J. Moll. Stud. (1987), **53**, 223–228.

PHOSPHOGLUCOMUTASE ALLOZYME EVIDENCE FOR AN OUTCROSSING MODE OF REPRODUCTION IN THE HERMAPHRODITIC BROODING BIVALVE *MYSELLA TUMIDA* (GALEOMMATACEA)

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(Received 30 December 1986)

ABSTRACT

Allozyme variation of a *Mysella tumida* population in Patricia Bay, B.C., Canada was investigated at the phosphoglucomutase (PGM) locus. Seven alleles were detected. The observed frequency of allelic combinations did not differ significantly from random mating expectations ($0.25 < P < 0.50$), however, heterozygosity levels were slightly greater than expected (Selander's $D = 0.022$). These results, together with previous work on the *M. tumida* reproductive cycle, indicate that natural populations of this bivalve rarely, if ever, self-fertilize.

INTRODUCTION

The superfamily Galeommatacea contains arguably the most diverse reproductive specializations in the Bivalvia (Ockelmann & Muus, 1978). Many of these specializations are present in the genus *Mysella*. Species of *Mysella* whose reproduction have been studied to-date are hermaphroditic and brood embryos in the suprabranchial chamber until they reach a straight-hinged veliger stage (Lebour, 1938; Miyazaki, 1936; Gage, 1968; Franz, 1973; Ockelmann & Muus, 1978; Fox, 1979; Ó Foighil, McGrath, Conneely, Keegan & Costello, 1984; Ó Foighil, 1985a). Exogonadal sperm storage is known to occur in 3 species: *M. bidentata* (Deroux, 1961; Ockelmann & Muus, 1978), *M. cuneata* (Gage, 1968; Fox, 1979) and *M. tumida* (Ó Foighil, 1985b). Spermatophore production has been recorded from *M. bidentata* (Deroux, 1961; Ockelmann & Muus, 1978) and *M. tumida* (Ó Foighil, 1985b), and the former species is also known to possess dimorphic sperm (Ockelmann & Muus, 1978). Due in part to the reproductive complexity of these species there is, however, little known about their basic reproductive modes, especially concerning the frequency of self-fertilization in natural populations.

Mysella tumida (Carpenter, 1864) occurs in the Northeastern Pacific Ocean (Abbott, 1974) where it lives in association with a variety of burrowing invertebrate hosts (Ó Foighil & Gibson, 1984; Ó Foighil, 1985b). This species is an hermaphrodite which retains sperm in the gonad during all stages of oogenesis, so could conceivably reproduce by self-fertilization (Ó Foighil, 1985b, 1986). However, several preliminary lines of indirect evidence suggest that self-fertilization is not the normal mode of reproduction. First, there is a temporal separation of approximately 1 month in the onset of the spawning of sperm and eggs in natural populations (Ó Foighil, 1985b). Second, the production of spermatophores and the exogonadal storage of sperm seems inconsistent with a high degree of self-fertilization in *Mysella* species (Ockelmann & Muus, 1978; Ó Foighil, 1985b). Finally, male allocation in terms of gonadal volume is estimated at 40–50% for this species (Ó Foighil, 1985b, 1986) which is in broad agreement with theoretical values expected in outcrossing simultaneous hermaphrodites (Heath, 1979; Fischer, 1981; Charlesworth & Charlesworth, 1981). However, these data do not rule out the possibility of facultative self-fertilization and do not give any clear insight into the importance of this potential process in the reproduction of *M. tumida*.

Ability to self-fertilize may be investigated by isolation experiments. Franz (1973) observed successful reproduction by isolated individuals of *Mysella planulata* and concluded that they

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had self-fertilized their broods. This may not be the case, however, if *M. planulata* engages in exogonadal sperm storage as do many of its congeners (Deroux, 1961; Gage, 1968; Ockelmann & Muus, 1978; Fox, 1979; Ó Foighil, 1985b).

