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Genetic improvement and selection in shellfish: A review based on oyster research and production

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SUMMARY - Despite the economic importance of shellfish, genetic improvement has not yet had a great impact on these species. Shellfish farming is traditionally based on wild species whose natural populations are often overexploited and do not fulfil market demand. Up to now, the most effective answer to disease problems or for the improvement of productivity has been the introduction of new species. However, the introduction of new species is constrained by their ecological impact and is also limited by the availability of suitable species. Genetic improvement of local species should therefore be of great importance as a viable alternative for the long term sustainability of the shellfish industry. The different approaches to genetic improvement include polyploidization, hybridization and selection. Polyploidization has the most potential for immediate application to commercial breeding as triploid induction has already been achieved for many shellfish species. Comparative performance of triploids versus diploids has been studied in detail in oysters: triploids show better growth performances due to a reduced reproductive output and reduced energy requirement associated with greater heterozygosity. Hybridizations among species can also be a source of novelty, but interspecific hybrids often show reduced performance compared to their parents. Experimental breeding programmes for disease resistance or growth performance show that heritable variation exists for these traits and that genetic improvement can be achieved by selection. At present, these breeding programmes still have to reach a scale enabling proper genetic diversity management, multi-site testing or effective improvement of the commercial stocks. Public research institutes, commercial bivalve hatcheries and shellfish farmers should cooperate to adopt selective breeding programmes as an integral part of the husbandry of shellfish species.

Key words: Oyster, introduction, polyploidy, hybridization, selective breeding.

RESUME - "Amélioration génétique et sélection chez les mollusques : Une revue basée sur la recherche et les productions ostréicoles". Malgré leur importance économique, l'amélioration génétique des espèces conchylicoles n'a encore eu que peu d'impact. La conchyliculture est traditionnellement basée sur l'exploitation d'espèces sauvages, dont les populations naturelles sont souvent surexploitées et ne parviennent pas toujours à satisfaire les demandes du marché. Jusqu'à présent, l'introduction de nouvelles espèces a été le moyen le plus efficace pour contrer les problèmes de maladies ou pour augmenter la productivité. Mais ces introductions sont limitées par leurs impacts écologiques et la faible disponibilité en espèces bien adaptées. L'amélioration génétique des espèces locales représente donc une composante importante de la pérennité des productions conchylicoles. Polyploïdisation, hybridation et sélection sont trois approches possible pour l'amélioration génétique. La polyplôidisation est la plus immédiatement applicable, car les méthodes d'obtention de triploïdes sont désormais disponibles chez de nombreuses espèces. Les performances comparées d'huîtres diploïdes et triploïdes ont été étudiées en détail : les triploïdes présentent une croissance supérieure, due à un effort reproducteur réduit et à un meilleur bilan énergétique. Les hybridations entre espèces sont une source possible de nouveauté biologique, mais les hybrides inter-spécifiques montrent souvent des performances moins bonnes que leurs parents. Des programmes expérimentaux de sélection, pour la résistance aux maladies ou pour les performances de croissance, montrent qu'il existe de la variabilité génétique pour ces caractères et qu'ils pourraient être améliorés par sélection. A ce jour, ces programmes de sélection n'ont pas encore atteint l'ampleur qui permettrait une bonne gestion de la variabilité génétique, des tests multi-sites, et un impact significatif sur les productions commerciales. Les instituts publics de recherche, les

éclosoirs et les producteurs doivent coopérer pour que l'amélioration génétique deviennent partie intégrante de la gestion des cheptels des espèces conchylicoles.

Mots-clés : Huîtres, introduction, polyploidie, hybridation, sélection.

Introduction

Shellfish farming is of major importance in marine aquaculture (Table 1). Despite this economic importance, genetic improvement has not yet had a great impact on shellfish species (except in localized areas, e.g., in parts of North America). Traditionally shellfish farming is based on wild species whose natural populations are often overexploited and do not fulfil market demand.

