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Hard Clam, *Mercenaria mercenaria*, Broodstocks: Genetic Drift and Loss of Rare Alleles without Reduction in Heterozygosity*

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ABSTRACT

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Two nursery stocks of the hard clam, *Mercenaria mercenaria*, selected for fast growth were compared to corresponding wild populations in regard to allele frequencies at seven polymorphic enzyme loci. Although as few as 30-60 parents were spawned at each of four generations to produce these two broodstocks, neither line exhibited any reduction in heterozygosity. Both lines, however, showed evidence of genetic drift and loss of rare alleles, suggesting that a cross between them could result in a third genetically distinct line.

INTRODUCTION

Considerable interest has focused recently on the genetic improvement of commercially important bivalve broodstocks (Newkirk, 1980, 1983; Wilkins, 1981). Multiple-locus enzyme heterozygosity has emerged as one of the primary areas of research (reviews by Mitton and Grant, 1984; Singh and Green, 1984; Zouros and Foltz, 1984; Gaffney and Scott, 1985). Evidence of a positive correlation between growth and heterozygosity has come from studies of naturally produced juvenile cohorts of several marine bivalve species (Zouros et al., 1980; Garton et al., 1984; Koehn and Gaffney, 1984). This relationship may disappear over time, however, as the populations become reproductive (Diehl and Koehn, 1985; Rodhouse et al., 1986). The absence of a heterozygosity/growth correlation in nursery crosses (Beaumont et al., 1983; Adamkewicz et al., 1984b) has been attributed to "genetic background and environmental noise" by Gaffney and Scott (1985).

Hybridization is one of the techniques often found useful in the production

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of improved varieties of commercially important species, perhaps because of associated heterozygosity increases (Frankel, 1983; Mitton and Grant, 1984). Thus, as part of a program to develop faster growing stocks of the hard clam, *Mercenaria mercenaria,* we have crossed individuals from the stocks maintained by two nurseries [Aquaculture Research Corporation (*ARC*) in Dennis, MA and the Virginia Institute of Marine Science (*VIMS*) at Wachapreague, VA] and compared their growth to that of purebred controls (Manzi et al., in press). A genetic characterization of the two nursery stocks involved in these crosses therefore became necessary. Here we report the results of a comparison of *ARC* and *VIMS* stocks to the wild populations that served as sources for the founding parents, using gene frequencies at seven enzyme loci.

METHODS

The original parents of the *ARC* nursery stock examined in this study were a mixture of clams from Massachusetts, Rhode Island, and New Jersey (G. Petrovits, personal communication, 1984). The fastest-growing of the F_1 offspring from this cross were mass spawned to produce an F_2 generation, and this process has been repeated twice again to produce the F_4 clams analyzed here. A small number of clams from the wild were also included in some of the subsequent spawnings. No records are available regarding the number of clams actually spawning at each generation, but a fair estimate would be 30–60 individuals, probably including more males than females.

The *VIMS* clams were derived from native Virginia stock maintained at the Wachapreague hatchery. Parental lines were first spawned in 1973 and two separate lines were founded by spawning the largest of these clams in 1975 and 1977. The individuals we examined in this study were a mixture of these two lines, some the result of four generations of selection for fast growth, others the result of five generations (M. Castagna, personal communication, 1984). The number of individuals spawning each generation is again unknown, but the 30–60 estimate mentioned above seems reasonable.

Wild populations for comparison with the two nursery broodstocks were collected in the spring of 1985 from Martha's Vineyard, Massachusetts, (*Mass*) and Hog Island, near Wachapreague, Virginia (*Va*).

We have found seven polymorphic enzyme loci consistently scorable in *M. mercenaria* muscle tissue. Inheritance at five of these loci has been demonstrated to be Mendelian by Adamkewicz et al. (1984a): glucose phosphate isomerase (GPI, EC 5.3.1.9), leucine aminopeptidase (LAP, EC 3.4.1.1), 6-phosphogluconate dehydrogenase (6PGD, EC, 1.1.1.44), and two phosphoglucomutase loci (PGMS and PGMF, EC 2.7.5.1). Superoxide dismutase (SOD, EC 1.15.1.1) was not examined by Adamkewicz et al., but has been previously reported polymorphic in *M. mercenaria* by Pesch (1974) and Hum-

phrey (1981). We discovered a seventh polymorphic locus, mannose phosphate isomerase (MPI, EC 5.3.1.8), in a survey of 28 enzymes over five buffer systems (Dillon, 1985).

