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THE BIOGENETICS OF MOLLUSCS AND CRUSTACEANS[1]

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J. A. H. Benzie (1991) The biogenetics of molluscs and crustaceans. *Bull. Inst. Zool., Academia Sinica, Monograph* **16**: 485-512. Sufficient genetic variation exists in natural populations of molluscs and crustaceans to sustain artificial selection programs. Measurements of the extent to which characteristics of importance to production are inherited indicate reasonable levels of genetic control and potential for positive responses to selection. Whole chromosome set manipulations are largely in experimental stages and the transfer of foreign genes into aquaculture species of molluscs and crustaceans has not been reported. Given the dependence of molecular approaches on aspects of traditional genetics for many practical applications, it is important to develop quantitative and population genetic methods in concert with molecular methodologies. Molecular genetics and whole chromosome set manipulations, particularly, will provide important research tools for reproductive biology.

The successful application of reproductive research to aquaculture depends upon recognition of the genetic consequences of breeding from closed populations. There is a need for an integrated approach to genetic and reproductive research given the interdependence of the two. The fact that aquaculture is at an early stage of development relative to agriculture, and the fact that so little is known, demands a research strategy for reproductive biology and genetics that is broad-based.

Key words: Molluscs, Crustaceans, Aquaculture genetics, Reproductive biology, Biogenetics.

Relatively little work has been carried out on the genetics of molluscs and crustaceans that are of importance to aquaculture. This is due, in part, to the difficulty of manipulating the reproductive process, a problem that is particularly relevant to crustaceans. The failure to fully exploit such manipulation results from the relative youth of many aquaculture industries where husbandry methods have been de-

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veloped only recently, and the fact that collecting either juveniles or gravid females from the wild is cheaper or more convenient than breeding in captivity. Furthermore, lack of pertinent research itself may also play a role in that any work must start from scratch, and the scope of the problem for a given application may therefore appear overwhelming.

The lack of information in these research areas highlights the need to develop a strategic approach to genetic work in crustacean and mollusc aquacultures. In considering the role of genetics in reproductive biology this situation also emphases the need to examine the general relationships between reproductive biology and genetics. The interdependence of reproductive biology and genetics research is deeply interwoven and occurs over a range of topics. It is not simply that one cannot develop genetic breeding programs without being able to control reproduction. If methods are developed that allow animals to reproduce in culture there will be genetic consequences arising from the production of progeny from such a closed population. A given husbandry technique may well have unintended and deleterious consequences for production, especially if relatively few animals contribute to the next generation. The prevention of these effects, and therefore the successful introduction of reproductive research to the industry, depends on integrating reproductive work with genetics.

In this paper, the relevance of different areas of genetics to aquaculture is examined. Examples from molluscs and crustaceans are used to highlight key issues, and the current state of research in these groups is reviewed. The reviews are intended to be representative rather than exhaustive, and to illustrate major points. The importance of each of these areas of genetics research to reproductive biology is then discussed in relation to the efficient application of research effort and the transfer of results to industry. Aquaculture is clearly in an early stage of development compared with agriculture, and a breadth of research approaches is required to achieve advances of use to industry. Although dealing with molluscs and crustaceans, the points concerning general strategy, and the relationship of reproductive biology and genetics, are pertinent to aquaculture as a whole.

METHODS

Four areas of genetics pertinent to aquaculture have been defined here as convenient headings under which to discuss the available data. These are population genetics, quantitative genetics, molecular genetics and whole chromosome set manipulations. Recent reviews of genetics as applied to crustacean and mollusc aquacultures can be found in Tiews (1987). Little has been published since then, and none of the later material alters the major conclusions expressed in that work.

POPULATION GENETICS

Population genetics is concerned with describing the genetic structure of populations. There are two major applications with respect to aquaculture. The first is identifying whether sufficient genetic variation occurs in natural population to sustain genetic improvement of animals as they are domesticated. The second is monitoring the genetic variation in cultured stocks to determine the degree of inbreeding that might occur under cultivation.

The level of genetic variation occurring within populations is not the only feature of importance with respect to the potential for genetic improvement. Genetic differences between populations within a species provide a useful source of variation that can be of advantage to aquaculture. For example, strains might differ in their capability to reproduce or grow in captivity, and mixing genetically different populations may improve yields through a 'hybrid vigour' effect. Surveys of genetic variation can identify genetically differentiated populations that could be utilised in breeding programs, or which might require some protection as a natural genetic resource for use in future aquaculture developments.

Population genetic analyses require counts of the frequency of occurrence of different forms of a gene (alleles), for each of several genes (loci). The data must be derived from clearly defined loci and for this reason morphological data are rarely used because of the difficulty of scoring the genotype unequivocally. In practice, gene frequencies of loci are quantified indirectly by identifying the frequency of gene products (proteins) that differ in their biochemical characteristics and in more direct fashion by quantifying DNA fragments.

Tissue samples are crushed and the liquid exudate placed in media

(*e.g.* starch gels) and subjected to electric currents. To detect and quantify different loci by protein products, the proteins are made visible by stains that react with specific enzymes, after the proteins are separated by charge in an electric field applied to the medium. The products of different alleles can then be counted. Pieces of DNA that result from the action of restriction enzymes, are separated by fragment size on acrylamide gels. As individuals differ in the number of sites cut by the restriction enzyme, the pattern of size fragments differs and is used to quantify restriction sites. Hillis and Moritz (1990) give details of protein and DNA techniques.

The level of genetic variation can be summarised in terms of the average number of alleles per locus, or as the average number of individuals carrying two different alleles at a locus (heterozygotes) as opposed to two copies of the same allele at a locus (homozygotes). The information available for crustaceans has been reviewed recently by Hedgecock (1987a) and that for molluscs reviewed by Blanc and Bonhomme (1987). Both authors observed sufficient levels of variation to conclude that genetic improvement in culture would be possible.

