J. Exp. Mar. Biol. Ecol., 1988, Vol. 116, pp. 79–86
Elsevier

JEM 01028

Enzyme heterozygosity and growth rate in nursery populations of *Mercenaria mercenaria* (L.)

Robert T. Dillon, Jr.[1] and John J. Manzi[2]

[1]*Department of Biology, College of Charleston, Charleston, South Carolina, U.S.A.*; [2]*Marine Resources Research Institute, Charleston, South Carolina, U.S.A.*

(Received 8 September 1987; revision received 12 November 1987; accepted 19 November 1987)

Abstract: Crosses between and within two lines of the hard clam *Mercenaria mercenaria* (L.) were reared to age 1 yr under typical commercial upflow nursery conditions. Within these crosses, little relationship was detected between shell length and heterozygosity averaged over seven enzyme loci. However, significant differences between the largest and smallest clams were detected at individual loci in 10 of 42 tests. Results were consistent neither with the hypothesis that the alleles themselves were affecting growth, nor with the hypothesis that these enzyme loci are tightly linked to other loci affecting growth. Rather, it appears that alleles are marking the entire genomes of their parents, and that variation in the growth rates of the offspring from individual clams may be obscuring any relationship with overall heterozygosity.

Key words: Growth; Heterozygosity; Linkage disequilibrium; *Mercenaria mercenaria*; Protein electrophoresis

Introduction

Much attention has been focused on the relationship between multiple-locus enzyme heterozygosity and growth rate in marine bivalves (reviews by Koehn & Gaffney, 1984; Mitton & Grant, 1984). Although a positive correlation has been convincingly demonstrated in wild populations of a number of species (Zouros *et al.*, 1980; Garton *et al.*, 1984; Koehn & Gaffney, 1984), the phenomenon has not been demonstrated in hatchery mass spawnings (Adamkewicz *et al.*, 1984b; Gaffney & Scott, 1984; Foltz & Chatry, 1986) or in pair matings (Beaumont *et al.*, 1983; Mallet *et al.*, 1986). The purpose of this investigation is to examine the reasons for the absence of a relationship between heterozygosity and growth in populations of the hard clam *Mercenaria mercenaria* (L.) spawned and reared under typical hatchery and nursery conditions.

Linkage disequilibrium has been cited as a factor both in the origin of a heterozygosity/growth correlation in wild populations of bivalves and in the dissolution of this relationship in the nursery. The hypothesis of "associative overdominance" suggests that transitory linkage disequilibria exist in natural populations between electrophoreti-

Contribution 221 from South Carolina Marine Resources Center.

Correspondence address: R.T. Dillon, Department of Biology, College of Charleston, Charleston, SC 29424, U.S.A.

cally detectable loci and potentially deleterious genes (Ohta, 1971; Mallet *et al.*, 1986). Thus in the wild, bivalves homozygous for enzyme loci might also tend to be homozygous for recessive deleterious alleles. But in the offspring of more limited hatchery spawns, extensive linkage disequilibrium between marker loci and other loci affecting growth obscures any effect of overall heterozygosity (Gaffney & Scott, 1984). Evidence for this second artificially generated sort of linkage disequilibrium is presented in this investigation.

In artificially spawned populations of bivalves, linkage disequilibrium can occur between alleles at independently assorting loci as well as between loci that are actually linked. ("Gametic phase disequilibrium" has been proposed as a less misleading term to describe all nonrandom associations of alleles within gametes.) The actual nature of the disequilibrium can be important, as in a directed breeding program, for example. An allele at an enzyme locus closely linked to a second locus affecting growth would be of considerable utility, while an allele simply marking the entire genome of a parent would not. In this study, we will present evidence suggesting that linkage disequilibrium can in fact occur between enzyme loci and loci affecting growth in hatchery populations of *M. mercenaria*, but we find no case where this linkage seems to be particularly tight.

Materials and Methods

Lines of *M. mercenaria* selected for fast growth over three or four generations have been developed by the Aquaculture Research Corporation (ARC), Dennis, Massachusetts, and by the Virginia Institute of Marine Science, Virginia (VIMS). A good deal of information regarding the genetics of these two stocks is available in Dillon & Manzi (1987). The wild New England and Virginia populations of *M. mercenaria* from which they were founded are almost indistinguishable at seven polymorphic enzyme loci. But both hatchery lines have diverged from wild populations, probably due to genetic drift, such that they now have different allele frequencies at five of these seven loci. Both the ARC and VIMS stocks also seem to have lost some rare alleles, possibly due to bottlenecking. Thus, some degree of linkage disequilibrium seems possible.