Cuttings of clam siphon were ground in 100 μl of 7% sucrose solution buffered at pH 7.4 with 0.05 Trizma and H_3PO_4. Samples were centrifuged and the supernatant separated electrophoretically in gels of 14% hydrolysed starch, a mixture of three parts Sigma starch and one part Electrostarch. An aminopropylmorpholine pH 6 buffer was used to resolve GPI, 6PGD, and PGMS, a Tris-Cit pH 6 buffer was used for MPI and LAP, and the Poulik discontinuous buffer system was used for SOD and PGMF (Dillon, 1985). Gels were sliced and stained using the recipes given in Dillon and Davis (1980) and Dillon (1982).

Chi-square goodness-of-fit tests were used to estimate the probability that observed genotype frequencies reflected Hardy–Weinberg equilibrium at all seven loci for each of the four populations. Pairs of populations were also compared using chi-square two-sample tests: *Mass* and *Va*, *Mass* and *ARC*, and *Va* and *VIMS*. Values of chi-square were corrected for continuity in 2×2 cases.

Two different methods were used to compare the heterozygosities observed in nursery stocks to those observed in the corresponding wild populations. In the first method, the number of heterozygous diploid genomes was totalled over all individuals and all loci for both nursery and wild populations. For example, the total number of diploid genomes sampled from the *ARC* nursery stock was 110+96+...+ =678. Of these genomes, 331 were heterozygous. This frequency of heterozygotes was compared to that observed in the *Mass* populations using a chi-square two-sample test, correlated for continuity.

However, heterozygosities are usually calculated by weighting all loci equally, and this first method weights loci by the number of individuals examined. Since the number of individuals sampled generally varied considerably from locus to locus in this data set, a second method of comparing overall heterozygosities was employed. The corrected number of heterozygous diploid genomes, weighting all loci equally, was calculated as

$$H_t = \sum_{i=1}^{7} \frac{N_t H_i}{7 N_i}$$

where N_i is the number of diploid genomes examined at the ith locus, H_i is the number of heterozygotes observed at the ith locus and N_t is the total number of diploid genomes examined from the population. Corrected values of H_t were computed for both nursery and wild populations, then compared using chi-square two-sample tests, corrected for continuity, as above.

RESULTS

Gene frequencies at seven loci for the four samples of *M. mercenaria* are given in Table 1. Genotype frequencies fit Hardy–Weinberg expectation very

TABLE 1

Allele frequencies and observed heterozygosities at seven enzyme loci in four samples of *M. mercenaria*; mean heterozygosities, both observed (H_0) and expected (H_E), are also given

Locus		*ARC*	*Mass*	*Va*	*VIMS*
GPI	*N*	110	79	93	89
	110	0.005	0.006	0.022	0.011
	105	0.017	0.019	-	-
	100	0.745	0.848	0.882	0.865
	90	0.009	0.019	0.027	0.028
	80	0.005	0.006	0.027	0.011
	70	0.195	0.063	0.031	0.084
	60	0.027	0.038	0.011	-
	H	0.509	0.278	0.226	0.258
MPI	*N*	96	65	58	57
	108	0.057	0.038	0.026	0.035
	105	0.307	0.485	0.457	0.395
	100	0.354	0.331	0.388	0.307
	95	0.281	0.146	0.129	0.263
	H	0.760	0.600	0.655	0.737
LAP	*N*	105	77	88	79
	104	0.081	0.084	0.131	0.089
	100	0.552	0.448	0.358	0.633
	96	0.362	0.448	0.500	0.228
	94	0.005	0.019	0.011	0.051
	H	0.648	0.623	0.603	0.582
SOD	*N*	89	78	88	42
	100	0.601	0.692	0.687	0.679
	90	0.399	0.308	0.313	0.321
	H	0.551	0.410	0.398	0.405
6PGD	*N*	105	78	85	80
	110	-	0.006	0.024	0.081
	100	0.714	0.647	0.653	0.525
	90	0.286	0.346	0.324	0.394
	H	0.438	0.602	0.518	0.712
PGMS	*N*	96	78	90	77
	103	-	0.019	0.006	0.019
	100	0.880	0.731	0.722	0.682
	97	0.052	0.027	0.056	0.032
	92	0.057	0.154	0.133	0.182
	87	0.010	0.071	0.083	0.084
	H	0.208	0.449	0.378	0.468
PGMF	*N*	77	77	89	44
	105	0.013	0.040	-	-
	103	0.162	0.104	0.174	0.057
	100	0.825	0.857	0.826	0.943
	H	0.247	0.260	0.303	0.068
	H_o	0.480	0.460	0.440	0.462
	H_E	0.432	0.436	0.437	0.438

well within samples. Only three of the 28 separate goodness-of-fit tests resulted in values of chi-square significant at the 95% level. Excess heterozygosity was detected at the 6PGD locus in *Mass* and *VIMS* and at the GPI locus in *ARC*. Because so many separate tests were performed, however, it is difficult to distinguish these results from type I statistical error.

