RICHARD J. NEVES

Newkirk 1980

Aquaculture, 19 (1980) 209—228

REVIEW OF THE GENETICS AND THE POTENTIAL FOR SELECTIVE BREEDING OF COMMERCIALLY IMPORTANT BIVALVES

GARY F. NEWKIRK

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1 (Canada)

(Accepted 29 July 1979)

ABSTRACT

Newkirk, G.F., 1980. Review of the genetics and the potential for selective breeding of commercially important bivalves. *Aquaculture*, 19: 209—228.

The domestication of species used in aquaculture will not be complete until we have control over all aspects of their biology including their genetics. For virtually all shellfish, we are at present far from having that control but significant advances have been made in the past few years towards understanding many aspects of their genetics. In this paper will be presented a review of recent advances in, and an assessment of the state-of-the-art of shellfish breeding. Breeding here is meant to imply that we are concerned with the genetic control, manipulation and improvement of traits of commercial interest.

The ultimate objective of the breeder is to produce strains which are genetically improved. There are several studies which give us an indication of the potential of such selective breeding both through heritability studies and experimental selections. These studies indicate that there is the expected potential and that what is needed next is a major commitment to develop selected lines. The various aspects of bivalve genetics such as Mendelian genetics, population genetics and quantitative genetics will be reviewed in the light of their roles in breeding for the improvement of the economic value of shellfish.

INTRODUCTION

The potential contribution of selective breeding to the development of aquaculture has been discussed in a number of places recently (e.g., Longwell, 1976; Glude, 1977; National Research Council, 1978). Very often parallels are made between the development of agriculture and that of aquaculture and examples are given of the genetic gains made in various agricultural species. As a general statement there is no arguing with the assertion that there is considerable potential for genetic improvement of aquaculture stocks. However, we do not seem to be making a concerted effort to realize this potential in molluscan shellfish even though we have had control of the life-cycle of all the commercially important species for a number of years.

A variety of approaches have been taken in the study of 'the genetics' of molluscs including Mendelian genetics, cytogenetics, quantitative genetics and hybridization studies. Not all of these approaches will contribute equally to

the immediate development of genetically improved strains. In this paper these various genetic studies will be discussed as they relate to selective breeding for commercial purposes. Several areas requiring immediate attention for the development of commercial shellfish breeding will also be discussed.

The first point to be emphasized is the distinction between selective breeding and genetics. Selective breeding is the genetic manipulation of a cultured species for the purpose of improving certain traits of interest to man. Genetics is the broad discipline covering any field which deals with inheritance. The various fields of genetics such as cytogenetics, population genetics, and Mendelian genetics, are often used as tools in selective breeding but by themselves do not produce the genetic gains needed. There is no substitute for the use of conventional selective breeding as practised in agriculture for the production of genetically improved stocks for culture.

Some of the approaches to molluscan genetics have limited potential for making immediate practical contributions but may be useful in the future when aquaculture breeding becomes more sophisticated. This is not to suggest that these studies should not be encouraged. I only want to emphasize that we must not be too optimistic about practical contributions from the more basic genetic studies in the near future. The studies which will have more immediate impact on the development of improved stocks are those which examine the quantitative genetics and selection response of the species used in aquaculture. Traits such as growth rate, mortality rates, and disease resistance are among the most important traits to the aquafarmer. The experience of agricultural breeders suggests that these kinds of traits are determined by a relatively large number of genes with considerable environmental influence. At the same time, improvement of these traits has been made in agriculture through selective breeding. The various kinds of genetic studies that have been done on marine molluscs will be discussed as they relate to selective breeding.

ELECTROPHORESIS GENETICS

Most of our knowledge of single locus genetics comes through the use of electrophoresis. We now have information about the frequencies and the distribution of alleles in the wild populations of some marine molluscs. These genes are necessarily a very small sample of the total genome and are presumably chosen at random. Nothing is known about the location of these loci on the chromosomes and in most cases the physiological effect of the different alleles is completely unknown. In other words, these are simply a random sample of genes of unknown relation to the fitness of the individual which provide some idea of the genetic variation within and between wild populations of the species.

The bivalve most extensively studies by these methods is the blue mussel, *Mytilus edulis*. Most of this work has been done on the northeast coast of the U.S.A. and has been reviewed recently by Koehn et al. (1976). There are

genetic differences between populations for certain loci of which the most extensively studied is leucine aminopeptidase (LAP). The frequency of certain LAP alleles is correlated with the salinity at which the population is found. (The details of this work will not be discussed here. See Koehn et al. (1976) for discussion and further references.) The hypothesis currently being tested is that this gene is involved in adaptation to salinity changes and the different alleles have different physiological optima under different conditions, presumably salinities. Ahmed et al. (1977) have shown that mussels in the U.K. have considerable electrophoretic variation, about the same as has been observed in other marine invertebrate species.

To date there has not been demonstrated any direct connection between an electrophoretic locus and any trait which would be of interest to the mussel farmer. Nevertheless, the genetic differences between populations as shown with enzyme loci *may* be correlated with genetic differences for other loci. If this is so, then in choosing seed sources for culture it may be necessary to consider the possible genetic differences between populations. This will be discussed further below.

Using serological techniques, Numachi (1962) showed that there were some antigenic differences between four races of *C. gigas* in Japan. The differences observed were in accordance with the degree of geographic isolation. Such antigenic differences are probably caused by a small number of genes.

The electrophoresis genetics of *Crassostrea gigas* is being studied at the University of Washington. Buroker et al. (1975) reported that there is considerable genetic variation in a population of *C. gigas* from Mud Bay in Puget Sound. Fifteen loci were examined of which eight were polymorphic and on average an individual was heterozygous at 21% of the loci. This is higher than the average for marine invertebrate species which is 10%.