In October 1984, 16 individuals from the ARC line (seven males, nine females) and 10 from VIMS (five males, five females) were spawned. The eggs and sperm were then combined within each line and reciprocally between the two lines. This process was repeated in March 1985 at which time 18 male ARC, eight female ARC, eight male VIMS, and seven female VIMS were spawned. Larvae were reared to metamorphosis using standard larval culture techniques (Loosanoff & Davis, 1963). Recently metamorphosed clams were cultured in recirculating downflow culture units (Manzi & Castagna, in press). After attaining a minimum size of 1 mm, the juvenile clams were transferred to a commercial scale upflow nursery system (Manzi, 1985). Data on growth in these lines collected over the first 2 yr of culture will be reported elsewhere.

In October 1985, large random samples were taken from all four of the first crosses,

here designated ARC1, AXV1, VXA1, and VIMS1. (Female parents are listed first in the outcrosses.) The purebred offspring from the second cross, ARC2 and VIMS2, were sampled in March 1986. Hard clams of the sizes sampled here are rarely reproductively mature (Eversole *et al.*, 1980), and thus the complication of energy allocated to gamete production was avoided (Rodhouse *et al.*, 1986). A great deal of size variation, measured as maximum shell length, was apparent in these six samples of clams (Fig. 1). The 62 largest and 62 smallest clams from each sample were selected for electrophoretic examination.

The entire individual was extracted from the shell and at least one-half the tissue ground in a buffer and sucrose solution. Procedures for the electrophoresis of the

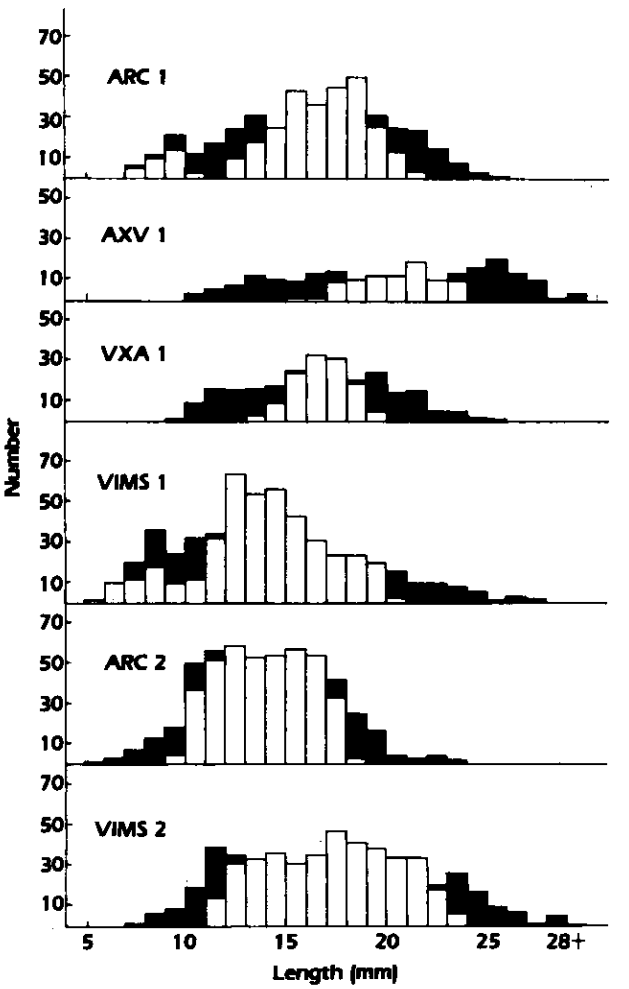

Fig. 1. Maximum shell lengths of *M. mercenaria* sampled from six crosses at age 1 yr. Darkened portion of each sample was examined electrophoretically.

supernatant and the demonstration of isozymes are described elsewhere (Dillon, 1982; 1985; Dillon & Manzi, 1987). Variation was scored at seven loci. Glucose phosphate isomerase (*Gpi* – seven alleles), leucine aminopeptidase (*Lap* – four alleles), 6-phosphogluconate dehydrogenase (*6pgd* – three alleles), and two phosphoglucomutase loci (*PgmS* – five alleles and *PgmF* – three alleles) have all been found to assort independently in *M. mercenaria* (Adamkewicz *et al.*, 1984a). Linkage relationships between these five loci and two others, superoxide dismutase (*Sod* – two alleles) and mannose phosphate isomerase (*Mpi* – four alleles), are unknown.