Molluscs generally have higher levels of variation than crustaceans, with approximately twice the average number of alleles per locus and two to ten times the average heterozygosity (Table 1). It is not clear why the two groups differ with respect to the levels of protein variation they display, and the result does not necessarily imply that molluscs have a greater potential for genetic improvement in culture. The data illustrate that differences in the average levels of genetic variation are observed among major groups of organisms (Table 1), and that the level of genetic variation displayed by a group is related to various aspects of their biology. For example, the molluscs *Crassostrea* and *Saccostrea* do not incubate their eggs and show higher levels of genetic variation than *Ostrea* and *Tiostrea* which do. *Tiostrea* species have a very short larval life span (about 1 day) compared with *Ostrea* species (2-3 weeks) and show less genetic variation than *Ostrea*. An interesting feature of mollusc population genetics is the strong relationship that is often observed between heterozygosity and physiological performance or growth rates

Table 1
Summary of genetic variation observed in genera of molluscs and crustaceans of importance to aquaculture. Data abstracted from Hedgecock (1987a) and Blanc and Bonhomme (1987) and for *Tridacna* from Benzie and Williams (unpublished data)

Taxonomic group	Number of species studied	Mean number of alleles per locus	Average Heterozygosity
Crustaceans			
Penaeidae			
Metapenaeus	6	1.26	0.019
Penaeus	14	1.47	0.038
Palaemonidae			
Macrobrachium	6	1.29	0.039
Molluscs			
Ostreidae			
Crassostrea	4	2.40	0.209
Saccostrea	2	2.46	0.192
Ostrea	3	1.69	0.132
Tiostrea	2	1.24	0.078
Tridacnidae			
Tridacna	2	2.75	0.286

that is yet unexplained (Hawkins *et al.*, 1989).

Australian penaeid prawns showed significant genetic differences between populations of *Metapenaeus bennettae* whose larvae develop in coastal lagoons and do not disperse widely, while species with oceanic larval phases such as *M. macleayi* and *Penaeus plebejus* showed no significant differences in gene frequency (Mulley and Latter, 1981). The giant clam *Tridacna maxima* does not differ in gene frequencies between neighbouring populations but some genetic structure is present (Fig. 1). As the geographical separation of populations of this species becomes greater so does the genetic distance between them, and if samples are obtained from populations sufficiently far apart, it is likely that significant genetic differences would be detected.

So the evolutionary history, breeding system, dispersal capabilities and many aspects of the biology of an organism contribute to the level and pattern of genetic variation which they display. However, the particular patterns of variation, and the particular numerical

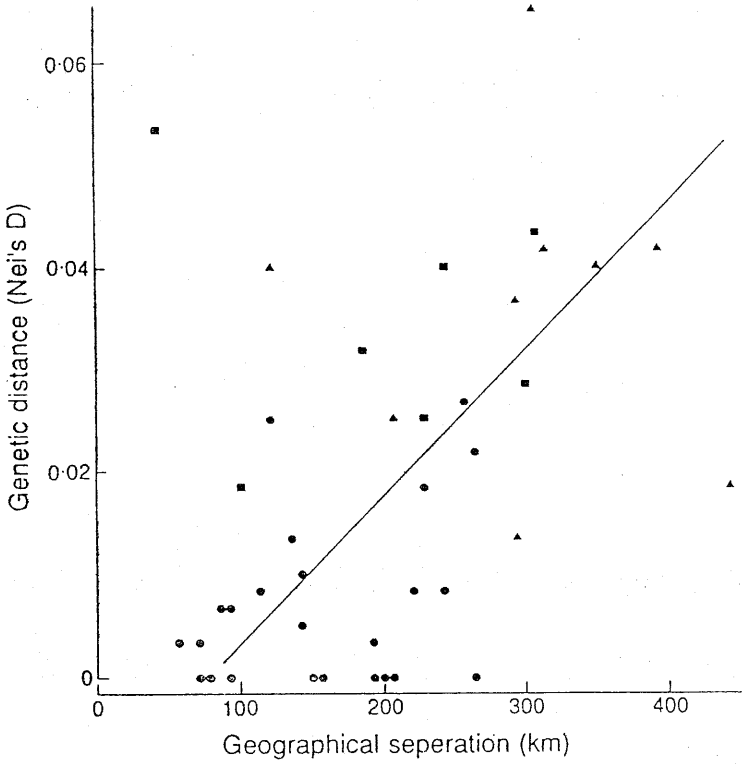

Fig. 1. The ralationship of genetic distance to increasing geographical separation in giant clam (*Tridacna maxima*) populations in the Western Coral Sea (Benzie and Williams, unpublished data).

values observed are secondary to the demonstration that variation exists, and the documentation of the occurrence and distribution of genetically differentiated populations that may provide a valuable genetic resource for breeding programs.

Population genetics also provides a means of monitoring the genetic variation in cultured stocks. Maintenance of reasonable levels of genetic variation in cultured stocks is important, not only as a basis for future genetic improvement, but to prevent the deleterious effects of inbreeding. Separation and breeding of a set of individuals results in a series of genetic changes that, on average, follow the predictions of population genetic theory. Key parameters in the equations are the number of individuals that contribute to the next generation, the sex ratio in the broodstock population, and the variation in the number of offspring in each family. If one does not wish to perform a specific genetic breeding program some general rules that need to be followed to prevent inbreeding are use of large numbers

of males and females to provide the next generation and the maintenance of 1:1 sex ratio. However, this does guarantee the desired result, and population genetic methods can be used to determine the changes actually taking place in a breeding population.