In a recent report on the breeding for disease resistance in *C. gigas*, Beattie et al. (1978) have examined several electrophoretic loci in full-sib families which were selected for resistance to summertime mortality. Unfortunately, the number of families was very small and they were not all assayed for all the loci reported. The authors suggest that, of the families that showed better survival than the controls, those that were monomorphic for LAP were more susceptible. However, there were only three families assayed for LAP (their table 1) and the controls which were most susceptible were in fact heteromorphic for all loci. Genotype frequencies were not presented for the families nor their parents. More extensive sampling is clearly necessary before such associations can be made.

In an earlier paper, Hillman (1964) presents some evidence based on free amino acids and/or small peptides that there are genetic differences between *C. virginica* from Long Island Sound and the James River, Virginia. But surprisingly little has been published on electrophoresic studies of the American oyster. There has been some work done on a population of this oyster in Prince Edward Island by Singh and Zouros (1978). They have observed a positive relation between the degree of heterozygosity at four loci and the

size at 1 year. More recent, as yet unpublished work has extended this to a more complete sample of the population and seven loci (E. Zouros, unpublished data, 1978). The regression of weight on heterozygosity and weight remains significant. At first it may appear that breeding for increased heterozygosity at these enzyme loci would be a quick method of increasing the mean size of the population, but two factors must be considered. First, there is, overall, a deficiency of heterozygotes in the population. This makes it difficult to explain how the polymorphisms are maintained in the population when there is apparently overdominance at these loci for size. Natural selection probably has produced the observed genotypic frequencies and may counteract attempts to increase the heterozygosity. Second, and most importantly, there remains the question of whether the overdominance observed at 1 year of age (mean size about 2.3 g) will disappear with age. We have data (Haley and Newkirk, 1977 and unpublished) which indicate that weight at 1 year is not very strongly correlated with weight at market size. The correlation between an individual's weight at 1 year (2—3 g) and that same individual's weight at 3 years (mean size 27 g) is 0.53. This means that of the variation observable at 3 years of age (which is considerable) only 28% is explainable by the variation in size at the end of the first year. Since these oysters are only about one-third market size, the correlation with market size will be even lower. These data were obtained from the same 'population', i.e. collecting site, as the oysters used by Singh and Zouros (1978). It may be that the correlation between heterozygosity and weight will increase with age as the genetic potential is expressed, but this remains to be shown. Until this kind of data are available we can take the observations of Singh and Zouros (1978) to indicate that it is possible that crossbreeding will be an effective means of increasing production, but the enzyme loci cannot be used as the sole criterion for establishing pure lines. It is most likely that overall heterozygosity was important in producing the overdominance and that the few enzyme loci were unimportant. In establishing pure lines it would be much better to develop selection criteria based on production traits.

The role of electrophoresis in shellfish breeding

Genetic variation within and between populations has been demonstrated by the use of electrophoresis. We can take these observations to indicate that there may also be genetic variation in the traits of interest to the shellfish breeder. But in the final analysis these traits will have to be studied themselves. Even apparently inbred strains of animals maintain genetic variation which can give rise to genetic gains through selection. Thus, the information from electrophoresis is not really surprising or enlightening.

The differences between populations will similarly have to be studied for the traits of interest to the breeder. Even where a correlation has been established between, say, heterozygosity and weight, this does not necessarily mean that hybridization of geographically separated strains will produce the

same overdominance. The observations of Singh and Zouros (1978), for example, could have been produced by the particular population structure and genetic structure of the oysters sampled. I will return to this point below.

The expense of using electrophoretic markers to keep track of individuals hardly seems justified at least for molluscs. It does not require much effort to glue a label on, or scratch a code in a shell. This gives an identifier which is easily observable and does not require sacrificing the individual which is most important if you intend to spawn it. In some cases the use of electrophoretic markers may prove useful, but if a laboratory has to be set up for the purpose of labelling individuals then perhaps more thought should go into developing an alternate method of labelling!

QUANTITATIVE GENETICS AND BREEDING

The traits of most interest from a production viewpoint are most likely controlled by a large number of genes. They are referred to as polygenic, or quantitative traits and include such things as growth rate, survival, meat yield, shape etc. The hope of finding a small number of genes which account for a major proportion of the variation in such traits is illusive. The years of experience in agricultural breeding would indicate that in most cases we are dealing with what appears to be a large number of genes, each having a small contribution. To deal with the genetics of such traits one works with means and variances, trying to partition the variance of the trait into genetic and environmental components and their interaction. The relative importance of the genetic variance is often expressed as a ratio of the genetic variance to the total phenotypic variance (which is composed of the genetic and environmental components). This ratio is called the heritability (h^2). The genetic component can be partitioned further into additive and nonadditive components. The amount of additive genetic variance can be estimated from an analysis of half-sib families yielding a half-sib h^2. The presence of additive genetic variance will allow continued progress in a mass selection program. The nonadditive genetic variance is included in h^2 estimates from the analysis of full-sib families along with the additive genetic variance. Thus, the difference between estimates from half- and full-sib families gives an indication of the amount of nonadditive variance. Nonadditive genetic variance can be exploited in a crossbreeding program, but will not produce a continued response to mass selection.

Innes and Haley (1977a) have published an analysis of the variation of larval growth rate among families of the blue mussel, *Mytilus edulis*. The experimental design was a factorial mating of six males and six females, replicates being raised at different salinities. Such a mating is accomplished by spawning individuals, then crossing every male with every female and vice versa. Thus with six males and six females, 36 families are produced. From their analysis can be obtained a half-sib h^2 of about 0.16 and a full-sib h^2 of 0.29. There was some interaction of the family means with salinity. G.F. Newkirk

et al. (unpublished data, 1978) have combined estimates from several similar experiments to get h^2 estimates of larval growth rate in *M. edulis*. The h^2 from half-sibs was 0.12 and from full-sibs was 0.62. The mating design used in these crosses gives particularly good estimates of the full-sib h^2 which are free of environmental and nongenetic maternal influences present in estimates from certain other designs. The results from these experiments indicate that there is some additive genetic variance for larval growth rate and considerable nonadditive genetic variance in this population.