In order to counter disease problems or to improve productivity, different approaches may be followed, principally:

(i) Introduction of new species. Suitable species need to be identified and their ecological impact should be carefully assessed.

(ii) Development of polyploids. The performance of triploids needs to be compared with that of diploid siblings.

(iii) Hybridization between species. Some between-species crosses are reported, but few data are available on their performance or fertility.

(iv) Selection. Heritable variation must be demonstrated for traits such as disease resistance or growth performance.

In this paper, we present a review of these different approaches in shellfish, and develop examples based on oyster research and production.

Introduction of new species

The introduction of new species for bivalve shellfish aquaculture has occurred frequently in the past. The best-known cases of introduction and implantation of new populations of molluscs are relatively recent, taking place this century. The most significant introductions have been reviewed by Chew (1990) and Grizel (1996). Among them, the Pacific oyster (*Crassostrea gigas*) is well known throughout the world (Lannan, 1972) because of its adaptability to a wide range of environments and its fast-growing ability. Originating from Japan, it is now of great importance on the Pacific coast of North America (major imports of seed occurred from 1920 to 1930, and again after World War II), in Australia, New Zealand and Europe (Grizel and Héral, 1991). In Europe, the Pacific oyster (*Crassostrea gigas*) with the Manila clam (*Ruditapes philippinarum*) are the most significant examples of introduced exotic species (Table 2).

Table 1. World aquaculture production of major marine bivalve species (world production > 5000 mt) (Source FAO, 1996)

Species	World production in 1994 (mt)	Main countries of production in 1994 (> 2000 mt)
Mussel	<i>Mytilus crassitesta</i>	Korea Rep.
	<i>Mytilus chilensis</i>	Chile
	<i>Mytilus edulis</i>	Canada, France, Germany, Ireland, Netherlands, Spain, Sweden, United-Kingdom
	<i>Mytilus smaragdinus</i>	Philippines, Thailand
	<i>Mytilus galloprovincialis</i>	France, Greece, Italy, South Africa
	<i>Mytilus canaliculus</i>	New Zealand
Oyster	<i>Ostrea edulis</i>	Spain, France
	<i>Crassostrea gigas</i>	Australia, Canada, China, France, Japan, Korea Rep., New Zealand, USA.
	<i>Crassostrea virginica</i>	Canada, Mexico, U.S.A.
	<i>Saccostrea commercialis</i>	Australia
	<i>Crassostrea iredalei</i>	Philippines
Scallop	<i>Pecten yessoensis</i>	China, Japan
	<i>Argopecten purpuratus</i>	Chile
Clam	<i>Arca spp</i>	Korea Rep
	<i>Anadara granosa</i>	China, Korea Rep, Malaysia, Thailand
	<i>Ruditapes decussatus</i>	Spain, Portugal
	<i>Ruditapes philippinarum</i>	China, Korea Rep., USA
	<i>Mercenaria mercenaria</i>	Asia, USA

Table 2. Marine bivalve introductions which had a significant impact on aquaculture

Species		Origin	Country
Oysters	<i>Crassostrea gigas</i>	Japan	North America: 1912 (voluntary) Australia: 1950 (voluntary) France: 1971 (voluntary) New Zealand: 1971 (unintentional)
	<i>Ostrea edulis</i>	Europe	North America: 1949 (voluntary)
Scallops	<i>Argopecten irradians</i>	USA	China: 1981 (voluntary)
Clams	<i>Ruditapes philippinarum</i>	Japan	France: 1973 (voluntary)

C. angulata and *C. gigas*: An example of two introductions of an alien species into Europe

Crassostrea angulata, known as the Portuguese oyster, was introduced into France from Portuguese populations in 1868, and became a major species for the oyster industry in Europe (cultivated mainly along the French Atlantic Coast) until the 1970's. From 1967 to 1972, Portuguese oysters became affected by a viral disease. In 1970 a decision was taken to conduct a massive introduction of *Crassostrea gigas*, in order to counter the crisis and sustain the industry with the substitute species. The oysters were brought from British Columbia (Canada) and Japan. Their implantation was a success and local oyster industry quickly recovered (Grizel and Héral, 1991). Populations presumed to be *Crassostrea angulata* have remained only along the southern coasts of Portugal and Spain. These populations could be of interest in terms of genetic resources and the basis of their relatedness with *C. gigas* should be investigated to provide a firm background for exploitation of any useful characters.