As reported by Humphrey (1981), very little divergence seems to have occurred between wild populations of *M. mercenaria* from Massachusetts to Virginia. Gene frequencies are not significantly different at six of the seven loci, setting aside or combining rare alleles for the purpose of chi-square tests. The only significant difference between *Mass* and *Va* samples was the absence of the allele PGMF 105 from the latter. Thus the founding of the *ARC* stock using clams from Rhode Island and New Jersey as well as Massachusetts may have had little effect on gene frequencies at enzyme loci.

The two hatchery stocks have, however, diverged from the wild populations. Highly significant differences between *ARC* and *Mass* were detected at the

GPI, MPI, and PGMS loci. The *VIMS* and *Va* populations were significantly different at the 6PGD, LAP, and MPI loci.

In addition, some loss of rare alleles seems to have taken place in the nursery stocks. No individuals with the GPI 60 allele were sampled from the *VIMS* stock, although the allele has an estimated frequency of 0.011 in the *Va* population. There is a fair chance that this allele is present in *VIMS*, but was missed in the sample, however. Given a frequency of 0.011, the chance of drawing no individuals showing the GPI 60 allele in a sample of 89 clams can be calculated as $0.989^{178} = 0.140$. The *ARC* stock seems to be missing two alleles found rarely in *Mass*, 6PGD 110 and PGMS 103. As was the case with *Va* and *VIMS*, there is a fair chance that an allele as rare as 6PGD 110 seems to be may have been present in *ARC* but missed in the sample. However, the PGMS 103 allele has an estimated frequency of 0.019 in *Mass*. The chance of completely missing such an allele in the sample of 96 individuals taken from *ARC* is just 0.025. Thus it seems likely that the *ARC* stock has indeed lost this allele.

Table 1 shows that the mean heterozygosities observed in the two nursery stocks were actually greater than the mean heterozygosities observed in the two wild populations. There is no evidence that these differences are significant, however. Regardless of whether the test was based on the observed number of heterozygous individual loci or on the corrected number weighing all loci equally, values of chi-square comparing wild and nursery populations were not significant.

DISCUSSION

Judging from gene frequencies at enzyme loci, the two nursery stocks have diverged considerably from wild populations of *M. mercenaria*. Selection would seem an unlikely explanation, considering that different loci seem to be involved in the two cases. Further, the amount of environmental difference between the nursery and the wild would not seem to be substantially greater than the difference between the Massachusetts and Virginia environments, where populations of clams have remained remarkably uniform genetically. Genetic drift due to bottleneck effects would seem a much more likely explanation for the alteration of gene frequencies in the nurseries.

The genetic consequences of population bottlenecks have been the object of some research in recent years. Theoretical treatments (Nei et al., 1975; Watterson, 1984; Maruyama and Fuerst, 1985) have suggested that genetic drift and loss of rare alleles should be more sensitive to bottlenecking than average heterozygosity. These predictions have been tested in natural populations only rarely, however, and with mixed results (Ayala et al., 1971; Taylor and Gorman, 1975; Bryant et al., 1981). A good deal more information is available regarding the genetics of hatchery populations of fish. Reduced heterozygosity, genetic drift, and reduction in number of alleles are all often apparent in fish

hatcheries (Allendorf and Phelps, 1980; Ryman and Stahl, 1980; Cross and King, 1983; Stahl, 1983; Taniguchi et al., 1983); although there have been some exceptions (Allendorf and Utter, 1979; Busack et al., 1979). Thus our finding that heterozygosity has remained unchanged in broodstocks of clams is somewhat unusual.

Since coefficients of inbreeding are based on heterozygosity, neither of the two nursery stocks examined here is demonstrably "inbred". Genetic divergence has occurred, however, so that the hybrid between these two stocks may indeed be expected to show some unique and possibly desirable properties.

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