These results are not of much practical value at present since mussels are not produced in a hatchery, and, as a result, there is not likely to be a breeding program for them. However, they serve as a model for other species and suggest that it will be worthwhile to look at the genetics of similar traits in other species with long planktonic larval stages.

Lannan (1972) has given an estimate of the h^2 of larval survival of 0.31 in *C. gigas* and h^2 of setting success of 0.09. Both these estimates are based on one set of matings of 11—15 full-sib families. (It is not stated in the paper how many families were used in the estimates of the larval traits.) These might indicate the presence of genetic variation but many more families are needed to give reliable estimates.

In *C. virginica*, there are two recent reports of heritability estimates of larval growth rate. In both cases the size of the larvae was measured on a particular day in crosses from a factorial mating. Losee (1978) reported the h^2 based on half-sib families on days 7, 14 and 21 as being 0.44, 0.40 and 0.55, respectively. These were averages taken from three separate experiments each with a few males mated to a few females. There were a total of eight males and eight females. (The h^2 from each experiment is given in the paper.) Newkirk et al. (1977) reported half-sib h^2 of larval growth to day 6 and 16 as being 0.33 and 0.50, respectively. These are averages from two experiments with a total of eight males and five females. Both of these papers taken together still leave us with a very small sample size, but the concistency of the estimates is encouraging. Most of the estimates from these two papers cluster in the range of 0.25—0.50 which indicates that there is considerable additive genetic variance in these natural populations which could be exploited in a selection program.

Newkirk et al. (1977) also reported full-sib h^2 for larval growth rate which were 0.43 and 0.60 on the 2 days. There is not much difference between the half-sib and full-sib estimates, suggesting that most of the genetic variance is made up of additive variance. If this is so, there appears to be a difference between these oysters and the blue mussels discussed above which had relatively more nonadditive genetic variance.

In the same paper, Losee (1978) gave h^2 estimates of spat length at 6 weeks postsetting based on two of the matings. The two estimates were 0.29 and 0.71. Again, we must be careful in making predictions because of the small size of these experiments but h^2 of this size should lead to rapid gains in a selection program.

Lannan (1972) gave estimates of h^2 of a number of spat traits at 12 months and 18 months of age in *C. gigas*. These estimates are all high at 12 months (0.31—1.17) and all decrease dramatically at 18 months (0.0—0.37). These are all traits which are expected to be highly correlated (length, width, weight etc.), thus it would be expected that the heritabilities would be similar. One would also expect each trait to be highly correlated over a 6-month period; thus, it seems unusual for the h^2 estimates to change so much in 6 months. These results do point out the problem of needing to know what trait is of interest and whether the expression of the genetic variance changes over time and if so at what stage would selection be most efficient. These problems were discussed by Haley and Newkirk (1977) with data on phenotypic correlations taken from *C. virginica*. Most of the maricultured species are luxury foods; thus, it behooves us to be concerned about what will happen to the quality of the product as we select for traits such as growth rate which will produce a greater quantity.

Some traits are determined solely or predominantly by a few loci but also have an environmental component. Such is sometimes the case when the phenotypic expression is as a few alternative morphotypes.

Chanley (1961) reported that the notata shell markings of *Mercenaria mercenaria* were inherited by a simple single locus mechanism. He used only two crosses in the first generation but subsequently had six crosses from the F1 generation of one of these initial crosses. The ratio of the marked and unmarked classes fit the proposed single locus model very well. But with so few families it is very possible that there is more than one gene producing these markings. It is not known whether there is any association of these shell markings with commercially important traits.

In *Mytilus edulis*, Mitton (1977) has observed different frequencies of shell color morphs in wild populations. The color differences, either brown, blue-black, or striped are due to the presence or absence of the blue pigment within the matrix of the shell. Innes and Haley (1977b) have proposed a single locus-2 allele mechanism for the color morphs. Actually there is a continuum of phenotypes since the pigment may vary in intensity and/or the stripes may vary in width. Unpublished data of mine confirm that there is a genetic basis for this shell coloring but it may not be as simple as Innes and Haley suggest.

I have also been accumulating data on mussels grown in the field and there is a consistent difference in the size of the color morphs when arbitrarily classified into two morph types, light and dark; the dark ones are larger. Mitton's observations were on intertidal animals of unknown age and he has postulated that the differences in color result in different absorption of radiant energy and thus influence metabolic processes. My observations were on cultured mussels of known age that were grown in suspended culture completely submerged such that incident radiant energy would have very little influence on the temperature of the animals. Whatever the physiological mechanism is, the dark animals were on average 10—20% larger. A genetic correlation between color and growth rate may have evolved in the intertidal

populations but it apparently does not depend on an interaction with sunlight. This correlation may have implications for the culture of this species since there are often large differences in the frequencies of these morphs in different populations even on a local scale.

Another trait that probably has relatively few genes controlling it is sex. So far there have been no sex chromosomes observed in any mollusc but this is not surprising since their presence is highly unlikely in species which are either hermaphroditic or can change sex. Haley (1977, 1979) has been following the frequency of sex changes in five full-sib families of *C. virginica*. Initially, (Haley, 1978), it appeared that the sex determination of these oysters could be explained by a three locus (two alleles each) model with certain genotypes as fixed males, others fixed females, and the remaining potential sex changers. His more recent results, based on a fourth year of observation, are probably better explained by more than three but still a limited number of loci.

Understanding and being able to control the sex of oysters could become a powerful tool in oyster breeding. If a sterile animal can be produced, growth and product quality may be increased dramatically.

POPULATION DIFFERENCES

Since the species with which we are concerned in aquaculture are wild and there are usually many sources of stock in the form of different natural populations we should consider the potential of these different populations as sources of different sets of genes (Newkirk and Haley, 1977; Hershberger, 1978). As was discussed above, the electrophoresis studies have shown us that some of the natural populations are genetically differentiated to some extent. If the populations experience different environments one might expect to see adaptations to local conditions evolving. The animals used in aquaculture should be studied to determine whether there are sufficient differences between populations to favor particular ones for culture or whether the culture conditions or sites should be changed to optimize conditions to suit particular adaptations in the animal.