The presence of these two conspecific taxa on opposite sides of the world, *C. gigas* in Japan and *C. angulata* in Portugal, poses the question of their true origin. The restriction-fragment length polymorphism of PCR-amplified mitochondrial DNA fragments was used to examine genetic differentiation between Portuguese oyster (*Crassostrea angulata*) and Pacific oyster (*Crassostrea gigas*) populations. Samples were collected from populations identified as *C. gigas* and from populations located in southern Portugal and Spain, identified as *C. angulata*. Among the 6 observed haplotypes, one was commonly observed in populations identified as *C. gigas* while another one was most frequent in populations identified as *C. angulata*. The analysis of haplotype diversity among populations showed that samples from southern Portugal and Spain cluster with the Taiwanese sample (Fig. 1). These results indicate Taiwan as the possible origin of European *C. angulata* populations. The ability to differentiate between these two previously indistinguishable taxa allowed us to identify a mixed population in Portugal. These results open new perspectives for the study of characters previously described as varying between *C. gigas* and *C. angulata*, such as growth performances and disease susceptibility.

Polyploidization

The induction of triploidy has been reported in mussels *Mytilus galloprovincialis* (Kiyomoto *et al.*, 1996), *M. edulis* (Yamamoto and Sugawara, 1988), *M. chilensis* (Toro and Sastre, 1995), oysters *Ostrea edulis* (Gendreau and Grizel, 1990; Hawkins *et al.*, 1994), *Saccostrea commercialis* (Nell *et al.*, 1994), *Crassostrea gigas* (Allen and Bushek, 1992; Allen *et al.*, 1986; Desrosiers *et al.*, 1993), *C. virginica* (Barber *et al.*, 1992), pearl oysters *Pinctada fucata* (Durand *et al.*, 1990), *P. martensii* (Jiang *et al.*, 1993), scallops *Pecten maximus* and clams *Tapes dorsatus* (Nell *et al.*, 1995), *Tapes philippinarum* (Utting and Child, 1994), *Mercenaria mercenaria*. Triploidy is generally induced in fertilised eggs at meiosis I or meiosis II by the suppression of polar body I or polar body II formation. The most frequently used method is based on treatment of fertilised eggs using cytochalasin B. Other protocols are based on pressure shock, heat shock, and chemical treatment using 6-dimethylaminopurine (6-DMAP) (Desrosiers *et al.*, 1993). An alternative method is based on the mating of tetraploids and diploids to obtain all-triploid stocks (Guo *et al.*, 1996). Tetraploids have been obtained in *C. gigas* (Guo and Allen, 1995; Guo *et al.*, 1994) and *M. galloprovincialis* (Komaru *et al.*, 1995; Scarpa *et al.*, 1993).