χ^2 two-sample tests, corrected for continuity in 2 × 2 cases (Siegel, 1956, p. 107), were used to determine whether the 62 largest and 62 smallest clams differed significantly in gene frequencies at each of the seven loci. Rare allelic classes were combined if possible or deleted. Overall heterozygosities of largest and smallest clams were compared by totalling the number of the $\approx 62 \times 7 = 434$ loci heterozygous in each group. Since similar numbers of individuals were examined at all loci, the correction factor of Dillon & Manzi (1987) was unnecessary. χ^2 tests, corrected for continuity, were then used to determine whether the proportions of heterozygous and homozygous loci differed significantly in large and small clams. Thus a total of eight χ^2 values were calculated for each of the six crosses.

RESULTS

Much of the allozyme variability available in the ARC and VIMS parental stocks was transmitted to the lines spawned for this experiment. Of 28 alleles at seven loci, the lowest number appearing in the experimental generation was 19, in ARC2. No monomorphic loci resulted from any cross. The lowest heterozygosity observed in any of the six crosses was 0.38 in ARC1, to be compared with 0.46 for the VIMS and 0.48 for the ARC parental stocks (Dillon & Manzi, 1987). Table I shows χ^2 values from comparisons of overall heterozygosity in the largest and smallest offspring of the six crosses. In only one of the six tests did the heterozygosity of the larger clams differ significantly from the heterozygosity of the smaller (Table I), with the larger clams being more heterozygous. This χ^2 value is barely significant at the 0.05 level, and rejection of the null hypothesis in this single case may be a Type I statistical error. The chance of one or more Type I errors at this level in six tests is 0.26.

On the other hand, the 42 values of χ^2 testing for allele frequency differences included 10 significant values, seven at the 0.01 level or better. Allele frequencies at these seven loci are given in Table II, using the nomenclature of Dillon & Manzi (1987). Interestingly, no significant differences were found between the largest and smallest offspring in ARC2, with 26 parents spawning. The significant χ^2 values were all obtained from crosses of 10–16 parents.

Some disequilibrium between isozyme alleles was in fact detected in these data. Although a complete analysis of the ≈ 20 alleles in all six crosses is beyond the scope

of this investigation, the data of Table II suggested that some disequilibrium might exist between the alleles of *Lap* and *PgmF* in the AXV1 cross, and between *PgmF* and *6pgd* alleles in the ARC1 cross. These suspicions were confirmed with χ^2 tests. Since Adamkewicz *et al.* (1984a) reported that these loci assort independently in Mendelian crosses, one would assume that the linkage disequilibrium observed here is loose.

TABLE I

χ^2 values and df comparing gene frequencies and overall heterozygosities in largest and smallest offspring from six crosses. One, two, and three asterisks designate values significant at 0.05, 0.01, and 0.001 levels, respectively.

	ARC1		AXV1		VXA1		VIMS1		ARC2		VIMS2	
$\bar{H}$	1.68	1	0.17	1	1.96	1	0.17	1	0.07	1	3.90*	1
Gpi	2.33	2	1.08	2	11.8**	3	9.27**	2	2.04	1	2.83	2
6Pgd	8.66**	2	0.02	2	4.73	2	1.85	2	1.67	1	1.11	2
Lap	0.46	1	10.7**	2	0.51	2	6.00*	2	2.02	2	6.10	3
Sod	0.53	1	2.47	1	1.91	1	2.08	1	0.92	1	0.01	1
Mpi	0.93	2	41.4***	2	3.47	2	4.99	2	1.23	2	6.63*	2
PgmS	0.74	1	8.51*	2	3.50	1	5.88	2	0.21	1	5.94	2
PgmF	26.5***	1	17.8***	2	0.64	2	0.55	2	2.10	1	2.39	1

TABLE II

Allele frequencies differing between largest and smallest clams at 0.01 level or better.

Cross	Locus	Allele	Large	Small	Cross	Large	Small
VXA1	*Gpi*	110	0.008	0.000	VIMS1	0.000	0.000
		100	0.712	0.590		0.885	0.746
		90	0.127	0.139		0.090	0.186
		70	0.110	0.090		0.016	0.068
		60	0.042	0.180		0.008	0.000
ARC1	*PgmF*	105	0.000	0.000	AXV1	0.145	0.158
		103	0.107	0.402		0.153	0.375
		100	0.893	0.598		0.702	0.467
ARC1	*6Pgd*	110	0.000	0.016			
		100	0.798	0.919			
		90	0.202	0.065			
AXV1	*Lap*	104	0.026	0.000			
		100	0.603	0.411			
		96	0.121	0.145			
		94	0.250	0.444			
AXV1	*Mpi*	105	0.380	0.041			
		100	0.398	0.508			
		95	0.222	0.451			

Discussion

It seems quite likely that a substantial portion of the size variance seen in Fig. 1 is genetic, rather than environmental, in origin. The individuals in each experiment were all spawned on a single date and maintained in upwelling units under uniform environmental conditions. But, as reported from previous nursery experiments, the relationship between size variation and heterozygosity is not striking. The significant differences in allele frequencies between largest and smallest clams apparent at individual loci in most of these experiments suggest a likely explanation for this absence of a growth rate/heterozygosity correlation.