An alternate method to determine reproductive mode is to investigate the population genetics of the species of interest. The genetic consequences of self- and cross-fertilization are distinct (Bell, 1982). Heterozygosity levels are very sensitive to self-fertilization because the diluting effect of random chromosome segregation during meiosis is not counteracted by the fusion of gametes originating from different genomes. Prolonged self-fertilization rapidly eliminates heterozygosity (Kallman & Harrington, 1964). In contrast, alleles segregating in a large randomly outcrossing population will approximate the Hardy-Weinberg-Castle equilibrium, provided the population is not subjected to significant selection or migration (Levinton & Koehn, 1976). Comparing observed population allozyme frequencies at given loci with those expected in a random mating system is one way of inferring whether the observed variation is Mendelian (Ferguson, 1980). The occurrence of self-fertilization in the population will lead to greater than expected homozygosity, as could other factors such as differential mortality and/or substantial immigration from neighbouring populations that contain a different set of allele frequencies (Wahlund effect) (Berger, 1983).

This study employs a protein phenotype approach for a single population of *Mysella tumida* and compares the observed heterozygosity level at the highly polymorphic Phosphoglucomutase (PGM) locus to the alternative expectations of outcrossing and self-fertilization.

MATERIALS AND METHODS

Mysella tumida adults were collected at Patrica Bay, British Columbia, in January 1986. After collection, non-brooding adult animals were stored at −70°C until analyzed electrophoretically. Electrophoresis was performed at Friday Harbor Laboratories using 13% starch gels, standard power supplies and horizontal electrophoretic apparatus. PGM gave the best resolution of the polymorphic isozyme phenotypes detected (PGM, phosphoglucose isomerase, leucine amino peptidase, esterase with α naphthyl acetate substrate, peptidase with glycyl-leucine substrate and peptidase with leucyl-glycyl-glycine substrate) and 111 individuals were characterized for this locus. Recipes for gel and bridge buffers and for the PGM staining solution were obtained from Tracey *et al.* (1975) and Eernisse (1984). Electromorph phenotypes were scored using standard techniques described by Tracey *et al.* (1975). The most common allele at the PGM locus (which migrated 26 mm from the origin) was arbitrarily designated as 100. Other alleles were defined by adding or subtracting their absolute millimeter difference in migration from the modal allele (100). Thus, allele 104 migrated 4 mm further, and allele 95, 5 mm less than the modal allele (100).

Allele frequencies and the effective number of alleles were calculated as described by Ferguson (1980). A log likelihood ratio (G) test (Zar, 1974) was carried out on observed phenotype combinations and those expected in a random mating system. Heterozygote deviations from random expectations were monitored using the 'D' statistic (Selander, 1970). Negative values of D indicate a deficiency, and positive values an excess of heterozygotes.

RESULTS

Seven different PGM electromorphs were detected, three of these occurring in <1% of the animals sampled. Each *Mysella tumida* produced either one or two bands (Fig. 1), indicating that this enzyme has a monomer subunit structure. The electromorph combinations observed are consistent with the hypothesis that each electromorph represents one allele of a shared locus. Therefore, single-banded individuals were assumed to be homozygous and two-banded animals heterozygous at the PGM locus. Allele frequencies and genotype distributions are presented in Tables 1, 2 respectively.

The calculated D value of 0.022 is greater than the expected value of 0. This indicates that there is a slight excess of heterozygotes. However, the observed frequency of allele combinations did not differ significantly from random mating expectations ($0.25 < P < 0.50$).

Table 1. Allelic frequencies at the PGM locus of Patricia Bay *Mysella tumida*.

Allele	Frequency ± 1 St. Error
95	0.1528 ± 0.0245
100	0.6296 ± 0.0329
104	0.1898 ± 0.0267
108	0.0278 ± 0.0112

Rare alleles (and frequencies): 92(0.0045); 110(0.0045); 112(0.0045).
Ne (effective number of alleles) = 2.21
Number of alleles screened = 222.