An excellent example of how apparently large populations may suffer inbreeding that dramatically affects production levels has been documented by Sbordoni *et al.* (1987). Despite the fact that several hundred individuals of *Penaeus japonicus* were present in the breeding population, measurements of protein variation over several generations showed a decline in genetic diversity that was consistent with an average of only four individuals contributing to the offspring of each generation (Fig. 2). The decline in genetic diversity paralleled a decline in productivity, given by mean hatching rates, of the population.

This example highlights the fact that reproduction in culture is not the only requirement for a sustained aquaculture. Appropriate genetic considerations should be included to monitor genetic variation to confirm the system is operating as expected.

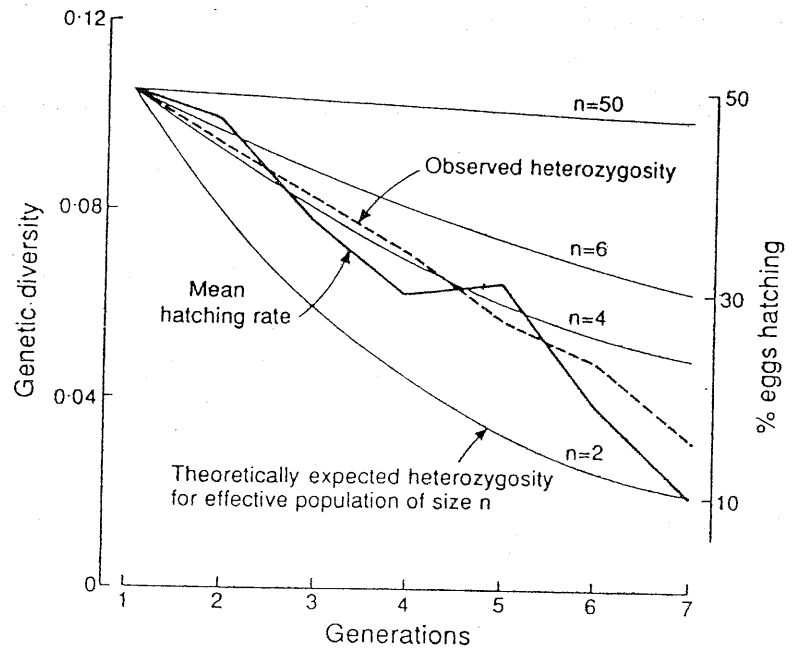

Fig. 2. Decreasing productivity (% eggs hatching) and decreasing genetic diversity (mean heterozygosity) in *Penaeus japonicus* populations compared with theoretical expectations for heterozygosity for populations with effective population sizes of *n* (after Sbordoni *et al.*, 1987).

QUANTITATIVE GENETICS

Quantitative genetics is concerned with describing and manipulating characteristics that show continuous variation and which are often under the control of a number of different genes. The methods of quantitative genetics, applied properly, are extremely powerful and sophisticated means of manipulating the genetic structure of cultured populations. Selective breeding provides the means of domesticating and adapting organisms to the culture system, and is one of the most effective means of producing organisms with improved yield, disease resistance, and enhanced hardiness to culture. The scope of application to aquaculture is obviously broad.

The basic methodology of quantitative genetics is the careful measurement of the characteristics of interest in a series of individuals whose genetic relationships are known. The individuals may be parents and offspring, siblings or other relatives. Some of the most useful information comes from groups of half brothers and sisters where the heritability, that is, the extent to which the characteristics expressed are a result of the genetic constitution of the individuals concerned as opposed to environmental influences, is estimated. The total variation observed (V_P) includes several components of genetic variation, additive (V_A), dominance (V_D) and epistatic (V_I) and environmental variation (V_E). The heritability (h^2), or breeding value, for a trait is equal to V_A/V_P, indicating the particular importance to breeding programs of additive genetic variation. In fact, the expected response to selection is simply the additive genetic variation times the strength of the selection applied. If one calculates the regression of the mean value for a trait in the offspring as a function of the mean value for that trait of the parents, the slope of the regression line equals h^2, and the covariance of offspring and adults in this case is $1/2V_A$. Similarly, one can calculate values for the correlation between values measured in other relatives. For example, the covariance of half-sibs is $1/4V_A$ and the slope of the correlation between half-sib groups is $1/4h^2$. The covariance of full families is $1/2V_A+1/4V_D+V_{Ec}$, and the slope of the correlation between families is equal to $1/2h^2$ only if the other non-additive genetic effects and environmental differences between families are zero.

An important element in estimating the heritabilities of useful characteristics for breeding programs is to establish the correlation between characters. If, for example, disease resistance were highly correlated with poor growth rates, selecting solely for disease resistance might produce animals of such poor growth performance as to be economically inviable. Information of the heritability of characters, their correlation with each other and responses to selection allows breeding plans to be optimized for overall production efficiency.

There are a variety of breeding methods (Falconer, 1981), some of which rely on increasing the frequency of genes that enhance the expression of the character concerned by making individuals more homozygous for those genes. These selected lines are maintained, and not diluted by crossing with others. However, the advantage of the increased genetic homogeneity for the genes of interest must be balanced against the risk of increased homogeneity for other, deleterious, genes that lead to inbreeding depression and decreased vitality. If several lines are available, they can be crossed to make use of the hybrid vigour likely to be expressed as a result of the increased heterozygosity of the crosses, and perhaps to make new lines that have some of the desired characteristics from each of the parental lines. Breeding programs clearly involve a balance between excessive inbreeding and excessive dilution of desired genes by deliberate introduction of new genetic material. An alternative uses a base population created from a variety of sources for maximal genetic variation coupled with a generalised "domestication" selection to adapt that population to culture. The advantage of this approach is that it does not require the expensive formation and maintenance of several lines. For animals recently brought under cultivation this provides a means of improving general performance in the absence of detailed information on the heritability and inter-relationships of individual characters.