There are many reasons why allele frequencies may vary with size within cohorts of clams as we have spawned them. One possibility is that some particular allozyme variant at one of the loci sampled affects growth under nursery conditions. But if this were true, the allele coding for this variant should appear unusually common in the largest or smallest clams from all six experiments. Table I shows that this is not the case for any allele at any locus. Nor has this phenomenon been reported in any other hatchery experiment to our knowledge (e.g., Gaffney & Scott, 1984).

A second possibility is that a particular enzyme locus may be tightly linked to another locus affecting growth, and that an allozyme allele is in linkage disequilibrium with a beneficial or deleterious allele at this second locus. For example, suppose that through population bottlenecking, the uncommon *Gpi* allele *60* is in linkage disequilibrium with a beneficial allele at a second locus in the ARC population. Then *Gpi 60* would be expected to appear unusually common in the largest clams sampled from both the ARC1 and ARC2 experiments. But because disequilibrium between two particular alleles is a chance event, it seems unlikely that the beneficial allele would also become linked to *Gpi 60* in the VIMS population. Thus, the largest clams in the VIMS1 and VIMS2 experiments would not necessarily be expected to show high frequencies of *Gpi 60*. They might show increased frequencies of some other *Gpi* allele, or they might show no relationships at all. To the extent that the beneficial allele is dominant, the larger AXV1 and/or VXA1 clams might also show high frequencies of *Gpi 60*. But Tables I and II show no evidence that any allele we have sampled is tightly linked to a beneficial or harmful allele. None of the relationships seen in ARC1 were repeated in ARC2, nor do VIMS1 and VIMS2 show any matches.

Loose linkage disequilibrium is the most likely explanation for significant differences in gene frequencies between large and small clams. Suppose, for example, that one particular ARC parent is endowed with a genome conferring increased growth in the nursery. Any number of loci could be involved, on any number of chromosomes. Suppose further that this individual is heterozygous for the unusual allele *Gpi 60*. Then half of the offspring produced by this individual will grow quickly and carry *Gpi 60*. Glucose phosphate isomerase *60* might appear unusually common in the largest clams simply because it marks an advantageous genome, not because it is beneficial itself or even because it resides on the same chromosome as a beneficial allele. The same

phenomenon would not be expected to reoccur in VIMS spawnings or even in a respawning of ARC, because different parents were involved. But to the extent that the beneficial portions of that single ARC genome, be they one allele or many, are "dominant", *Gpi 60* might appear more common in the AXV1 cross (if the ARC clam with the good genome were female) or the VXA1 cross (if the clam were male).

This third explanation seems to fit the observations reported in Table I. The great majority of the significant results appear but a single time. The remainder occur in one purebred line and in either AXV1 or VXA1. For example, Table II shows that the *PgmF* allele *103* was strikingly common in the smaller clams of both ARC1 and AXV1. This suggests that a small number (perhaps just one) of the nine ARC females spawned in October 1984 produced slow-growing offspring and carried *PgmF 103*. The offspring of this parent may account for a large fraction of the size variance in the ARC1 and VXA1 experiments.

All six of the experiments reported here are 'mass spawnings" by the criterion of Gaffney & Scott (1984). However, it would appear that even in spawnings of 10–16 parents, the contributions of individual clams to the size variance seen in the offspring can be substantial. Thus it is not surprising that little relationship was detected in these experiments between multiple-locus enzyme heterozygosity and growth. The absence of the heterozygosity/growth correlation in nursery crosses does not seem to be attributable to tight linkage disequilibrium, but rather to very loose disequilibrium between individual isozyme alleles and the entire genomes of the parents.

ACKNOWLEDGEMENTS

We thank D. McLean, N. Hadley, and the staffs at the James M. Waddell, Jr. Mariculture Center and the Marine Resources Field Station for their technical assistance. We are grateful to V.G. Burrell, Jr. and E. Wenner, and to J. Hilbish, W. Anderson, and L. Greene. Samples of *M. mercenaria* spawned for this analysis were provided by M. Castagna and G. Petrovits. This work was sponsored by NOAA, National Sea Grant College Program Office, U.S. Department of Commerce Grant R/A-15, and the South Carolina Sea Grant Consortium.