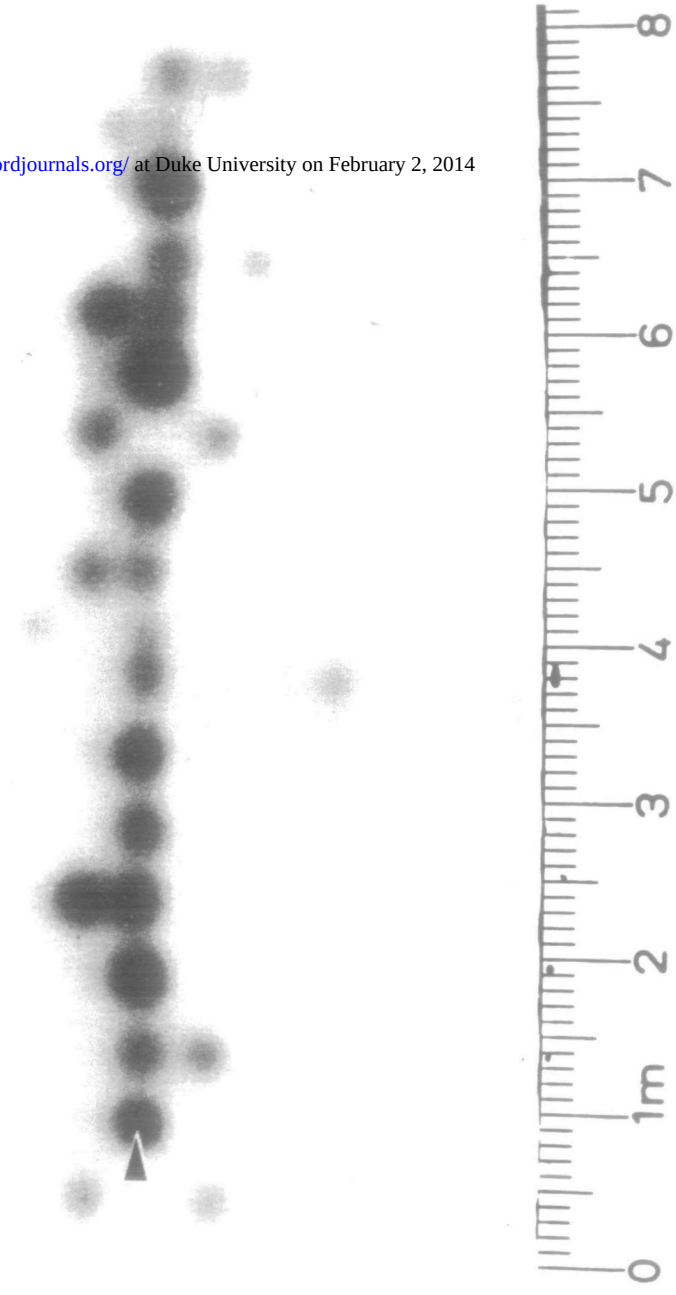

Fig. 1. PGM electromorphs for 18 Patricia Bay *Mysella tumida* individuals showing 6 of the 7 alleles detected in this study. Arrow points to the modal allele (100). Scale is in cm and is placed at the origin.

Table 2. Genotype distributions at the PGM locus for Patrica Bay *Mysella tumida*. Alleles with frequencies <0.01 excluded.

Genotype	Observed Frequency N	H-W-C Expected N Frequency
95/95	0	2.5209
95/100	24	20.7781
95/104	8	6.2638
95/108	1	0.9168
100/100	43	42.8149
100/104	22	25.8142
100/108	4	3.7781
104/104	5	3.8910
104/108	1	1.1389
108/108	0	0.0834

Observed proportion of heterozygotes (Ho) = 0.555.

Expected proportion of heterozygotes (He) = 0.543.

D = 0.022 when D = (Ho-He)/He (Selander, 1970).

G = 7.0481, df = 6, $0.25 < P < 0.50$ when G is the Log-Likelihood Ratio.

