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Genetic Variability and Selective Breeding for Traits of Aquacultural Interest in the Pacific Oyster *(Crassostrea gigas)*

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The most significant genetic improvement for production of Pacific oyster *(Crassostrea gigas)* has been obtained through the breeding of triploids, especially since the development of tetraploids. Quantitative genetics studies suggest that significant gains, for disease resistance or for other traits of aquacultural interest, could be obtained using this approach. However, the limited extent of hatchery-propagation (versus natural recruitment) and/or various technical difficulties and biological characteristics of the species have slowed the development of selective breeding programs. Recently, in the USA, Australia and New Zealand, family-based selective breeding programs have been initiated to improve growth and yield. In Europe, where both natural and hatchery-propagated spat are farmed, no large-scale selective breeding programs have been initiated. However, special attention has been paid to "summer mortalities", for which the causal factors are still unclear. Our studies have shown that family-based selective breeding can improve spat survival, with no impact on growth. However, a genetic trade-off between survival and reproductive allocation was shown in adults, but was influenced by environmental variation. This might explain how additive genetic variance for fitness-related traits is maintained in wild populations. Practical difficulties in breeding large numbers of families are a major constraint for family-based selective breeding in oysters. Genetic variability exists for several larval traits, which increases the imbalance in reproductive success between breeders in hatchery-propagated populations. Multiplexed-microsatellite markers can be efficiently used to trace parentage in mixed-family breeding programs. Finally, a new means of introgression of traits of interest from genetically improved diploids to polyploids will allow the combination of selective breeding and polyploidization.

"Aquacultural production of the Pacific oyster *Crassostrea gigas* is of increasing economic importance, but little genetic improvement has been made on this species . . ."

Introduction

Aquacultural production of the Pacific oyster *Crassostrea gigas* is of increasing economic importance, but little genetic improvement has been made on this species to date (see Sheridan[1] for review). In many countries, wild spat is collected and raised, which offers little or no possibility of controlled genetic improvement, although natural selection might contribute to the adaptation of stocks to local environmental conditions. This effect could be more pronounced in countries where

C. gigas was recently introduced for aquaculture and is now successfully established (e.g., Australia, France, New Zealand). However, such local adaptation is poorly documented, probably because of the high plasticity of oyster species and the difficulty of setting up comparative experiments between introduced and native stocks (but see Soletchnik et al.[2]). Furthermore, temporal and spatial variation, natural and aquaculture-induced gene flow and large effective population sizes (but see Li and Hedgecock[3]), are likely to make natural adaptation rather slow. Natural selection will primarily act on fitness-related traits, but it will not always work in favour of aquaculture. Trade-offs between growth, survival and reproduction can lead to shifts in resource allocation strategies from survival to reproductive effort and growth when resource abundance increases.[4] In such cases, natural selection would favour genotypes with low survival but high growth and reproductive allocation, and this might not be the best selective direction for aquaculture.

Triploidy as a "Single-Step" Genetic Improvement

The most significant genetic improvement for the production of Pacific oyster to date has been obtained through the breeding of triploids. Polyploid *C. gigas* have become increasingly important following findings that they grow quicker than their natural diploid counterparts, probably due to their high level of infertility.[5] This is a clear demonstration that traits which are counter-selected in the wild (such as infertility) can be beneficial for aquacultural production of oysters. The development of tetraploid oysters has eased the production of triploid oysters, now bred by crossing diploid females with tetraploid males.[6] Additionally, these "natural" triploids show better growth and survival than chemically induced triploids (see Eudeline[7]). However, triploidy is a "single-step" genetic improvement and further genetic improvement should be considered.

Field testing of the "Morest" families in the Marennes-Oléron Bay (France).

