

VARIATION IN GENOTYPE FREQUENCIES DURING THE LIFE HISTORY OF THE BIVALVE, *DREISSENA POLYMORPHA*

WENDELL R. HAAG¹ AND DAVID W. GARTON²

¹U.S. Department of Agriculture, Forest Service, Forest Hydrology Laboratory, P.O. Box 947, Oxford, Mississippi 38655 and Department of Zoology, Ohio State University, Columbus, Ohio 43210

²Department of Biological and Physical Sciences, Indiana University, 2300 South Washington St., Kokomo, Indiana 46904 and Department of Zoology, Ohio State University, Columbus, Ohio 43210

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Populations of marine benthic invertebrates often show geographic and temporal genetic heterogeneity, even though these species have widely dispersing planktonic larvae that tend to homogenize genetic structure of populations. Much of this heterogeneity is attributed to localized selection or immigration of genetically different larvae (Koehn et al. 1980; Gartner-Kepkay et al. 1983; Johnson and Black 1984; Hilbish 1985). However, these studies have been handicapped by an inability to examine genetic variation in the larvae themselves. Selection may be most intense on larvae and newly settled individuals (Johnson and Black 1984; Hilbish 1985; Hilbish and Koehn 1985; Gosling and McGrath 1990) because extremely high mortality may occur during the pelagic stage and in the transition from planktonic larvae to sedentary juveniles (Lewandowski 1982a; Powers et al. 1991). Therefore, selective mortality of larvae may largely determine the genetic structure of adult populations, and selection on adults may be relatively unimportant in producing and maintaining local heterogeneity (Ayre 1990).

Despite the potential importance of processes acting on early ontogenetic stages, no studies have examined genetic variation throughout the development of a cohort of benthic invertebrates. This is because of unique difficulties associated with the determination of genetic structure of larval populations. The small size of larvae of most species renders traditional allozyme analyses difficult or impossible. Additionally, the large number of morphologically similar larvae in marine systems makes identification to species level difficult. Hu et al. (1992) were able to resolve phosphoglucose isomerase allozymes in laboratory cultured oyster larvae, but studies of larvae from wild populations are lacking. The recent introduction of the freshwater bivalve *Dreissena polymorpha* into the North American Great Lakes provides an opportunity to examine genetic variation throughout the life history of a cohort. *Dreissena polymorpha* is native to the Caspian and Black Sea drainages (Wiktor 1963) and is thought to have entered the Great Lakes via ballast water discharge from European shipping traffic. *Dreissena* is a recent secondary colonizer of freshwater (Barrington 1979) and is similar to marine bivalves in having a planktonic veliger

larva. Gametes are shed into the water where fertilization occurs, and larvae remain in the plankton for approximately 10 d before settling (Walz 1978; Lewandowski 1982b). The Great Lakes population appears to be derived from a single founding group (Griffiths et al. 1991); thus, migration from other, genetically distinct populations can be discounted as an explanation for any genetic differences observed among life history stages. Furthermore, *D. polymorpha* is the only mollusk in most of the Great Lakes with a planktonic larval stage thus, problems associated with identification of larvae are avoided. We examined allozyme frequencies throughout the life history of *D. polymorpha* in the Great Lakes in order to directly compare the genetic structure of the planktonic larvae with that of benthic stages.

MATERIALS AND METHODS

Phosphoglucose isomerase (*PGI*, EC 5.3.1.9) genotype frequencies were determined for individuals from four life-history stages of a putative cohort of *Dreissena polymorpha* in the Bass Islands of western Lake Erie during 1990. In ontogenetic sequence, these stages are (1) the parent population for 1990 recruits, (2) planktonic larvae, (3) newly settled post larvae, and (4) 1- to 2-mo-old juveniles. The study was conducted at The Ohio State University's F.T. Stone Laboratory at Put-in-Bay, Ohio (fig. 1). The site consists of a shallow, rocky reef and adjacent mud flats in water 5 m deep or less. *Dreissena polymorpha* colonized the area in 1988, and in 1990 was present at very high densities.

Genotypes were determined using cellulose acetate electrophoresis, which requires less tissue than traditional starch-gel techniques (Hebert and Beaton 1989). Phosphoglucose isomerase was chosen because it is a highly polymorphic system in Great Lakes *D. polymorpha* (Hebert et al. 1989; Garton and Haag 1991) and is a robust enzyme that yielded easily scorable results even with small tissue masses of larvae. For all samples, a standard was run on each gel for reference. This standard consisted of a large (22 mm shell length) *D. polymorpha*, ground in 5 ml of distilled water. This volume was divided into smaller aliquots and frozen for later use. The standard animal was of the *cld* genotype (see below for description of the *PGI* locus).