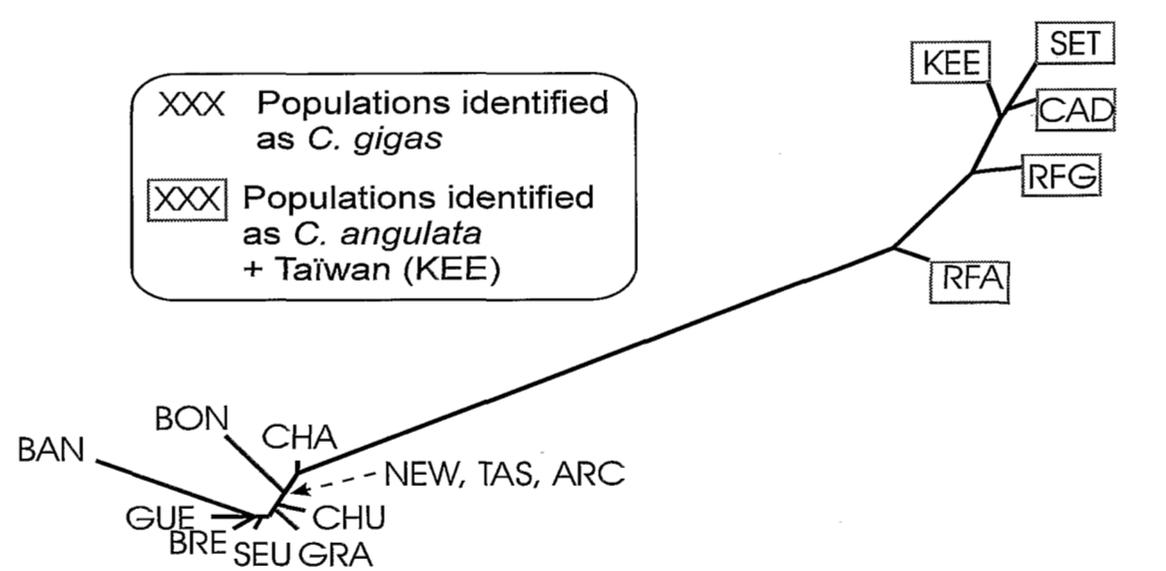


Fig. 1. Neighbour-joining tree summarizing the genetic relationships among populations, based on the nucleotide divergence matrix for mtDNA PCR-RFLP analysis among 16 cupped oyster populations (total number of oysters studied = 253). 6 Haplotypes were observed for the cytochrome oxidase C subunit I gene digested with four polymorphic endonucleases.

The higher performance of triploids over diploids can be explained by two factors:

(i) Triploidy induces sterility or reduced gametogenesis. The energy normally allocated to reproduction can then be reallocated to growth. Consequently, reserves are accumulated as glycogen and are not transformed into unpalatable lipids (e.g., in

Saccostrea commercialis: Cox *et al.*, 1996, in *Mytilus galloprovincialis*: Kiyomoto *et al.*, 1996, in *Mercenaria mercenaria*: Eversole *et al.*, 1996, in *Crassostrea gigas*: Gouilletquer *et al.*, 1996).

(ii) Triploidy potentially induces higher mean heterozygosity, a character which has been shown to be positively correlated with growth in many shellfish species (for a review see Zouros, 1987, but also Britten, 1996). Indeed, in *Ostrea edulis*, Hawkins *et al.*, (1994) report faster growth in meiosis I triploids than in meiosis II triploids or in their diploid siblings.

Comparative performance of triploid and diploid *C. gigas*: A multi-site field experiment

A multi-site comparative study of performance of triploid and diploid oysters was initiated by IFREMER in 1992. Triploid and diploid sibling oysters were produced in the hatchery in 1992. The triploidy level in the triploid stock was above 90%, as estimated by image analysis (Gérard *et al.*, 1994). These oysters were tested in 4 French sites from spring 1993 to late autumn 1994. The main results of the multi-site field experiment are summarized in Table 3 (Gérard, 1994; Gérard *et al.*, in prep). The mean individual total weight was significantly higher in triploids compared with diploids in all sites. There was a clear north-to-south cline for the difference in total weight from Port-en-Bessin in the North through Marennes-Oléron and Arcachon to Thau Lagoon in the South. This result could be due to a positive correlation between summer temperature and reproductive effort in this species. The limited gametogenesis in triploid versus diploid oysters leads to a higher glycogen content and lower lipid content during the summer period. These characteristics give triploid oysters a much better quality of flavour in summer.