Family-based Selective Breeding: Present and Prospects

Many quantitative genetics studies suggest that significant heritable variation exists for traits of aquacultural interest in oysters, such as disease resistance or growth (for review see Dégremont[8]). Additionally, heterosis and the use of non-additive genetic variation have also been investigated[9] (see also http://hmsc.oregonstate.edu/projects/wrac/). Consequently, significant gains should be obtained by selective breeding in diploid oysters. However, the limited extent of hatchery-propagation (versus natural recruitment), some technical difficulties, and certain biological characteristics of the species, have retarded the development of selective breeding programs. Until recently, most hatcheries ei-

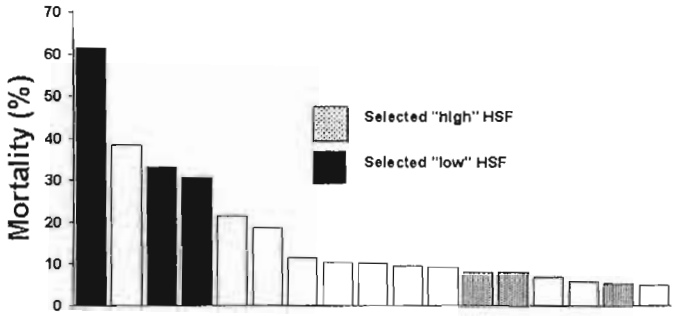


Figure 1
Variation of mean mortality among G1 half-sib families (HSF) and identification of the selected "high" and "low" families for the breeding of the second generation ($P < 0.001$).

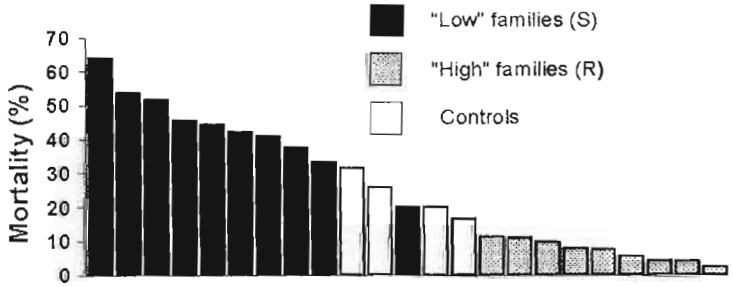
ther chose their breeding oysters from within commercial batches or else bred from offspring that were selected by mass selection, most often on weight and morphology. Recently, in the USA, Australia and New Zealand, countries where hatcheries are a major source of *C. gigas* juveniles, family-based selective breeding programs have been initiated to improve growth and yield [10,11] (see also http://hmsc.oregonstate.edu/projects/mbp/).

In Europe, where both natural and hatchery-propagated spat are farmed, no large-scale selective breeding programs have yet been initiated. However, special attention has been paid to the role of genetics in "summer mortalities", for which the causal factors are still unclear. A national multidisciplinary program, named "Morest" (see http://www.ifremer.fr/francais/defis/defi6/morest.ht_Hlt73855766m _Hlt735855766) and coordinated by IFREMER, was initiated in France to help understand the causes of the summer mortalities in *C. gigas* and to find possible solutions.

As a first step, 15 families (5 males crossed with 3 females each, following a nested half-sib design) of one-year-old oysters were studied under controlled (IFREMER's facilities in Bouin) and natural (farming site close to La Rochelle) rearing conditions, showing contrasting food availability. The aim was not only to estimate genetic variability of survival, growth and reproductive allocation, but also the genetic component of their plasticities, giving a highly informative combined data set.[4] Phenotypically, high food abundance led to lower survival but higher growth and reproductive effort. Heritability estimates were low for growth and its plasticity, but high for survival and plasticity of reproductive effort. Interestingly, estimates of genetic correlations between reproductive effort and survival changed sign according to food abundance (they were positive when food availability was high and negative when it was low). These results clearly illustrate that multi-trait approaches should be favoured to better understand genetic variation for resource allocation traits in oysters. Trade-offs and environmental influence on genetic correlations might explain why additive genetic variance for fitness-related traits is maintained in wild populations subjected to spatial and temporal variations. However, this also makes it difficult to predict correlated response to selection for a given trait.