Newkirk (1978a) has shown genetic differences between populations of *Crassostrea virginica* in the growth of larvae at different salinities. Adult oysters were sampled from two sites in each of two separate estuaries and spawned. The two sites were a high and a low salinity site. Mass crosses were then made between all four populations. The larvae were grown at four salinities from 30 to $12^{0}/_{00}$. The results indicate that there were greater genetic differences between the populations of oysters in the same estuary than between the geographically isolated populations at similar salinity. The expression of these genetic differences, however, depended on the environment (salinity) in which the larvae were raised. There is interaction of the genes and the environment such that it is not always possible to extrapolate the results from one situation to another.

Results of Mallet and Haley (1979) show that there are differences among

the growth of spat from *C. virginica* adults taken from different estuaries and then spawned in a hatchery. The spat are being grown in several estuaries including the source localities of the parents. After 1 year there are differences between the population crosses and in a particular estuary the offspring of local parents are not necessarily the fastest growing oysters.

H.H. Haskin (personal communication, 1978) has been maintaining several geographic strains of *C. virginica* by hatchery spawning individuals and growing all stocks in the same environment in Delaware Bay. Even after several generations in the same environment there are differences between these strains in growth rate and temperature threshold for gonad maturation. The oysters from northern sources grow faster and spawn earlier than southern oysters.

Such population differences may be very useful in a breeding program if there is a separate genetic control for growth rate and gonad maturation in response to temperature.If a low threshold for growth from the northern oysters can be combined with a high threshold for gonad maturation it may produce a much faster growing oyster in cool or cold waters. This would first require considerable research to determine the genetic control of these traits and then the designing of the proper selection program.

Such population differences should be considered when choosing sources of spat. The biology as well as the economics have to be considered, however. In the long run it might be cheaper to draw from a particular source of spat, perhaps because of quantity, than to go somewhere else that will provide a faster growing oyster but require much more work to collect. These considerations may have consciously or unconsciously gone into the selection of traditional sources of spat; but if they have not, it may be time to look into the problem.

HYBRIDIZATION

One method of improving production is to produce a hybrid animal which has a better performance than either of the parental stocks because of heterosis or hybrid vigor. As noted earlier there appears to be evidence of heterosis in the electrophoretic studies of Singh and Zouros (1978). The presence of some nonadditive genetic variance in the heritability studies also suggests crossbreeding as a potential breeding scheme.

In the study of population differences, Newkirk (1978a) also showed that some of the hybrid crosses (between populations) were superior to the within population crosses; but the expression of the hybrid genotype varied with the environment. Mallet and Haley (1979) observed a similar pattern in the spat and adults of the population crosses they produced.

The production of a heterotic set of offspring depends on there being the right alleles in each of the parental lines. Thus, the failure to observe heterosis in one or more cases does not mean it will not be possible in other cases. Alternatively, the demonstration of heterosis in one case does not mean it will

be present in all cases. This is illustrated in the results of Newkirk (1978a). The crosses between populations of the same estuary produced heterosis when the larvae were raised at a salinity of $24^0/_{00}$ but the crosses between populations from different estuaries did not. This may be explained by increased genetic differences between geographically isolated populations such that although some genes will tend to produce a superior hybrid, other genes are to some extent incompatible and produce an inferior hybrid. What we observe in these cases is an average over the whole genome. It is possible with the right kind of breeding program to select lines that when crossed produce a superior hybrid.

Geographic isolation resulting in genetic differentiation may explain some of the results of Menzel's (1977) hybridization experiments with *Mercenaria mercenaria*. It is difficult to assess the importance of the differences he has observed between crosses because no standard errors or statistical tests are given. The irregularity of crossing also makes comparisons difficult.

Another application of hybridization taken one step farther is to produce interspecific crosses. Here one is reasonably sure that considerable genetic differences have accumulated between the two groups. The more distantly related the groups are, presumably the greater the genetic differences. However, if there is too large a genetic difference between the species it may be impossible to produce a viable hybrid.

There has been considerable interest in hybridizing *Crassostrea virginica* and *C. gigas*. The hope is to combine the fast growth of *gigas* with the quality of *virginica*. (There are those, however, who say that the quality of *gigas* is already competitive with that of *virginica*!) The work of Stiles (1978) and Davis (1950) indicate that these two species do not readily hybridize and produce viable adults. Menzel (1971), however, produced hybrids which metamorphosed and grew. His data do not indicate what percentage of attempted crosses were successful. Unpublished work of N. Windsor (1978) indicates that these species can be hybridized readily and produce fertile offspring.

At present it is difficult to asses the potential of these interspecific hybridizations because of the limited size of the successful experiments and the inconsistency in ability to produce viable hybrids. This inconsistency may arise from genetic variation in interspecific compatibility in which case it may be possible to select for increased compatability. Before such an endeavor is started, however, the potential for within-species improvement should be explored more fully. The problem of introducing nonindigenous species would certainly not make such a hybridization program easy and, if there is no evidence that the gains to be made are much greater than the gains from an equal effort spent on the indigenous species, it is certainly not worth the risk involved in introducing the nonindigenous species.

INBREEDING

Another subject of concern in developing a breeding program is that of

inbreeding which can be both beneficial and detrimental. Inbreeding can be beneficial when used as a means of 'purifying' lines to be used as is or as parental stock in a crossbreeding program. But inbreeding can also produce a deterioration in vigor and survival because of what is known as inbreeding depression. There are several explanations for the loss of vigor on inbreeding based on either the presumption that heterozygosity per se is necessary to produce normal vigor or that many deleterious recessive alleles are expressed on inbreeding which are usually masked at the normal level of heterozygosity.