Information on the heritability and response to selection of even the most basic characteristics is limited for both crustaceans and molluscs (Hedgecock *et al.*, 1982; Malecha, 1987; Wada, 1987). The paucity of information on crustaceans once again reflects the lack of control over reproduction in this group. Lobsters and prawns show a high

Table 2
Summary of the heritability of characters of commercial importance in crustaceans and molluscs. Data abstracted from references in Hedgecock *et al.* (1982), Wada (1987) and Lester (1988)

Taxonomic group	Character examined	Family structure	Heritability
Crustaceans			
Lobsters			
Homarus americanus	weight at 100 days	full-sibs	0.33 (0–0.58)
	weight at 90 days	full-sibs	0.38 (0.26–0.50)
Marine prawns			
Penaeus vannamei	protozoea	full-sibs	0–0.64
	mysis	full-sibs	0–0.18
	post larvae	full-sibs	0.15–0.36
Penaeus stylirostris	protozoea	full-sibs	1.27–1.31
	mysis	full-sibs	0.64–1.09
	post larvae	full-sibs	0.84–1.02
Freshwater prawns			
Macrobrachium	weight at 311 days unsexed	unknown	0.15–0.16
	weight at 311 days male	unknown	−0.14–0.19
	weight at 311 days female	unknown	0.35
Molluscs			
Oysters (*Crassostrea*)			
C. virginica	larval growth	full-sibs, half-sibs	0.07–0.85
	juvenile length	full-sibs, half-sibs	0.29–0.71
C. gigas	larval survival	full-sibs	0.31
	size at 1.5 years	full-sibs	0.15
	shape at 1.5 years	full-sibs	0.13
	meat weight at 1.5 years	full-sibs	0.37
	total weight at 1.5 years	full-sibs	0.33
	settling success	full-sibs	0.09
Mussel (*Mytilus*)			
M. edulis	larval growth at 16 days	half-sibs	0.12–0.78
Pearl oyster (*Pinctada*)			
P. fucata martensii	shell width at 3 years	half-sibs	0.13–0.47
	shell convexity at 3 years	half-sibs	0.13–0.37
Clam (*Mercenaria*)			
M. mercenaria	size at 1 year	half-sibs	0.37 (±0.12)

range of values for given traits (Table 2). High levels of error were associated with single estimates of heritabilities of size in penaeid prawn larvae (Lester, 1988) and illustrate the difficulty of making reasonable estimates of heritabilities without designs incorporating half-sib groups. Median values for the heritability of characteristics such as growth rate or size for crustaceans are similar to those of other animals and indicate sufficient additive genetic variance is present for selection to be worthwhile. Although Dobkin *et al.* (1975) and Malecha (1977) have attempted selection on *Macrobrachium* the first lacked control lines and the second included variance enhancement as a result of the "bull-runt" phenomenon. Thus, the response to selection of crustaceans of importance to aquaculture remains to be determined (Table 3).

In molluscs few useful estimates of heritability and response to selection have been obtained (Wada, 1987). Earlier estimates of heritabilities were often based on small numbers of full-sib families and were therefore unreliable, but more recent work on oysters and clams using half-sib designs also show considerable fluctuations in the heritability estimates where several experiments have been reported (Table 2). The variation in results has been attributed to the difficulties in designing the large scale rearing experiments needed for this work (Wada, 1987). Work on the inheritance of *Mercenaria* (Rawson and Hilbish, 1990) provides an excellent example of the difficulties of undertaking work in this area, and of the care required to obtain reliable results. Several components of genetic variance for growth rate were estimated for a series of half-sib families, but initial experiments did not control culture density which significantly impacts on non-additive genetic variation in females, but has little effect on the additive genetic component, estimated as 0.72 ± 0.32 to 0.91 ± 0.17. The source of variation in density was not considered a result of replicate specific mortality or of the culture environment as replicate variation was a small source of variation and consistent across replicates within females. More subtle genotype-environment interactions or possibly some maternal effect may have been involved.

Several selection experiments have been carried out on oysters, mussels and pearl oysters for enhanced growth, enhanced disease resistance and shell type. Improved

Table 3

Summary of selection experiments on crustaceans and molluscs of importance to aquaculture. Data abstracted from Hedgecock *et al.* (1982) and Wada (1987)

Taxonomic group	Character selected	Number of generations	Parental stock	Performance of selected line relative to control
Crustaceans				
Freswater prawn				
M. acanthurus	size	2	unknown	no control (larger than parents)
Molluscs				
Oysters				
C. virginica	weight at 2 years	1	8-20 individuals	5-9% heavier
	susceptibility to MSX disease	6	6-16 strains	60% less mortality
C. gigas	resistance to summer mortality	3	4-6 families	60% less mortality
O. edulis	weight at 2 years	2	15 individuals	9-28% heavier
Pearl Oyster				
P. fucata martensii	shell width at 3 years	3	6-22 individuals	14% larger
	shell convexity at 3 years	3	12-23 individuals	13%
	frequency of white nacre shells	3	8-18 individuals	300% greater

performance for these traits has usually been observed in the selected compared with control lines (Table 3). Reduced performance at times within selected lines has been attributed to inbreeding depression or genetic drift. To date, all of the selection work appears to have been restricted to experimental scales and none have given rise to industry-wide commercial breeding programs. The lack of commercial programs arises from the difficulty in manipulating matings and rearing families separately, and, perhaps more importantly, doubt about the value of the results of selection given the extra costs involved.

In summary, the information on heritability and response to selection is limited in quantity and quality for molluscs and crustaceans, but the order of values for growth are consistent with other organism. The levels of genetic control are likely sufficient to provide a sound foundation upon which to base selection programs, with generally positive responses to selection in experimental programs.