DISCUSSION

Although data are available for only one locus, the observed allelic frequencies do not differ significantly from random mating expectations ($0.25 < P < 0.50$). This indicates that the Patricia Bay population of *Mysella tumida* consists of randomly outcrossing individuals and does not experience a detectable Wahlund effect. The observed heterozygosity level of 0.556 for *Mysella tumida* is similar to that found at the PGM loci of outcrossing oysters [0.462–0.670 (Buroker, 1982; 1983)] and scallops [0.265–0.684 (Beaumont & Beveridge, 1984)]. Selfing in the population would lead to a rapid drop in heterozygosity at all loci in the genome (Kallman & Harrington, 1964; Selander & Kaufman, 1973; Bell, 1982; Bucklin, Hedgecock & Hand, 1984; Eernisse, 1984). The high degree of heterozygosity at the *M. tumida* PGM locus, therefore, indicates that if selfing occurs in the Patrica Bay population, it is a rare event. In addition, the positive D value of 0.022 is slightly higher than expected, a result incongruous with the occurence of significant selfing rates in the population. Wilkins (1978) reported similar high heterozygosity levels at the glucose phosphose isomerase locus of the hermaphroditic scallop *Pecten maximus*, indicating that this species also rarely self-fertilizes.

Hermaphroditic marine invertebrates that normally outcross experience severe inbreeding depression when selfed, resulting in reduced offspring viability (Barnes & Crisp, 1956; Gee & Williams, 1965; Sastry, 1965; Sabbadin, 1971; Beckwitt, 1982; Beaumont & Budd, 1983). Inbreeding depression can result from the expression of deleterious recessive homozygous alleles and reduced offspring viability may also be due to lowered heterozygosity in the offspring, a trait that has been associated in bivalves with lowered growth rates (Zouros, Singh & Miles, 1980; Koehn & Gaffney, 1984), lowered juvenile survival (Zouros, Singh, Foltz & Mallet, 1983), higher metabolic costs (Koehn & Shumway, 1982; Garton, Koehn & Scott, 1984), less ability to withstand stress (Rodhouse & Gaffney, 1984; Diehl, Gaffney & Koehn, 1986) and lowered fecundity (Rodhouse, McDonald, Newell & Koehn, 1986). Selfing individuals will be at a reproductive advantage only in populations that are already highly inbred (Charlesworth & Charlesworth, 1981; Strathmann, Strathmann & Empson, 1984). Data presented here showing a high degree of polymorphism and heterozygosity at the PGM locus suggest that this is not the case for *Mysella tumida*. The 3–4 week planktotrophic larval development found in this species (Ó Foighil, 1986) may play a major role in maintaining this level of genetic diversity in natural populations. It is probable that any *M. tumida* offspring which might be produced by occasional selfing would experience a marked inbreeding depression and lowered survival rates. This factor may, however, conceal the presence of a small proportion of selfed offspring in predominantly outcrossed broods [as Beckwitt (1982) observed in spirorbid pair matings], because only adult animals were analysed in this study.

Definitive isolation experiments have not been performed with *Mysella tumida* to determine if this species is capable of selfing. To establish unambiguously whether or not *M. tumida* can viably self-fertilize would require isolating newly-metamorphosed juveniles and their resulting progeny, if any, for at least 2 generations. A positive result in these isolation tests would not establish the degree to which *M. tumida* self-fertilizes in natural populations. Indirect evidence from the PGM locus suggests that if self-fertilization occurs in the Patrica Bay population, it is a rare event. To test this directly, however, would require sampling brooding individuals from the population and raising their progeny until they were large enough to be analysed electrophoretically.

Analysis of the progeny, parent and population genotypes at a number of polymorphic loci should in principal yield a quantitative estimate of the frequency of self- and cross-fertilization in the population. However, in practice, these estimates are often highly variable (Bell, 1982).

Although it can be extremely difficult to accurately estimate the reproductive mode of a potentially facultative self-fertilizer, evidence presented here on the large number of alleles, high heterozygosity and the maintenance of random mating expectations by *Mysella tumida* at the PGM locus, strongly support the hypothesis that natural populations reproduce predominantly by outcrossing.

ACKNOWLEDGEMENTS

This work was supported by a University of Victoria graduate student fellowship to D.Ó. Foighil and N.S.F. grant OCE-8415258 to R.R. Strathmann and D.J. Eernisse. We thank the Director of Friday Harbor Laboratories, A.O.D. Willows for the use of facilities and an anonymous reviewer for suggesting improvements to this paper.

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