An extreme form of inbreeding is self-fertilization. This is theoretically possible in those species that are hermaphroditic but is usually prevented by avoiding simultaneous release of sperm and eggs. Self-fertilization has been accomplished in the bay scallop, *Argopecten irradinas*, by Castagna and Duggan (1971) and apparently resulted in normal development.

Another way of producing self-fertilization is the preservation of sperm in those species that change sex and the use of the sperm to self-fertilize the eggs of individuals that spawn first as males and then as females. Lannan (1971) has done this with *Crassostrea gigas* by cryopreserving sperm and then 6 months later respawning the animals. Two females were obtained which had previously been males. The fertilization rate and survival of the matings involving cryopreserved sperm were low when compared to contemporaneous crosses of the same females with fresh sperm. There were only two self-fertilized families produced thus it is impossible to say how much of an effect this level of inbreeding will have on the oysters. (No data were presented on relative growth rates of the crosses.)

Stiles (1978) has presented data on attempts to produce parthenogenetic development of *C. virginica* by using irradiated sperm. The irradiated sperm is used to stimulate the development of the egg but if sufficiently damaged it does not contribute its chromosomes. Stiles has been working with different radiation intensities to determine the optimal level. The frequency of parthenogenisis was increased greatly at doses over 15,000 R. It was not indicated whether these larvae survived past the straight hinge stage.

In earlier work, Longwell and Stiles (1973) showed that inbred oyster larvae produced by brother—sister matings of *C. virginica* had lower viability and growth rate than contemporaneous outbred crosses, but there were considerable differences between individual inbred crosses. The pronounced inbreeding depression in these crosses suggests that if inbreeding were to be used as a breeding method in these oysters it could not be very intense.

Longwell and Stiles (1973) have proposed a genetically determined incompatibility reaction in these oysters which promotes outbreeding in the species. This is supported by their data from the inbred crosses and other data from outbred crosses. Such a mechanism should increase the level of heterozygosity in a population but the data of Singh and Zouros (1978) on a completely isolated population from that sampled by Longwell and Stiles show an excess of homozygotes. Further analysis is required to determine whether the incompatability mechanism and/or the excess of homozygotes occur in all populations of the species.

Inbreeding has been practised in agriculture on a number of species of crops but in livestock has proved economically useful only in poultry. Inbreeding is used to make lines more homozygous either to produce more predictable performance or for outcrossing to produce hybrid offspring as market animals. Inbreeding in aquaculture may not prove very useful until enough selection has been performed to produce strains which have an accumulation of 'better' genes. At that point inbreeding as a means of purifying or for crossing may prove useful. Inbreeding of wild stock at this time would be essentially taking small random samples of genes from the population. Such a procedure might be productive if a very large number of inbred lines could be produced and tested, but this seems unlikely in the near future. In the meantime inbreeding will be useful as an experimental tool.

SELECTION

In planning a selection program, estimates of a number of parameters are needed to plan the most efficient program. Examples of the information needed are: the heritability of the traits concerned, the correlation (both phenotypic and genotypic) between traits, and the relative economic values of the various traits if more than one trait is being considered. The importance of these data in decision making with examples of data from the American oyster was discussed by Haley and Newkirk (1977).

The aim of selective breeding is to produce strains which are genetically improved for certain economic traits. Genetic studies of various kinds, especially estimates of heritability, provide an idea of the potential rate of progress but the best indication of the potential is a series of selection experiments which actually show progress being made. Selection experiments have the advantage of producing improved strains as well as usually being more efficient than most other heritability studies (Hill, 1971).

Selection for disease resistance in shellfish has been done by natural selection in the past. The rehabilitation of the oyster industry in eastern Canada was through natural selection for oysters resistant to Malpeque disease (Logie et al., 1961). The same is true for the Delaware oysters and MSX. For a number of years Dr. H.H. Haskin has been using artificial selection for resistance to MSX in Delaware Bay on a number of hatchery reared lines. The results of four generations of selection have recently been reported (Haskin and Ford, 1978). Artificial selection has increased resistance much faster than natural selection of the wild stocks. After four generations of artificial selection the percentage survival was 8.9 times the average of susceptible (unselected) stocks. These susceptible stocks are imported stocks not previously exposed to MSX (at least at the intensity that occurs in lower Delaware Bay). The corresponding ratios for the first, second and third generation selected lines are 4.4, 5.0 and 6.6, respectively. Offspring of native Delaware Bay oysters had 2.3 times higher survival than the susceptible lines. All lines were hatchery reared and grown in a common environment. The increased

survival of the selected stocks could be due to specific resistance to MSX or due to selection for 'hardier', generally more vigorous animals. Valiulis (1972) found that these MSX resistant stocks were also more resistant to *Labyrinthomyxa marina.* The Cape Shore, where these selected stocks have been maintained, is considered by Haskin and Ford to be a harsher environment than the planted grounds, where most oysters are cultivated. As a result several physiological traits may have been selected which may or may not include specific resistance to MSX.

These results are significant in that they show a rapid, continuous and repeatable response to selection in the oyster. In addition they show how artificial selection can be more efficient at improving a specific trait than natural selection. This is probably explained by the fact that natural selection acts on total fitness but a particular trait (or set of traits) may not be monotonically related to fitness. Another explanation would be that the wild populations are continuously subjected to immigration from other populations where the selection may be less intense. These results are especially encouraging since disease resistance is usually considered to have a low heritability (Falconer, 1960) and thus be rather slow to respond to selection.