Selection occurs in culture even if no deliberate attempt is made to select for particular characteristics. Such "indirect selection" is well-illustrated in amphipod curstaceans, where populations which had been reared in the laboratory for more than 20 generations (but not deliberately selected) performed far better using a variety of criteria than animals taken from the wild and acclimated to the artificial system (Doyle and Hunte, 1980). However, where animals are recently brought into culture the management system used to obtain broodstock can result in selection for characteristics that are detrimental to desired goals. Using a combination of computer modelling and data from *Macrobrachium* populations Doyle *et al.* (1983) demonstrated that different management regimes can result in relatively strong, unintended, selection for size, and this need not be in the direction desired by the farmer. In this case if animals being used as broodstock for the next generation were taken from the pond at 3 months there was a positive selection for weight at harvest of 28%, but if they were taken at 6 months the selection for weight at harvest was −20%. In the first case the practice selects for larger animals, in the second case the practice selects for smaller animals. Present practices in the prawn farms are likely to

result in zero slightly negative selection for weight at harvest. Reproduction in culture is not sufficient to prevent inbreeding or negative selection, let alone maximise returns.

MOLECULAR GENETICS

This area of genetics has developed relatively recently and refers to the technology that allows the isolation and manipulation of specific fragments of DNA, or given genes, using recombinant DNA methods. The most obvious application to aquaculture is the production of transgenic animals: those with genes from other sources incorporated in their DNA by artificial means. Genes of interest are, for example, those controlling growth or disease resistance. Molecular genetics will also benefit other areas of aquaculture such as research in reproductive physiology.

Molecular manipulation involves the insertion of a specific gene from one organism, perhaps in combination with other genes or sequences of DNA regulating their expression, into the genome of the recipient. Several steps are required in this process: Purified DNA fragments are spliced into a vector such as a plasmid within bacteria. The vectors are induced to replicate rapidly to maximise the amount of the cloned DNA necessary for further analyses. Further analysis may include sequencing the DNA concerned, or introducing the material into other cells. The vectors carrying the particular gene(s) of interest can be identified by molecular probes developed for that gene. The colony of bacteria showing expressing the gene can then be isolated and the cloned genes can be produced in large quantities. The cloned gene can then be cut out, fused with DNA which will enhance the expression of the gene, then inserted into cells by microinjection, or by pulsed electric fields. Details of the methods used are provided by Maclean and Penman (1990).

The insertion of a growth hormone gene from one organism into another to enhance growth rates has obvious aquaculture potential. Work on fish, which has general relevance to the application of transgenics to aquaculture, has been reviewed by Chen *et al.* (1987, 1991) and Hew *et al.* (1991). To date there are no published reports of attempts to insert genes into crustaceans or molluscs of aquaculture potential.

This area of genetics has received a great deal of attention

because of the promise of it holds for manipulating genetic material, and the potential improvement in animal and plant production. However, practical application will likely take several years to decades, for two reasons. Firstly, although the methods use contemporary sophisticated biochemical techniques, they are nevertheless extremely crude with respect to the complexity of the biological system being manipulated. At present, overdoses of fragments of DNA are placed in cells in the hope that some will be incorporated, without control over either the number of copies of the gene incorporated, or the position within which they are incorporated in the genome. Current techniques lack both control on expression of the foreign gene and capability to tune the effects of the introduced gene with other important developmental or control processes that may be affected. Secondly, several years may be required to establish that the inserted gene is inherited stably by subsequent generations. Finally, genetic lines produced must be maintained, using techniques of quantitative genetics. The time scales for the application of molecular genetic methods to industry are at least as long as those for standard quantitative breeding programs.

WHOLE CHROMOSOME SET MANIPULATIONS

The term whole chromosome set manipulations is used here to describe the formation of interspecific hybrids, the formation of clones through gynogenesis or androgenesis, the manipulation of ploidy levels, and sex reversal. The applications to aquaculture include combining the desired characteristics of two species in a single animal; the production of clones with desired production traits which, because they are composed of genetically identical individuals, have more predictable production control; enhancing growth through triploidy, and because triploids are non-reproductive, producing animals that are not a threat to natural gene pools if they escape; and enhancing production in single sex populations where one sex is aggressive or has poorer growth. A number of the techniques can produce material of considerable utility as research tools in other areas of aquaculture research, particularly reproduction and physiology.

The manipulations examined here effect major changes to the

genome structure with the expectation that some will result in regulatory or developmental changes that will be of use. The rationale is that major chromosomal reorganisations, and the mixing or replication of chromosomal sets, have been identified as potent sources of evolutionary change (Grant, 1981). The outcome of such major changes to the genome cannot be predicted. Many results in inviable products. Hybrid lines may have characteristics intermediate to those of their parents, or may express the characteristics of one parent for some traits and the characteristics of the other parent for other traits. At one level the approach is crude, but it has been responsible for some of the greatest advances agricultural production, particularly in the creation of new and highly productive strains of plants. One advantage of the methods that produce clones from a single parent is the immediate expression of all the deleterious or lethal genes. Any clones that perform well have been purged of such genes. In addition, because the method involves the whole genome, rare alleles and coadapted gene sets that can be lost in standard breeding approaches are preserved.

Interspecific hybrids

The goal in producing interspecific hybrids is to obtain animals that have the desired characteristics of one species combined with those of another. Interspecific hybrids can be produced by natural service matings between the male of one species and the female of another, but more often relies on artificial insemination. If chromosome numbers of the species involved differ, the successful development of hybrid lines also requires manipulation of ploidy levels to balance chromosome sets.