Hybridization

Studies on between-species hybridization fall into two groups:

(i) Hybridization between species with adjacent natural geographic distributions. In this case, most of the studies deal with the maintenance of hybrid zones (e.g., quantification of gene flow and fitness of hybrids). The best documented cases concern hybridization of *Mytilus edulis* x *M. galloprovincialis* (Hybrid zone: France) (Coustau *et al.*, 1991; Gardner, 1995; Gardner, 1994; Sanjuan *et al.*, 1994) and *Mercenaria mercenaria* x *M. campechiensis* (Hybrid zone: Florida, USA) (Bert *et al.*, 1993, Arnold *et al.*, 1996).

(ii) Hybridization between closely related species following introduction to new geographic areas, or experimental crosses: these cases are poorly documented, mainly due to the lack of suitable genetic markers. When such markers exist, they provide tools for assessing hybrid status (e.g., *C. gigas* x *C. virginica*: Allen *et al.*, 1993).

Table 3. Comparative performance of triploid versus diploid *C. gigas* in 4 French sites

	North		Sites				South	
	Port-en-Bessin		Marennes-Oléron		Arcachon		Thau lagoon	
	2N	3N	2N	3N	2N	3N	2N	3N
Number of oysters studied	1200	1200	1200	1200	1500	1500	750	750
mean individual total weight (g)	109	132	59	77	90	122	139	192
3N versus 2N individual total weight (%)	121.1%		130.5%		135.6%		138.1%	
Survival in the field (%)	90.0	79.0	71.9	76.7	74.4	68.0	94.8	95.4
Glycogen content (% of dry weight) (July 94)	/	/	0.5	8.4	/	/	0.64	5.4
Lipid content (% of dry weight) (July 94)	/	/	14.0	11.2	/	/	13.4	10.9

In cupped oysters, inter-specific hybridization has been reviewed by Gaffney and Allen (1993). Table 4 presents the present knowledge about hybridization ability among *Crassostrea* species. There is little information about the performance or fertility of these hybrids, but in most cases, inter-specific hybrids show intermediate or reduced performance compared with their parents (Allen, pers. com.; personal observations). Some of these cross-compatible taxa are presently sympatric (e.g., *C. gigas* and *C. sikamea* along the west coast of the USA; *C. gigas* and *C. angulata* in southern Europe), which might already have led to spontaneous hybridization in the wild.

 Table 4. Hybridization among *Crassostrea* species (according to Gaffney and Allen, 1993; and personal results)

	<i>C. gigas</i>	<i>C. rivularis</i>	<i>C. angulata</i>	<i>C. sikamea</i>	<i>C. virginica</i>
<i>C. gigas</i>		FLH	FLH	FLH	FX
<i>C. rivularis</i>			FL?	FL?	FX
<i>C. angulata</i>				FLH	FX
<i>C. sikamea</i>					FX

F = fertilization; L = viable larvae; H = viable hybrids; X = result reported as negative

Selection

Many experimental breeding programs show that heritable variation exists for traits of interest, and that genetic improvement can be achieved by selection (Table 5 shows results obtained for oysters).

In most studies, the results are based on a single generation of selection, and the genetic variability in the selected populations is sometimes limited. Furthermore, in most studies, improper control of the environmental variation leads to reduced heritability values.

Table 5. Review of experimental breeding programs in oysters (* FS = full sib)

Authors	Species	Methods	Main results
(Lannan, 1972)	<i>C. gigas</i>	comparison of 11 FS* families 1 generation	Heritability for 14 traits related to growth (e.g., total weight at 18 mo, $h^2 = 0.37 \pm 0.2$)
(Beattie <i>et al.</i> , 1980)	<i>C. gigas</i>	comparison of 13 FS* families 1 generation	Progress in resistance to summer disease
(Toro and Newkirk, 1990)	<i>O. edulis</i>	Mass selection 1 generation	Realized heritability for live weight and shell length (e.g., at 18 mo, $h^2 = 0.19 \pm 0.07$)
(Toro and Newkirk, 1991)	<i>T. chilensis</i>	Mass selection 1 generation	Realised heritability for decreasing shell length: $h^2 = 0.34 \pm 0.12$
(Toro <i>et al.</i> , 1995)	<i>T. chilensis</i>	Mass selection 1 generation	Realized heritability for shell length and live weight (at 27 mo, $h^2 = 0.43 \pm 0.18$)
(Sheridan <i>et al.</i> , 1996)	<i>S. commercialis</i>	Mass selection 2 generations	Progress in testing methods
(Newkirk and Haley, 1982)	<i>O. edulis</i>	Mass selection	Progress in live weight at the first generation, lack of response to selection in the second generation due to loss of variability
(Newkirk and Haley, 1983)		2 generations	