In Washington state there is a program to develop stocks of *C. gigas* which are resistant to summertime mortality. In a recent report, Beattie et al. (1978) gave the results of the program so far. The approach in this case is different from that of Haskin and Ford (1978). Beattie et al. have been producing full-sib families from parents which are selected for increased resistance to summertime mortality whereas Haskin and Ford have been producing mass spawned lines, 8—20 individuals per spawning. So far there have been only seven families tested, three that set in 1974 and four that set in 1975. The controls in these tests have been Japanese stock grown in local water, and a local population. There is some variability in the results of replicate tests, e.g. in some tests the selected families show increased survival and in some they were no better than the controls, but on the whole it appears that some of the families are showing increased resistance. One difficulty with the control population is that they were not reared in the same environment as the families until the last year before testing, and no data are presented on the percentage survival from setting to testing in either the control populations or the families; thus, one cannot determine whether these groups have been subject to selection before the tests. There is, however, an indication that some of the families are genetically improved for resistance to this disease. Hopefully, many more families will be produced to give a better sample size.

Chanley (1961) gave the results of an experimental selection for growth rate in the clam, *Mercenaria mercenaria.* The results can be taken only as a preliminary indication of the potential response to selection in the clam because of the small size of the experiment; there was only one cross made and this mating was made between full-sibs. There was, however, a substantial increase in the growth rate of the selected clams.

Recently we have been conducting selection experiments in our laboratory

on both the American and the European oyster. L.E. Haley (in preparation) will be reporting the results of a selection experiment on the American oyster. Adults from several year-classes and whose growth rates had been monitored for 3 years were selected, spawned and mass matings were made with 8—20 individuals per group. Contemporaneous controls were raised at both of the spawning times. Some of the results are presented in Table I. As we have discussed before, the reliability of selection will vary with the age of the animals. So far the results of these year-classes show some indication that the response to selection will not be as great in the younger year-class. However, the offspring of the selected parents from the two older year-classes (1972 and 1973) showed much faster growth over the 2 years than the control groups. The relative difference between the selected lines and the control lines may increase but probably will not decrease as the animals grow larger.

TABLE I

Preliminary results of selection for growth rate in *Crassostrea virginica* (L.E. Haley, unpublished data)

Year-class	Age at selection (years)	Mean weight (g) of offspring after 2 years
1974	2	13.6
1973	3	18.1
Control 1		12.9
1972	4	20.0
Control 2		18.4

In the summer of 1977, as part of our oyster breeding program at Dalhousie, a sample of 460 European oysters, *Ostrea edulis*, were taken from 2-year-old hatchery stock and measured. From these several selected groups and controls were chosen and spawned. At the same time a sample of the parents of this year-class were respawned as primary controls since the year-class as a whole had been subjected to some selection as a result of routine hatchery procedure. The offspring of these lines were grown in the laboratory over winter and samples were placed out in the field during the summer of 1978. The mean weights of one set are given in Table II. These data are preliminary and will be subject to further analysis to consider such factors as differences between replicates and will be reported elsewhere. The five lines A—E are offspring of the individuals from the 1975 year-class. The P line is the result of the respawning of the older stock. The mean size of 15.5 g given in the table as the weight of the parents of the P line is the mean size of the 1975 year-class at the time of selection since we do not have comparable data for the actual parents of P. Because these oysters were mass spawned using the same techniques as Hidu and Richmond (1971), and Walne (1974), and because the females brood the larvae it is not known

TABLE II

Preliminary results of selection for growth rate in *Ostrea edulis*. (G.F. Newkirk, unpublished data)

Line	Mean weight (g) of parents at selection	Range of parental weights (g)	Offspring weight (g) after 1 year
A	36.4	32.0—42.2	30.2
B	30.0	28.9—30.9	28.2
C	26.3	25.9—27.0	29.9
D	16.4	9.8—24.1	31.0
E	12.5	6.7—24.4	25.7
P	15.5*	1.5—42.2*	24.4

*Data for 1975 year-class, including parents of A—E. P parents are a sample of the parents which produced the year-class and no data are available on them.

which individuals actually contributed genes to the offspring. This is a problem in groups D and E where there was a larger range in the parental sizes than in groups A, B and C. The data for this sample of lines grown in one environment indicate that the lines which had a positive selection differential, A—D, were larger than the other lines, E and P. Five more mass spawned lines were produced during 1978 as well as a set of selected families from single pair matings but the spat are too small at present to given any meaningful data.

These selection experiments in different species in different regions give definite evidence of the potential response to selection. Heritability studies are extremely important in providing the information required to plan selection experiments, but as predictors of the response to selection they are sometimes slightly biased. The selection experiments for growth rate have gone for only one generation but selection for MSX resistance has proceeded for four generations and the response to selection has not diminished significantly.

Roosenburg (1978) has called for the organization of a breed association in the oyster industry with a system of certification of seed performance. This suggestion, regardless of its merits, is certainly premature. When selected stocks have been established, then perhaps such a system will be useful although the establishment of 'breeds' should be based solely on the economic value of the traits. Many breeds of livestock are maintained as purebreeds solely for aesthetic or historical reasons (cf. Lerner and Donald, 1966). A breed association with an oyster registry could be either a stimulus to promote oyster breeding or an unnecessary attempt to monopolize oyster breeding by a small number of individuals who want to protect their control of the market. As Roosenburg (1978) has said, we must first define what the end product should be in terms of individual traits and response to different en-

vironments. These definitions should develop naturally through market demand and may be helped by, but should not be arbitrarily imposed by a governmental agency or a self-appointed breed association.

CONCLUSION

The genetic and breeding work that has been done so far on shellfish has brought out three important points: (1) there are genetic differences between populations; (2) there is evidence of inbreeding depression; and (3) there is a clear potential for rapid response to carefully controlled selection. Other results have been discussed but these three points are of most immediate concern to shellfish culture and the development of selective breeding programs.

The genetic differences between populations, as demonstrated in a number of ways in a number of species, has implications for present culture practices and future development. As discussed above, in choosing natural seed sources the potential genetic differences between populations for traits of economic interest should be considered. Relatively simple experiments involving transplants of samples from different sources and subsequent monitoring should be adequate to solve the problem on a local scale. In the long term, such population differences are sources of genetic variability that will be needed in future breeding programs. Consideration should be given to maintaining the natural populations for such purposes.