Interspecific hybridization in crustaceans and molluscs has been reviewed recently by Hedgecock (1987b) and Menzel (1987) respectively. The difficulty in controlling reproductive processes in crustaceans has meant relatively few interspecific crosses have been reported and most have been achieved after techniques for artificial insemination were developed. Most of the interspecies crosses within genera of freshwater prawns (*Macrobrachium*) and penaeid prawns (*Penaeus*) resulted in low hatch rates, low larval survival, few animals reared to maturity and few reports of their ability to breed (Table 4). Hybrid lobsters (*Homarus*) had larval survival and growth

Table 4
Summary of interspecific hybrids obtained in molluscs and crustaceans of importance to aquaculture. Data abstracted from Hedgecock (1987b), Menzel (1987), and Lin *et al.* (1988). All crosses are given female×male

Species hybridized	Fertilisation	Hatching	Stage of development reached (and survival)	Fertility of adult hybrid
Crustaceans				
Lobsters				
Homarus				
H. americanus×*H. gammarus*	+	?	adult	males sterile females fertile
H. gammarus×*H. americanus*	+	?	adult	males sterile females fertile
Marine prawns				
Penaeus				
P. setiferus×*P. schmitti*	+	<1%	post larvae (50%)	—
P. setiferus×*P. stylirostris*	+	<1%	juvenile (50%)	—
P. stylirostris×*P. setiferus*	+	<1%	post larvae (9%)	—
P. monodon×*P. penicillatus*	+	32%	adult (<1%)	sterile
P. penicillatus×*P. monodon*	+	33%	adult (<1%)	sterile
Freshwater Prawns				
Macrobrachium				
M. acanthurus×*M. carcinus*	+	?	adult	sterile
M. asperulum×*M. shokitai*	+	?	post larval	—
M. rosenbergii×*M. malcomsonii*	+	?	post larval	—

Table 4. Continued.

Species hybridized	Fertilisation	Hatching	Stage of development reached (and survival)	Fertility of adult hybrid
Molluscs				
Oysters				
Crassostrea				
C. angulata×*C. gigas*	High	—	adult	fertile
C. angulata×*C. iredalai*	Good	—	early larval	
C. angulata×*C. rhizophorae*	High	—	adult	unknown
C. angulata×*C. virginica*	High	—	adult	unknown
C. brasiliana×*C. rhizophorae*	Low	—	no rearing	—
C. brasiliana×*C. virginica*	Low	—	no rearing	—
C. columbiensis×*C. rhizophorae*	High	—	adult	fertile
C. columbiensis×*C. virginica*	High	—	adult	fertile
C. gigas×*C. iredalei*		—	early larval	—
C. gigas×*C. rhizophorae*		—	late larval	—
C. gigas×*C. rivularis*	Low	—	—	—
C. gigas×*C. virginica*	+	—	adult	probably not fertile
C. iredalai×*C. rhizophorae*	High	—	early larval	—
C. iredalai×*C. virginica*	High	—	early larval	—
C. rhizophorae×*C. virginica*	High	—	adult	fertile

similar to that of their parents, but lower hatch rates. Adult hybrid males were sterile. Hybrid females backcrossed to the parental species spawned successfully, but the young were only reared to early juvenile stages.

Greater success has been achieved with molluscs because the external fertilization used by these species facilitates procurement of large quantities of eggs and sperm which can then be mixed as desired. Intergeneric crosses of several species of *Crassostrea*, *Ostrea*, *Saccostrea*, and *Striostrea* resulted in no fertilization, except in the case of crosses between *Crassostrea gigas* and either *Saccostrea echinata* or *Saccostrea glomerata*, where fertilization rates were low and no progeny survived to sexual maturity (Menzel, 1987). Intrageneric crosses of *Crassostrea* species were more successful but the results were variable ranging from no to high (>95%) rates of fertilization (Table 4). Chromosomal examination of those hybridising easily has suggested these taxa may be conspecific (*i.e.*, *C. angulata*=*C. gigas* and *C. columbiensis*=*C. rhizophorae*=*C. virginica*) and that progeny from crosses of *C. angulata* with *C. rhizophorae*, *C. angulata* with *C. virginica* and *C. gigas* with *C. virginica* are likely to be sterile.

The most successful results have been obtained with quahog clams, with northern (*Mercenaria mercenaria*) and southern (*M. campenchiensis*) species hybridising readily, and possessing commercially important characteristics that are superior to both parents. The hybrid had the better growth rates of *M. campenchiensis* and the good shell closure of *M. mercenaria* that reduces spoilage after harvesting.

Gynogenesis, androgenesis and ploidy manipulation

Most of the organisms used in aquaculture are diploid, and therefore have two sets of chromosomes, one derived from each parent. In normal cell division (mitosis) the number of chromosomes is doubled with each chromosome having two identical copies which, when still joined together, are called sister chromatids. The genetically identical chromatids separate to give a complete and identical chromosome set to each daughter cell. When the gametes are produced each chromosome replicates itself but the identical copies do not separate and, in addition, the matching (homologous) chromosomes from each parent bind closely with each other.

After exchanging genetic material with their homologous partner the sister chromatids need not be identical. The homologous chromosomes are then separated at the first meiotic division, and in females one set is usually ejected as a first polar body, and the chromatids of the other set are retained. One set of these chromosomes is discarded as a second polar body in females at this second meiotic division. The other provides a cell with half the number of chromosomes (haploid) of the parent, and has a unique mixture of parental genes created by the genetic exchange between homologous chromosomes earlier in the process. In males both products of the first meiotic division undergo a second division to produce four cells each with a haploid chromosome set. If either the first or the second meiotic division is suppressed, the normal diploid number of chromosomes will be retained, and will have been derived from one parent.