¹ Address for correspondence.

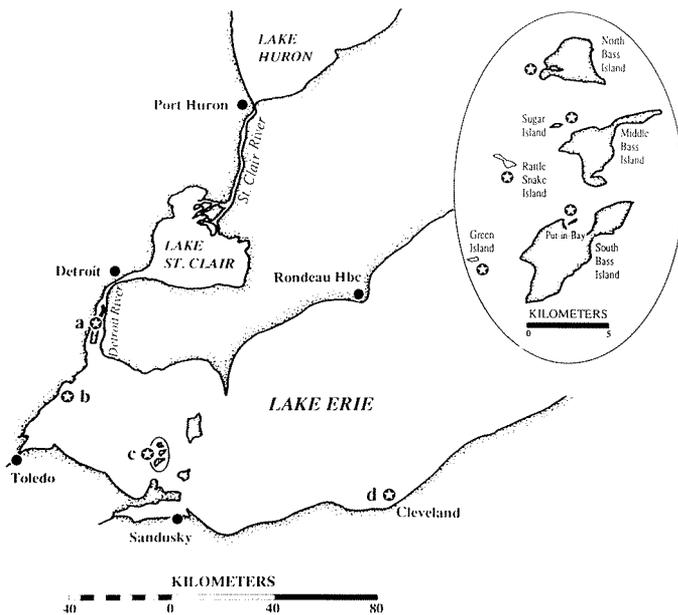


FIG. 1. Map of western Lake Erie showing sample sites for collections of *Dreissena polymorpha*. Site a, Detroit River, Michigan; site b, LaPlaisance Bay, Michigan; site c, Bass Islands, Ohio, representing five sample sites indicated in the inset; and site d, Cleveland, Ohio.

Adults considered as the parent population for recruits to Put-in-Bay were collected from eight widely separated sites in Lake Erie. This was done to insure that any differences observed among life-history stages could not be attributed to migration of larvae from other, genetically distinct populations. Because of the rapid flow of water through western Lake Erie (30 d residence time; Garton and Haag 1993) and the ability of planktonic larvae to postpone settlement for several weeks (Sprung 1989), larvae recruiting to Put-in-Bay could have originated from many locations. Five sites were located in the Bass Islands region of the western basin (fig. 1), all within an 8-km radius of Put-in-Bay. The other three sites were located at LaPlaisance Bay, Michigan; Detroit River, Michigan, and Cleveland, Ohio (fig. 1). Animals greater than 10 mm in length were considered adult and reproductively mature (Walz 1978). At each site, approximately 100 adult mussels were collected and frozen at -70°C for later allozyme analysis. *PGI* genotype frequencies were determined from a piece of adductor muscle and mantle tissue for each individual. Mean shell length of animals collected at each site ranged from 19.4 to 23.1 mm. Adults from the eight study sites showed no among-population heterogeneity for *PGI* genotype frequencies ($F_{ST} = 0.0003$, not significantly different from zero, $P < 0.995$, χ^2 , $df = 1$; F_{ST} calculated according to Nei and Chesser [1983] for multiallele systems) and were pooled for later analysis.

Veliger larvae were collected from plankton samples on four dates from August 4 to September 15 when high abundances of larvae were observed (fig. 2). Larvae were collected by making vertical plankton hauls with a 30.5 cm diameter net with 64 μm mesh. Whole plankton samples were transported to the laboratory in buckets and sorted immediately under a dissecting microscope. Live larvae were transferred

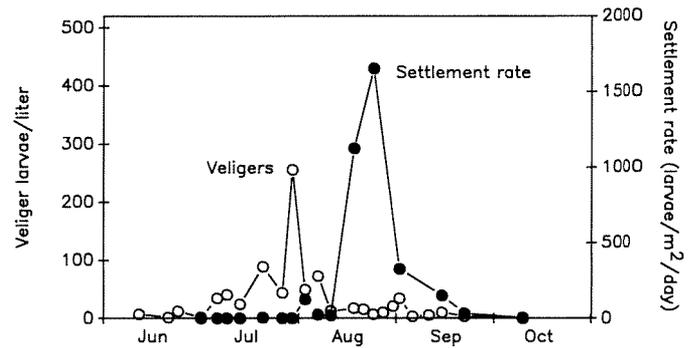


FIG. 2. Abundance of planktonic larvae and settling rate of *Dreissena polymorpha* in Lake Erie at Put-in-Bay, Ohio in 1990. From Garton and Haag 1993.

to electrophoresis loading trays with a micropipette, placing a single larva into each well. Individual larvae were immediately ground with fine forceps in 5 μl of distilled water under a dissecting microscope, and the entire volume of the well was applied to a cellulose acetate gel. After electrophoresis for approximately 30 min, gels were stained at 37°C for up to 1 h before scoring (normal staining time is 5–10 min.). Mean length of larvae electrophoresed was 0.199 mm. *PGI* genotypes were homogeneous among the four larval samples ($P < 0.43$, $G = 15.23$, $df = 15$) and these samples were pooled for ontogenetic analysis.