The second step, in 2001, concerned 72 bi-parental families ("G1"), produced in 3 sets of 24 (for each set, 6 males were crossed with 4 females each, following a nested half-sib design).[8] Due to variable larval survival and settlement success, heritability estimation of spat survival in the field during the first summer was determined using only 17 half-sib families (corresponding to 43 full-sib families). Families were monitored at 3 sites (in Normandy: Baie des Veys, in Brittany: Rivière d'Auray, and on the Atlantic

Figure 2
Variation of mean mortality in the outbred G2 families (divergent selection) in Brittany ($P < 0.001$).



coast in the Marennes-Oléron bay: Ronce), previously known to show different survival rates for juvenile oysters. A variance component analysis revealed that among the studied factors, 45% of the observed variance was due to variation among families. The heritability estimate for survival during the first summer was remarkably high. To confirm this result and to assess whether selection could be efficient, a second generation ("G2") was produced in 2002 by breeding the best and worst G1 half-sib families (Fig. 1) in order to evaluate responses to divergent selection. Both within (inbred) and between (outbred) family crosses were performed using G1 oysters that were protected from field mortality pressures (i.e., selection was strictly at the family level). Results confirmed the high heritability of survival of spat over their first summer. "High-selected" families (named "R" for "resistant") showed much higher survival than "low-selected" ones ("S" for "sensitive"). For example, in Rivière d'Auray (Fig. 2), summer mortality of outbred G2 "S" oysters was 43%, while it was only 7% for "R" progenies (the unselected control was intermediate: 24%). Similar results were found in 2003, in the third generation ("G3"), which replicated the outbred G2s without making any further selection. In Rivière d'Auray, summer mortality of G3 "S" oysters was 73%, while it was only 27% for "R" progenies (the unselected control was intermediate: 48%). In 2003, triploid progenies were also produced by crossing unselected tetraploid males with G2 "R" or "S" diploid females. Triploid "S" showed a higher mortality (58%) than triploid "R" progenies (36%), while the triploid control was intermediate (50%). Interestingly, no correlated response was observed between growth and survival in spat. Additionally, survival over the second summer was similar for "R" and "S" G1 and G2 progenies in Ronce and Rivière d'Auray, indicating that the first summer is the critical sensitive period. However this remains to be confirmed in Normandy, where mortalities mostly affect adult oysters.

These families are currently being studied to investigate their genetic, physiological and immunological characteristics further, as part of the multidisciplinary research project "Morest". Special attention is being paid to reproduction and resource allocation, which are not as easy to study on spat compared with larger oysters. Consequently, molecular approaches are being developed.[12]

Mixed-Family Approaches: Feasibility and Constraints

These results and others using similar approaches are encouraging and promising for the development of selective breeding in oysters. However, practical difficulties in breeding large numbers of families are a major constraint for family-based selective breeding in oysters. Mass selection can be considered as a more practical approach (e.g., Naciri-Graven et al.,[13] Nell et al.[14]), but genetic variability of the selected populations should be carefully monitored to ensure long-term improvement and limit inbreeding. Indeed, many studies have demonstrated that hatchery-propagated bivalve stocks have low effective population sizes,[15] which is commonly due to having a limited number of (highly fertile) parents (e.g., Launey et al.[16]). This can be easily overcome in species where reproduction and crossing are fully controlled. However, other aspects, such as density effects, are not so easy to control.

Our studies have shown that genetic and non genetic components exist for several larval traits, which increases the imbalance in reproductive success between breeders in hatchery-propagated populations at early stages. Firstly, sperm competition at fertilization tended to increase the imbalance in reproductive success between males.[17] Consequently, separate pair mating prior to common larval

rearing should be favoured to maximise effective population size at early stages. Secondly, a family-based study allowed the estimation of breeding value and genetic correlations for several early life-history traits.[18] Larval survival, development rate, size and success at settlement showed significant heritable variation. Negative genetic correlations ("trade-offs") were observed between development rate and success at settlement. Consequently, common practices in oyster hatcheries, such as selective sieving during larval rearing and at settlement (i.e., discarding the smallest and/or slow developing larvae) are likely to be significant selective pressures in hatchery-propagated stocks.