Gynogenesis uses sperm whose genetic material has been destroyed (usually by irradiation) to induce egg development, and the female chromosome number is restored to diploid number by blocking the second meiotic, or the first subsequent mitotic division. In androgenesis, the genetic material in the egg is destroyed prior to fertilization and the full chromosome complement restored by blocking meiotic or immediately subsequent mitotic divisions. Blockage of cell division is achieved by temperature, or pressure or chemical shock at critical periods in the cell division cycle. Chourrout (1987) and Longwell (1987) give summaries of the techniques used.

Interspecific crosses fail if the species differ in chromosome number, which leads to failure of the normal meiotic process and sterility. A balanced number of homologous chromosomes can be restored by blocking the first mitotic division of the hybrid. Similarly, the fusion of diploid female and haploid male nuclei can be used to produce triploids, and the suppression of the second meiosis division after an interspecific cross can produce hybrid triploids. Tetraploids can be produced by blocking the first embryonic mitosis. Tetraploid lines can then be mated with diploids to create new triploids, or with other tetraploid lines to create novel tetraploids. Continuous commercial production of triploids (usually sterile) is usefully achieved by mating a given tetraploid and diploid lines, rather than by the inhibition of meiosis by

physical or chemical shocks which only provides a proportion of triploid zygotes from the fertilised eggs treated.

Interest in triploids by the aquaculture industry has been stimulated by the fact triploids have retarded gonad development, and concommitant enhanced growth. An additional advantage of their lack of reproduction is that escapes from culture are unlikely to breed and become a self-sustaining environmental problem. The disadvantage is the continuing need to produce triploid individuals.

Again the difficulty in controlling crustacean reproduction has resulted in few attempts to manipulate chromosome sets in this group. The only published report concerns the production of polyploids of the prawn *Sicyonia ingentis* reared to early larval stages (Xiang *et al.*, 1990).

Triploids have been produced in a number of molluscs including oysters (*Crassostrea*), soft-shell clams (*Mya*), and scallops (*Argopecten*) (Allen, 1987a), manila clam (*Tapes*) (Gosling and Nolan, 1990; Beaumont and Contaris, 1990), and pearl oyster (*Pinctada*) (Wada *et al.*, 1990). Gonadal formation was reduced, while growth rates and glycogen reserves were enhanced, in triploid *Mya arenaria* and *Crassostrea gigas* (Allen, 1987b). In *C. gigas*, the commercial production of triploids has been successful. Although superior growth rates were observed in other triploids, the success rate in inducing triploidy was low, and the technique is still largely in experimental stages.

Sex reversal

The principal genetic goal of sex reversal is to produce clonal lines through mating an individual with itself. Applications to aquaculture also include improvements in production for organisms in which the sexes differ markedly in size or growth rates by restricting production to the growing sex. Similarly, in cases of strong social interaction by one sex that either restricts the number of animals that can be held, or reduces growth rates as a result of aggressive interactions, rearing the alternative sex would be more productive. Malecha *et al.* (1988) produced reproductively competent phenotypic males from sex-reversed genetic females of freshwater prawns *Macrobrachium*. No reports of efforts in this area were found for molluscs.

OVERVIEW

The previous four sections have dealt with areas of genetics that are separated by the techniques employed, and in orientation of research questions. Each has a role to play in a general industrial strategy aimed at genetic improvement.

Molecular methodologies allow individual genes to be identified and transferred from one species to another. However, application to many commercial problems demands a greater knowledgs of the genetic background of the lines into which genes are introduced, and will depend to a greater degree on quantitative genetic methods for line testing and maintenance, than has been recognised to date. Whole chromosome techniques offer the opportunity to establish isogenic lines instantaneously, with the immediate purging of lethal genes, whereas quantitative methods of producing inbred lines take several generations, are never truly isogenic, often loose rare alleles in the process, and show the effects of lethal or deleterious genes after some time. However, many such clones need to be produced to obtain the few of commercial value, and these are identified by a combination of inter-line selection programs, and subsequent inter-line crosses using the methods of quantitative genetics.

Although quantitative breeding programs do not offer the possibilities of instantaneous success, they are also less prone to an all or nothing, high risk result. Small, and usually consistent, improvements are the norm with significant improvements in production over several generations. Once achieved, genetically improved strains derived from breeding programs offer a more sound foundation within which to place engineered genes. Finally, it is important to monitor the results of any breeding program, and to obtain information cheaply and reliably on the genetic resources that could be utilised in first setting up broodstock populations, and it is here that the methods of population genetics come into play.

AQUACULTURE COMPARED WITH AGRICULTURE

A comparison of the research carried out on molluscs and crustaceans with animals and plants of importance to agriculture shows clearly how little has been attempted in mollusc and crustacean genetics (Table 5). An inability to assess

Table 5
The current state of knowledge (number of papers) of genetics in molluscan and crustacean aquaculture and the general development of aquaculture relative to agriculture

	Aquaculture (Molluscs, Crustaceans)	Agriculture
Population genetic studies	several 10's	100's
Quantitative genetic studies	10's	100's-1,000's
Molecular transfers/ Chromosome manipulation	<10	100's
Husbandry techniques	early development	advanced
Reproductive control	limited to non-existent	advanced

response to selection, or the likelihood of producing commercially useful clones results from the lack of basic information for molluscs and crustaceans, rather than the inapplicability of genetic methods to these groups. Even a cursory examination of the history of agriculture clearly demonstrates the practical and commercial advances in productivity and marketable quality achieved by genetic means.