Newly settled larvae (settlers) were collected from August 18–23, during a peak in settling intensity (fig. 2). Settlers were collected from glass slides that had been allowed to develop a coating of periphyton before exposure in the lake. Slides were held in wooden racks holding five to eight slides each and suspended 0.5 m off the bottom in water 5 m deep. Slides were removed and examined under a dissecting microscope, and live settlers were prepared immediately for electrophoresis using the method described for larvae. Mean length of settlers was 0.288 mm.

Juvenile mussels were collected at 2–3 wk of age (September 5) and again at approximately 2 mo of age (October 23). These animals were collected from settling plates constructed of two 20 \times 20 cm pieces of untreated plywood held by a central bolt and suspended 0.5 m off the bottom. Plywood plates were placed in the water during the peak in settlement observed in mid-August. Juveniles collected at 2–3 wk and 2 mo had mean lengths of 1.094 mm and 3.200 mm, respectively. Juveniles were frozen in liquid N_2 and later ground whole for electrophoresis. *PGI* genotypes were homogeneous among the two juvenile samples ($P < 0.94$, $G = 1.20$, $df = 5$) and these samples were pooled for ontogenetic analysis.

DATA ANALYSIS

Electrophoretically detectable alleles were designated *a* through *e*, depending on their relative mobility with *a* migrating the slowest and *e* the fastest. Alleles *a* through *e* had relative mobilities of 56, 80, 100, 147, and 200, respectively. These mobilities correspond closely to previously reported data for the *PGI* locus in Great Lakes *Dreissena* (Spindle et al. 1994). The *a* and *b* alleles were present at mean frequen-

TABLE 1. *PGI* genotype frequencies of four life-history stages of *Dreissena polymorpha* from Lake Erie.

	c/c	c/d	c/e	d/d	d/e	e/e	N
Adults	.1084	.3544	.1264	.2190	.1219	.0113	443
Larvae	.1076	.3233	.0775	.2268	.1701	.0416	529
Settlers	.1346	.3269	.0897	.2371	.1410	.0128	156
Juveniles	.1000	.3095	.1190	.2381	.0714	.0048	210

cies (for all life history stages combined) of 0.0026 and 0.0250, respectively, and were excluded from this analysis. Comparisons of genotype and allele frequencies were made using $R \times C$ G-tests of independence (Sokal and Rohlf 1981). In comparisons involving multiple testing, significance levels for G-tests were adjusted for experiment wise error rates using χ^2 critical values for unplanned multiple comparisons (Rohlf and Sokal 1981) and a sequential Bonferroni technique (Lessios 1992). Because no standard methodology exists for multiple comparisons of contingency tables, the most conservative significance level was used in instances in which they yielded different results. Genotype frequencies of each life-history stage were tested for agreement with Hardy-Weinberg expectations using goodness-of-fit tests with significance levels adjusted for multiple testing (Rohlf and Sokal 1981; Lessios 1992).

RESULTS

There was a significant overall difference in genotype frequencies among all life-history stages ($P < 0.05$, $G = 28.87$, $df = 15$) (table 1). To identify the life-history stage(s) responsible for this high G value, genotypes for the four life-history stages were pairwise tested in ontogenetic sequence (table 2). In this analysis, larvae were significantly different from the parent population ($P < 0.01$, $G = 19.10$, $df = 5$), but comparisons between subsequent life-history stages were nonsignificant. When larvae were excluded from the overall analysis, there was no difference among adults, settlers, and juveniles ($P < 0.68$, $G = 7.43$, $df = 10$).

The difference between adults and larvae was the result of small fluctuations in the frequency of genotypes with the e allele. There was no overall difference in allele frequencies among all life history stages ($P < 0.27$, $G = 7.65$, $df = 6$) but comparison of allele frequencies between adults and larvae resulted in a high, though nonsignificant G -value ($P < 0.059$, $G = 5.62$, $df = 2$). Genotype frequencies of each life-history stage, including larvae, were in agreement with Hardy-Weinberg expectations. However, when each genotype was individually excluded from the comparison of adults and larvae, excluding the genotypes ele and c/e , each yielded nonsignificant results (table 3). Individually excluding all other genotypes gave a significant difference between adults and larvae, indicating that variation in the frequency of the ele and c/e genotypes caused the observed difference between adults and larvae.