The demonstration of inbreeding depression in oysters has been somewhat limited but it is in line with what one would expect to observe considering the biology of the animal. Inbreeding depression is observed in domestic livestock which is initially more inbred than wild oysters. Inbreeding should be of concern to commercial hatchery operators (and their customers) as it may occur inadvertently and in spite of apparently large numbers of broodstock through genetic drift. This was discussed by Newkirk (1978b).

The rapid response of oysters to selection for disease resistance and growth rate is compelling evidence that the development of genetically superior strains can be accomplished. The evidence of Haskin and Ford (1978) shows that artificial selection for one trait can be much more efficient than natural selection. We cannot, as yet, make concrete predictions of the genetic gains possible because of the preliminary nature of some experiments and the small scale of others. However, based on these studies it is not unreasonable to expect gains of 10—20% in the mean per generation. If gains at this rate can be continued it will take about four to seven generations to double the mean.

The next step in the development of shellfish breeding is to define realistic goals and implement programs to achieve them. I would like to define three areas as needing immediate attention as they will set the stage for further development.

First, we must encourage the involvement of geneticists in all areas of

shellfish culture. This involvement will vary with the intensity of culture from such roles as screening natural populations for genetic differences in economic traits and designing hatchery broodstock maintenance programs to implementing selective breeding programs in commercial hatcheries. There are genetic implications to any management of shellfish stocks regardless of the intensity of culture. Someone with a clear appreciation of population genetics *and* breeding should be available for consultation in any region where shellfish are cultured, in the same capacity as the agricultural extension service.

The second area that needs attention is the qustion of how much improvement must be made in selected lines of shellfish to make hatchery production of seed competitive with natural seed. There are at present a limited number of growers that use hatchery seed primarily because in most regions natural seed is much cheaper to obtain. In such cases the need to develop genetically improved strains may seem minimal because of the expense of hatchery operation. One of the reasons hatchery seed cannot presently compete with natural seed is that it is not much better in quality. But if the quality of the seed were improved enough to reduce the cost of grow out, the initial cost of the seed would be tolerable. We need to determine how much better in terms of, say, growth rate, survival and meat yield the hatchery seed will have to be to justify the higher cost to the grower. (Obviously the other costs and problems associated with hatchery seed will have to be considered). Then the cost of developing such improved strains will have to be weighed against the economic gains to be made. Some of the information required for this assessment would come from the third area of concern to be considered.

The third need is for selective breeding programs for the species and in the regions which presently depend on hatchery seed. It should be the role of government to initiate such programs akin to the agricultural experiment stations which have been so vital to the development of agriculture. It must be realized that the success of a breeding program can be measured only in terms of gains per generation and that, with the tremendous variability shown by most shellfish, a clear indication of progress may be forthcoming only after several generations. Consequently these programs will require a long term committment. Another extremely critical consideration is the scale of such a program. The major difficulty with much of the work published to date in this field is the limited size of the experiments. There have been severe constraints on the physical or human resources during most of this work such that optimal experimental design has not been achievable. These constraints in many cases are a result of inadequate or inconsistant funding. (Although in some cases better experimental designs could have been employed.)

It would be a mistake to suggest that a breeding program can be successful on its own. Rather it should be integrated with research on other aspects such as nutrition, pathology and technological development. The interaction of these studies is necessary for the efficient development of strains which

will perform well under the systems being developed for culture. The first problem in any breeding program is to define the breeding goals. This can be done only with the cooperation of breeders and other researchers, growers and marketing personnel. It is no use for the breeder to develop a strain which in the end will not be acceptable on the market. The traits to be improved by selection must be chosen carefully at the start of the program.

These suggestions are not meant to be exhaustive. Certainly basic genetic research into all aspects of molluscan genetics should be encouraged. My intention has been to emphasize that we need definite action in certain areas. However, it is not sufficient for the geneticist alone to be making these recommendations. Commercial interest, which will be realizing the immediate gain of selection programs, should also be encouraging, perhaps badgering, government to initiate such programs. The potential of selective breeding is being demonstrated but will not make an impact on the industry until the presently small research projects are scaled up to concentrated breeding programs.

REFERENCES

Ahmed, M., Skibinski, D.O.F. and Beardmore, J.A., 1977. An estimate of the amount of genetic variation in the common mussel, *Mytilus edulis*. Biochem. Genet., 15: 833—846.

Beattie, J.H., Hershberger, W.K., Chew, K.K., Mahnken, C., Prentice, E.F. and Jones, C., 1978. Breeding for resistance to summertime mortality in the Pacific oyster (*Crassostrea gigas*). Washington Sea Grant Report WSG 78-8, 13 pp.

Buroker, N.E., Hershberger, W.K. and Chew, K.K., 1975. Genetic variation in the Pacific oyster, *Crassostrea gigas*. J. Fish. Res. Board Can., 2471—2477.

Castagna, M. and Duggan, W.P., 1971. Mariculture Experiments with the Bay Scallop, *Argopecten irradians*, in Waters of the Seaside of Virginia. Am. Malacol. Union Bull., 21 pp.

Chanley, P.E., 1961. Inheritance of shell markings and growth in the hard clam, *Mercenaria mercenaria*. Proc. Natl. Shellfish Assoc., 50: 163—169.

Davis, H.C., 1950. On interspecific hybridization in *Ostrea*. Science, 111, No. 2889; 522.

Falconer, D.S., 1960. Introduction to Quantitative Genetics. Oliver + Boyd, Edinburgh, 365 pp.

Glude, J.B. (Editor), 1977. NOAA Aquaculture Plan. Natl. Mar. Fish. Ser., U.S. Dep. Commerce, Washington, DC, 41 pp.

Haley, L.E., 1977. Sex determination in the American oyster. J. Hered., 68: 114—116.

Haley, L.E., 1979. Genetics of sex determination in the American Oyster. Proc. Natl. Shellfish Assoc, 69: 54—57.