General trends in animal and plant production began with a move from hunting and gathering to limited forms of husbandry that gradually intensified over hundreds of years (Fig. 3). Much of the

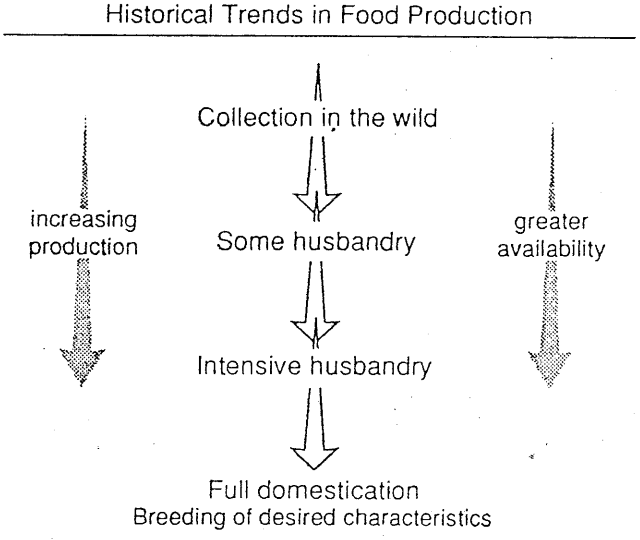

Fig. 3. The general patterns of increase in productivity in agriculture with first some husbandry, intensification of cultivation and finally full domestication.

improvements over recent decades are the result of genetic improvement through selective breeding programs. Aquaculture now lies between limited husbandry and the first attempts at more intensive methods. The stage is equivalent to that for cereal grains several millenia prior to the ancient Egyptians. Although providing a daunting vision of the task in hand, recognition of this fact should assist the development of general strategies to bring aquatic species into culture, and emphasise the shortened time scales in which present developments in aquaculture have been achieved.

The advances made in domesticated species have resulted from the application of a combination of genetic techniques. For example in cereal crops, many useful strains were developed by whole chromosome set manipulations followed by more sophisticated manipulation of single chromosomes through constructing a number of lines. However, the few commercially successful strains were not developed through the formation of chromosomal lines alone. Many thousands of such clones, or lines, were produced and tested in a range of environments, and subsequently crossed and selected. The end result was achieved, therefore, through a combination of major genome reorganisation combined with inter-line and quantitative breeding programs, Similarly, of the well-known domesticated animals, there are no transgenic populations in commercial production, despite the considerable knowledge of mammalian systems and the availability of lines of known genetic background within which to place well-characterised genes.

It is possible that gene transfers and the formation of clones or triploids will, in particular cases, produce instant commercial successes. However, a sound strategic approach for the aquaculture industry demands development of a broad base of genetics research and quantitative breeding programs. Selective breeding will produce, slowly but surely, improvements to production and a suitable framework within which to introduce the benefits that will undoubtedly emerge from molecular work.

RELATIONSHIP OF GENETICS AND REPRODUCTIVE BIOLOGY

It is against the framework outlined above that the relationship of genetics and reproductive biology

must be viewed. As with genetics, knowledge of reproductive biology of many aquaculture organisms, and almost all molluscs and crustaceans of importance to aquaculture, is limited. Knowledge of the molecular and biochemical basis for the control of reproduction of these species is limited although some recent advances have been made (Laufer and Downer, 1988; Geraerts *et al.*, 1991; Joosee *et al.*, 1991; Van Herp and Payen, 1991).

Many research tools remain to be developed. For example, cell lines have not been developed for molluscs or crustaceans. Lines of genetically identical individuals that would remove variation complicating the interpretation of experimental results do not exist. Identification and isolation of the biochemical factors mediating physiological change can be rapidly achieved by the isolation and characterisation of the DNA responsible for their production, rather than the peptides themselves. It is in this area of reproductive research that the methods of genetic engineering will prove particularly useful in the short-term.

Knowledge of the mechanisms controlling reproduction will improve the capability of industry to manipulate reproductive processes. However, many species of interest to aquaculture do not reproduce in captivity at all, or with great difficulty, and general research on the environmental conditions and husbandry techniques necessary for given species requires attention as much as the more sophisticated approaches. Nevertheless, genetics provides a source of research methodologies of considerable utility to reproductive biology. Increasing knowledge, and control, of reproduction will broaden the scope for applications of genetics to the

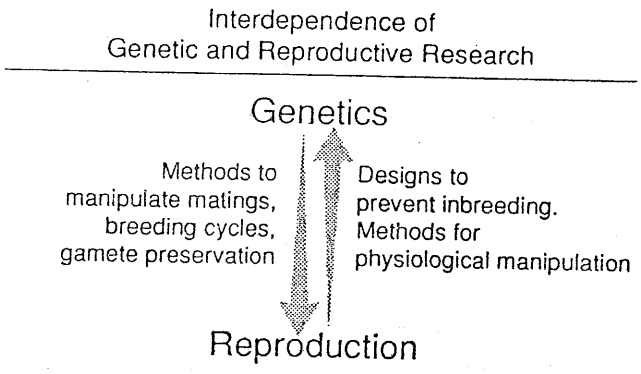

Fig. 4. The interdependence of genetics and reproductive research.

aquaculture industry.

The inter-relationship of genetics and reproductive biology is not simply the mutual provision of research tools (Fig. 4). If the goal of a research program in reproductive biology is to improve commercial production, it implies that research findings are implemented effectively. As has been discussed in the present paper, reproduction in culture will not necessarily improve production, and will certainly not optimise production, if appropriate genetic considerations are not an integral part of the broodstock management. Equally, the success of a research program on reproductive biology will lead to a greater number of taxa being brought into culture once the barriers to their reproduction in artificial environments are overcome. Environmental sensitivity to releases of cultured organisms, commercial considerations concerning the acquisition of the best strains, and the long-term need to protect wild genetic resources upon which the continued improvement of culture populations may depend, all demand early attention to the survey and documentation of these resources. Both these goals are best achieved by a close association of genetics and reproductive research. The need for a broad-based and integrated approach to genetics and reproductive research is displayed nowhere more clearly than in the molluscs and crustaceans, which, in terms of monetary value and tonnage include some of the most important aquaculture species in the world.

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