DISCUSSION

It has long been speculated that selection pressures or other processes may be very different in planktonic larval stages of benthic invertebrates than in adult populations. However,

TABLE 2. Results of $R \times C$ G-tests of independence among genotype frequencies of sequential life-history stages of *Dreissena polymorpha*. Each comparison has 5 degrees of freedom. Significance levels were adjusted for multiple comparisons using a sequential Bonferroni technique (Lessios 1992).

	G	P
Adults versus larvae	19.10	<0.01
Larvae versus settlers	5.18	NS
Settlers versus juveniles	3.14	NS
Juveniles versus adults	4.88	NS

previous studies of population genetics of organisms with widely dispersing larvae have been unable to directly examine genetic variation in larvae. Unique circumstances surrounding the introduction of *Dreissena polymorpha* into Lake Erie have made it possible to examine genetic variation throughout the ontogeny of a cohort. May and Marsden (1992) report the occurrence of another species of *Dreissena* in the Great Lakes (*Dreissena bugensis*), but this species is present at very low densities in Lake Erie (Mills et al. 1993). Furthermore, the eight populations used as the putative parent population showed no genetic differentiation and did not deviate from Hardy-Weinberg expectations as would be expected by the lumping of two genetically differentiated species (the Wahlund effect). Thus, we have been able to directly compare genetic variation of larvae to their corresponding parents and later life-history stages.

In Lake Erie *D. polymorpha*, planktonic larvae showed different genotype frequencies than the parent population. Early benthic life history stages were not different from parents but also did not differ from larvae. This implies that settling represents a transitional stage in which genotypes are intermediate between planktonic larvae and benthic juveniles and adults. For a sessile, benthic organism, the pelagic larval stage represents a brief, but radically different, mode of existence and may be subject to entirely different selection regimes.

Variation in viabilities of genotypes during ontogeny has been invoked to explain the heterozygote deficiencies that are frequently observed in marine invertebrates having planktonic larvae (Zouros and Folz 1984; Singh and Green 1984; Gaffney et al. 1990). Heterozygous larvae may suffer higher mortality because of greater food requirements associated with higher growth rates (Singh and Green 1984). Accord-

TABLE 3. Results of $R \times C$ G-tests of independence among genotype frequencies of adult and larval *Dreissena polymorpha* by individually excluding each genotype from the analysis. Each comparison has 4 degrees of freedom. Significance levels were adjusted for experimentwise error rate (Rohlf and Sokal 1981; Sokal and Rohlf 1981).

Genotype excluded	G	P
<i>c/c</i>	19.19	<0.01
<i>c/d</i>	17.87	<0.01
<i>c/e</i>	12.50	NS
<i>d/d</i>	19.04	<0.01
<i>d/e</i>	14.72	<0.05
<i>e/e</i>	10.19	NS

ingly, oyster larvae from inbred matings, with presumably lower heterozygosity, had higher viabilities than larvae from outcrossed matings (Mallet and Haley 1983). In contrast, heterozygosity is positively correlated with fitness characters in benthic life history stages of mollusks including survival in post larval oysters (Zouros et al. 1983) and growth rate in adult *D. polymorpha* in Lake Erie (Garton and Haag 1991). Thus, consistent heterozygote deficiencies may be explained by a reversal of selection during ontogeny, by which selection operates against heterozygotes during the larval stage, but favors heterozygotes after settlement (Mallet et al. 1985).

Dreissena polymorpha larvae did not show significant departure from Hardy-Weinberg expectations. However, fluctuations in frequencies of genotypes containing the *e* allele are clearly responsible for the overall difference in genotype frequencies between larvae and adults. Adults with the *e* allele have higher mass-specific metabolic rates and lower fecundity than other genotypes (A. Stoeckman and S. Kendall-Eagleson pers. comm. 1993). Fitness differences related to these genotypes may be manifested as differential survival as larvae, but these small effects are not detectable by conventional tests for Hardy-Weinberg expectations that typically have a high probability of type-II error (Lessios 1992).

This study provides a first look at the genetic structure of planktonic larvae in a natural, sexually reproducing population. Our results show that planktonic larval stages of aquatic organisms may be genetically different from parents. Furthermore, these differences are not the result of migration from other, genetically different populations. This implies that larvae may be subject to entirely different selection regimes than adults and processes acting at the larval stage play an important role in structuring adult populations.

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Corresponding Editor: J. Hilbish