Haley, L.E. and Newkirk, G.F., 1977. Selecting oysters for faster growth. In: J.W. Avault (Editor), Proceedings of the Eight Annual Meeting, World Mariculture Society, pp. 557—565.

Haskin, H.H. and Ford, S.E., 1978. Mortality patterns and disease resistance in Delaware Bay oysters. Proc. Natl. Shellfish Assoc., 68: 80.

Hershberger, W.K., 1978. The use of interpopulation hybridization in development of coho salmon stocks for aquaculture. In: J.W. Avault (Editor), Proceedings of the Ninth Annual Meeting, World Mariculture Society, pp. 147—156.

Hidu, H. and Richmond, M.S., 1972. Commercial Oyster Aquaculture in Maine. Maine Sea Grant Bull., 2: 59 pp.

Hill, W.G., 1971. Design and efficiency of selection experiments for estimating genetic parameters. Biometrics, 27: 293—311.

Hillman, R.E., 1964. Chromatographic evidence of intra-specific genetic differences in the eastern oyster, *Crassostrea virginica*. Syst. Zool., 13: 12—18.

Innes, D.J. and Haley, L.E., 1977a. Genetic aspects of larval growth under reduced salinity in *Mytilus edulis*. Biol. Bull. (Woods Hole, Mass.), 153: 312—321.
Innes, D.J. and Haley, L.E., 1977b. Inheritance of a shell-color polymorphism in the mussel. J. Hered., 68: 203—204.
Koehn, R.K., Milkman, R. and Mitton, J.B., 1976. Population genetics of marine pelecypods. IV Selection migration and genetic differentiation in the blue mussel,*Mytilus edulis*. Evolution, 30 (1): 2—32.
Lannan, J.E., 1971. Experimental self-fertilization of the Pacific oyster, *Crassostrea gigas*, utilizing cryopreserved sperm. Genetics, 68: 599—601.
Lannan, J.E., 1972. Estimating heritability and predicting response to selection for the Pacific oyster, *Crassostrea gigas*. Proc. Natl. Shellfish Assoc., 62: 62—66.
Lerner, I.M. and Donald, H.P., 1966. Modern Developments in Animal Breeding. Academic Press, London, 294 pp.
Logie, R.R., Drinnan, R.E. and Henderson, E.B., 1961. Rehabilitation of disease-depleted oyster populations in eastern Canada. Proc. Gulf Carib. Fish. Inst., 13: 109—113.
Longwell, A.C., 1976. Review of genetic and related studies on commercial oysters and other pelecycpod mollusks. J. Fish. Res. Board Can., 33: 1100—1107.
Longwell, A.C. and Stiles, S.S., 1973. Gamete cross incompatibility and inbreeding in the commercial American oyster, *Crassostrea virginica* Gmelin. Cytologia, 38: 521—533.
Losee, E., 1978. Influence of heredity on larval and spat growth in *Crassostrea virginica*. In: J.W. Avault (Editors), Proceedings of The Ninth Annual Meeting, World Mariculture Society, pp. 101—107.
Mallet, A. and Haley, L.E., 1979. Strain differences and the absence of heterosis in strain crosses of the American oyster. Presented at The Tenth Annual Meeting, World Mariculture Society.
Menzel, R.W., 1971. Selective Breeding in Oysters. In: K.S. Price and O.L. Maurer (Editors), Conference on Artificial Propagation of Commercially Valuable Shellfish (1969). University of Delaware, Newark, DE, pp. 81—92.
Menzel, R.W., 1977. Selection and hybridization in quahog clams (*Mercenaria* spp.). In: J.W. Avault (Editor), Proceedings of the Eight Annual Meeting, World Mariculture Society. pp. 507—521.
Mitton, J.B., 1977. Shell color and pattern variation in *Mytilus edulis* and its adaptive significance. Chesapeake Sci., 18 (4): 387—390.
National Research Council, 1978. Aquaculture in the United States, Constraints and Opportunities. Board on Agriculture and Renewable Resources, Commission on Natural Resources. Natl. Acad. Sci., Washington D.C., 123 pp.
Newkirk, G.F., 1978a. Interaction of genotype and salinity in larvae of the oyster *Crassostrea virginica*. Mar. Biol., 48: 227—234.
Newkirk, G.F., 1978b. A discussion of possible sources of inbreeding in hatchery stock and associated problems. In: J.W. Avault (Editor), Proceedings of the Ninth Annual Meeting, World Mariculture Society. pp. 93—100.
Newkirk, G.F. and Haley, L.E., 1977. The role of natural populations in the genetic improvement of species used in aquaculture. In: J.W. Avault (Editor), Proceedings of the Eight Annual Meeting, World Mariculture Society. pp. 567—578.
Newkirk, G.F., Haley, L.E., Waugh, D.L. and Doyle, R.W., 1977. Genetics of larvae and spat growth rate in the oyster, *Crassostrea virginica*. Mar. Biol., 41: 49—52.
Numachi, K., 1962. Serological studies of species and races in oysters. Am. Nat., 96 (889): 211—217.
Roosenburg, W., 1976. Can the oyster industry learn from livestock breeders? Proc. Natl. Shellfish Assoc., 66: 95—99.
Singh, S.M. and Zouros, E., 1978. Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). Evolution, 32 (2): 342—353.
Stiles, S., 1978. Conventional and experimental approaches to hybridization and inbreeding research in the oyster. In: J.W. Avault (Editor), Proceedings of the Ninth Annual Meeting, World Mariculture Society, pp. 577—586.

Valiulis, G.A., 1972. Resistance of *Crassostrea virginica* to *Minchinia nelson* and *Labyrinthomyxa marina*. Proc. Natl. Shellfish. Assoc., 63: 6 (abstract).
Walne, P.R., 1974. Culture of Bivalve Molluscs. 50 Years Experience at Conwy. Fishing News (Books) Ltd., Surrey, 173 pp.