation for the pattern of genetic differentiation, which we term the “cohort” hypothesis, centers on the population dynamics of zebra mussels. Although zebra mussels are broadcast spawners (Ludyanskiy et al. 1993), spawning is not always well synchronized among localities or individuals at sites (Garton and Haag 1993). Effective population sizes are therefore probably orders of magnitude lower than suggested by the often staggering densities of *D. polymorpha*, which can be upwards of 50 000/m² (Dermott et al. 1993). As a consequence, allele frequencies can differ among larval cohorts due to genetic drift, with the scale of this effect being inversely proportional to the number of parents contributing to cohorts (Tracey et al. 1975). If conditions are such that larval cohorts do not mix prior to settlement, then local genetic differentiation can result. For instance, effective population sizes in the range of about 160–370 would generate the F_{ST} values observed within lakes in our study (0.0063–0.0027). In addition, if larval cohorts do not tend to mix, then many new recruits at sites will be genetically related. This will heighten levels of inbreeding when these individuals reach sexual maturity and interbreed, further contributing to genetic differences among cohorts. Of course, patterns of mating are likely to be variable through time. Such demographic instability will produce highly dynamic population genetics, as evident in the “chaotic” nature of allozyme loci displaying significant variation within lakes in the current study.

If the dynamics of zebra mussel mating and dispersal are affecting population structure, then there must be both spatial and temporal genetic variation among settling larval cohorts. Unfortunately, technical limitations make it difficult to score zebra mussel larvae for allozymes. Nevertheless, Haag and Garton (1995) were able to score larval populations for

one locus (GPI) using cellulose acetate electrophoresis. They found that planktonic larvae had different GPI genotype frequencies than parental populations, a difference that they attributed to natural selection. However, given our results, it seems more likely that such differences could be due to population demography rather than to selection. Regardless, resolution of the cohort issue requires the simultaneous scoring of a number of different loci from the same set of individuals. The small size of zebra mussel veligers will always limit the number of allozyme loci that can be resolved. A possible future approach would therefore be to develop molecular DNA markers for *D. polymorpha* based on polymerase chain reaction technology. Such a strategy has recently been used to examine mtDNA variation among samples of larvae from the Pacific oyster, *Crassostrea gigas*, in Dabob Bay, Wash. (Li and Hedgecock 1998). Significant mtDNA haplotype frequency variation was observed among oyster cohorts during a single spawning season. This differentiation was ascribed to genetic drift owing to a large variance in adult reproductive success through the season, similar to what we propose for zebra mussels.

The general deficiency of heterozygotes from Hardy-Weinberg equilibrium could be interpreted as corroborative evidence for population substructuring and inbreeding in zebra mussels, supporting the cohort hypothesis. However, heterozygote deficiencies were not uniform across loci (Q test for heterogeneity of F_{IS} values by metaanalysis = 144.0, $P < 0.0001$, 12 df). It is therefore not possible for a single inbreeding coefficient to explain the observed genotypic deviations by a Wahlund effect. In addition, the rank order of F_{IS} values for loci was consistent across sites (Kendall congruence coefficient (W) = 0.289, $\chi^2 = 62.47$, $P < 0.0001$, 12 df). ODH, PEPGEN1, PEPGEN3, and EST2 generally showed the largest heterozygote deficiencies at sites, while MEP, MDH2, EST1, and PEPD displayed the smallest deviations. These results suggest that other factors, in addition to population subdivision and inbreeding, are contributing to the observed heterozygote deficiencies.

It has recently been suggested that departures of marine invertebrates from Hardy-Weinberg equilibrium could be explained by the presence of allozyme null alleles (Gaffney 1994; Hoare and Beaumont 1995; David et al. 1997). We cannot rule out this possibility for zebra mussels. In fact, we detected a null allele at MDH2 that ranged in frequency from about 0.011 to 0.053 at sites (K.M. Lewis et al., unpublished data). Since this particular null allele was able to dimerize, null heterozygotes gave a distinct double, instead of triple, banding zymogram pattern in gels. Interestingly, MDH2 was the only locus that displayed a significant excess of heterozygosity in our study, perhaps the result of us mis-scoring MDH2 null homozygotes as unresolved individuals. If the null heterozygotes are removed from consideration, then the F_{IS} value for MDH2 is not significantly different from 0 (-0.022 ± 0.276). The relatively large departures from Hardy-Weinberg proportions observed for the loci ODH, PEPGEN1, PEPGEN3, and EST2 could therefore be due to undetected null alleles. We estimate that null alleles segregating at frequencies of 0.036–0.118 would account for the ODH, PEPGEN1, PEPGEN3, and EST2 results. These estimates are based on the formula $r = D/(2 - D)$, where r is the frequency of the null allele at a locus and D is the

heterozygote deficiency (Chakraborty et al. 1993). Of course, this estimate assumes that the deficiencies are only caused by null alleles, which is clearly not the case, as population subdivision also exists. Furthermore, ODH, PEPGEN1, PEPGEN3, and EST2, the loci with the largest heterozygote deficiencies, are all monomers in zebra mussels. Consequently, null alleles would be difficult to detect at these loci, as null heterozygotes would not display an unusual banding pattern but would appear as homozygotes for the alternate allele at the locus. Further work is needed to assess the prevalence of null allozyme alleles in zebra mussel populations.

In conclusion, zebra mussels display significant genetic heterogeneity on a local scale within lakes, even though populations remain relatively homogenous on a larger regional scale between lakes. This pattern of differentiation is similar to that reported for a number of marine invertebrates. We suggest that fine-grained genetic differentiation for zebra mussels is the product of a dynamic population demography rather than natural selection. Additional genetic studies of larvae at the molecular level are needed, however, to verify that local patterns of allozyme variation for zebra mussels are due to asynchrony in spawning and incomplete mixing of veliger cohorts.

Acknowledgments

We express our thanks to the following people who assisted in the completion of this project. Gerry Bouchard, Brian Bouchard, Tom Horvath, Bill Perry, Steve Beaty, Melanie Vile, Bill Webb, and Eileen Smith aided in various zebra mussel collections. Randy Owens and Marty Berg provided zebra mussels from 30 Mile Point, N.Y., and Chicago, Ill., respectively. Jerry Castor and Art Brooks at the University of Wisconsin-Milwaukee helped arrange the Milwaukee Harbor survey. Becky Bruckert and Sheila Meehan assisted with processing and electrophoresis of zebra mussels. Two anonymous reviewers and an Associate Editor provided helpful comments on an earlier version of this manuscript. This research was funded through a cooperative agreement with the U.S. Environmental Protection Agency as part of the EPA Introduced Species Research Program (CR 820290-02).

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