REFERENCES

ADAMKEWICZ, L., S.R. TAUB & J.R. WALL, 1984a. Genetics of the clam *Mercenaria mercenaria*. I. Mendelian inheritance of allozyme variation. *Biochem. Genet.*, Vol. 22, pp. 215–219.

ADAMKEWICZ, L., S.R. TAUB & J.R. WALL, 1984b. Genetics of the clam *Mercenaria mercenaria*. II. Size and genotype. *Malacologia*, Vol. 25, pp. 525–533.

BEAUMONT, A.R., C.M. BEVERIDGE & M.D. BUDD, 1983. Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). *Mar. Biol. Lett.*, Vol. 4, pp. 151–161.

DILLON, JR., R.T., 1982. The correlates of divergence in isolated populations of the freshwater snail, *Goniobasis proxima*. Ph.D. thesis, University of Pennsylvania, Philadelphia, Pennsylvania, 183 pp.

DILLON, JR., R.T., 1985. Correspondence between the buffer systems suitable for electrophoretic resolution of bivalve and gastropod isozymes. *Comp. Biochem. Physiol.*, Vol. 82B, pp. 643–645.

DILLON, JR., R.T. & J.J. MANZI, 1987. Hard clam, *Mercenaria mercenaria*, broodstocks: genetic drift and loss of rare alleles without reduction in heterozygosity. *Aquaculture*, Vol. 60, pp. 99–105.

EVERSOLE, A.G., W.K. MICHENER & P.J. ELDRIDGE, 1980. Reproductive cycle of *Mercenaria mercenaria* in a South Carolina estuary. *Proc. Natl. Shellfish. Assoc.*, Vol. 70, pp. 22–30.

FOLTZ, D.W. & M. CHATRY, 1986. Genetic heterozygosity and growth rate in Louisiana oysters. *Aquaculture*, Vol. 57, pp. 261–269.

GAFFNEY, P.M. & T.M. SCOTT, 1984. Genetic heterozygosity and production traits in natural and hatchery populations of bivalves. *Aquaculture*, Vol. 42, pp. 289–302.

GARTON, D.W., R.K. KOEHN & T.M. SCOTT, 1984. Multiple locus heterozygosity and the physiological energetics of growth in the coot clam, *Mulinia lateralis*, from a natural population. *Genetics*, Vol. 108, pp. 445–455.

KOEHN, R.K. & P.M. GAFFNEY, 1984. Genetic heterozygosity and growth rate in *Mytilus edulis*. *Mar. Biol.*, Vol. 82, pp. 1–7.

LOOSANOFF, V.L. & H.C. DAVIS, 1963. Rearing of bivalve molluscs. *Adv. in Mar. Biol.*, Vol. 1, pp. 1–136.

MALLET, A.L., E. ZOUROS, K.E. GARTNER-KEPKAY & K.R. FREEMAN, 1986. Genetics of growth in blue mussels: family and enzyme-heterozygosity effects. *Mar. Biol.*, Vol. 92, pp. 475–482.

MANZI, J.J., 1985. Clam aquaculture. In, *Crustacean and mollusk aquaculture in the United States*, edited by J. Huner & E. Brown, AVI, Westport, Connecticut, pp. 275–310.

MANZI, J.J. & M. CASTAGNA, in press. Nurseries. In, *Clam mariculture in North America*, edited by J. Manzi & M. Castagna, Elsevier, Amsterdam, The Netherlands.

MITTON, J.B. & M.C. GRANT, 1984. Associations among protein heterozygosity, growth rate, and developmental homeostatis. *Annu. Rev. Ecol. Syst.*, Vol. 15, pp. 479–499.

OHTA, T., 1971. Associative overdominance caused by linked detrimental mutations. *Genet. Res.*, Vol. 18, pp. 277–286.

RODHOUSE, P.G., J.H. MCDONALD, R.I.E. NEWELL & R.K. KOEHN, 1986. Gamete production, somatic growth and multiple-locus enzyme heterozygosity in *Mytilus edulis* L. *Mar. Biol.*, Vol. 90, pp. 209–214.

SIEGEL, S., 1956. *Nonparametric statistics for the behavioral sciences*. McGraw-Hill, New York, New York, 312 pp.

ZOUROS, E., S.M. SINGH & H. MILES, 1980. Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution*, Vol. 34, pp. 856–867.