As individual tagging is impossible at early stages of life, marker-based parentage analysis of mixed families is likely to ease the evaluation of such phenomena. In order to reduce costs associated with genotyping, we have developed a set of multiplexed-microsatellite markers[19] that has been used to monitor the effect of sieving (i.e., selection of fast growing larvae) on the genetic composition of a hatchery-bred population. As expected, selective sieving of fast growing larvae leads to earlier and less variable time to settlement. More interestingly, this also leads to increased imbalance in reproductive success and, consequently, to reduced effective population size.[19] Similarly, effects of environmental conditions (e.g., temperature and food availability) during larval development are currently being investigated.

Such marker-assisted parentage analysis could also be of great use in mass selection breeding programs, in order to optimise management of the genetic resources by the minimising inbreeding or to evaluate genetic parameters on the progeny reared in a common environment.[20,21]

From Selected Diploids to Selected Triploids?

It is likely that triploidy will remain a major and expanding genetic improvement for oyster farming in the future. However, the incorporation of polyploids makes selective breeding programs more complex and slower compared to diploids. Therefore the use of polyploids might delay their contribution to the sustainability and the development of oyster farming. Furthermore, selection on tetraploids is also restricted because strict quarantine conditions must be observed for their culture in some countries. It is therefore unlikely that selective breeding of tetraploid oysters will efficiently contribute to the development of selected stocks in the near future.

10-month-old oysters from selected families

Improved 4n lines would, however, be an important step towards the production of genetically improved triploids. It is therefore necessary to have a viable means of generating tetraploids de novo from improved 2n stocks or, alternatively of integrating genetic material from 2n animals into existing 4n oyster lines. Using eggs from 3n females relies on the fertility of these maternal genitors and the quality of their

gametes. The character of fertility in 3n animals is not desirable in aquaculture and the deliberate selection of fertile 3n to supply parents for 4n stocks may represent a problem of fertility in subsequent 3n progeny of the 4n thus produced. Indeed there are already indications that the characteristic of high fertility can be passed from triploid to tetraploid.[6] An alternative means of generating tetraploids in bivalve mollusks is directly from diploids by inhibiting the expulsion of both polar bodies.[22] The use of diploids would allow animals grown and selected under normal culture conditions to be converted directly into tetraploids. However, direct induction from diploids has generally shown low percentages of tetraploids produced per cross and low survival.[22,23]

Another means of exploiting genetic characters from diploids in tetraploid lines would be the introgression of this material into 4n families using a recently developed technique.[24] This method produces tetraploid *C. gigas* by cytochalasin B inhibition of polar body 2 expulsion in diploid females crossed with tetraploid males. This offers a means of introgression of genetic characters directly from selected diploid to tetraploid lines, avoiding a triploid step. Viable tetraploids were found at 4 and 6 mo using this method, indicating that the technique is successful.

"Improved 4n lines would . . . be an important step towards the production of genetically improved triploids."

Conclusion

Multiple directions exist for progress in genetic improvement of oyster stocks, be these via family or group selection. Molecular tools, which will be developed alongside future selection programs, represent a manner of continuously monitoring the progenies issued from any chosen path of selection and can therefore represent an aid in decision making. In practical terms, diploid oysters are the simplest to work with, even if the final objective is integration of traits of choice into commercialised polyploids. Future work should also include an evaluation of the relevance of selection on diploid progenitors relative to performance in future polyploid offspring. The choice of traits for improvement and manner and age of selection in any program should be made in the light of knowledge about the correlation structure of life history and resource allocation traits that has recently been found.

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"The choice of traits for improvement and manner and age of selection in any program should be made in the light of knowledge about the correlation structure of life history and resource allocation traits . . . "

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