Selection for bonamiosis resistance in the flat oyster *Ostrea edulis*

Flat oyster production has decreased drastically in Europe since the late 1970's following the accidental introduction of 2 parasites, *Bonamia ostreae* and *Martelia refringens* (Renault, 1996).

Natural selection has not yet lead to economically significant tolerance levels. This might be due to the fact that spontaneous infestation only induces heavy mortalities in 3 to 4-year old oysters. Because the farmers reduce losses by selling their stocks before the age of 3, and because in France the spat most probably comes from cultivated parents, the heavy infestations have not led to strong selection pressures. In 1985, a program was initiated to select for bonamiosis resistance based on: (i) the fact that bonamiosis is present in all production sites while marteliosis is only present in estuaries; and (ii) the ability to purify the *Bonamia* parasite and to infect oysters (not yet available for marteliosis), which enables the selection cycle to be shortened.

Mass-mating of a large number (>40) of individuals older than 3 years were performed in 1985 to produce G1 populations, on which mass-selection was applied. The oysters were grown for five years in a heavily *Bonamia*-infested area (Baie de Quiberon, Brittany, France), and parents for the following generation were selected from among the survivors. A second ("S89-G1-non inoc") and a third ("S89-G1-inoc") G1 populations were initiated in 1989 (S89-G1-inoc descending from a mass-mating of a group of *Bonamia*-inoculated parents). Table 6 presents the main characteristics of 3 populations of flat oysters under selection. Selected individuals from each population were crossed by pair in 1995 in order to generate 73 full-sib families.

Table 6. Main characteristics of 3 populations of flat oysters under selection

	Selected populations		
	Pop 85	Pop 89 non-inoc	Pop 89 inoc
Mass selection	cycle 1 (85-90) no inoculation		cycle 1 (1988) by inoculation
	cycle 2 (90-95) no inoculation	cycle 1 (89-95) no inoculation	cycle 2 (89-95) no inoculation
Combined individual and full-sib family selection	cycle 3 (95-98) by inoculation	cycle 2 (95-98) by inoculation	cycle 3 (95-98) by inoculation
Number of parents used in 1995	58	32	24

The level of susceptibility to *Bonamia* in the selected populations, and that of the crosses were tested both by natural infection in heavily infested areas, and by laboratory inoculations. The performances observed in the different trials could not be compared directly, because the survival in the wild was tested under different conditions and controls came from different sources. Hence realized heritabilities cannot yet be calculated. But all the trials do show higher survival of the selected populations compared with their respective controls (survival in the selected populations was 1.04 to 4.54 times higher than the control populations), as well as lower prevalence in the selected populations tested in the wild (Naciri *et al.*, in press). This indicates genetic diversity and genetic improvement for this trait.

As pointed out by Newkirk and Haley (1982), mass-mating in *Ostrea edulis* does not enable us to know the effective number of parents and the quantitative contribution of each. Recent microsatellite analyses (Naciri *et al.*, 1995) on the selected individuals used as parents for the full-sib families in 1995 show that the real number of founders of the three populations above can be estimated to be 15, 10 and 3 respectively (Launey *et al.*, in prep). In 1995, 97 full-sib families were produced, and the selection program was shifted to a combined within and between-families selection scheme. Structuring the population in full-sib families will enable the calculation of heritability estimates for several traits such as resistance and growth, as well as a proper management of the genetic drift and of inbreeding.

Conclusions

Past introductions of shellfish species have had a important economical impact. Their ecological impact on the local environment has not been assessed precisely, but the displacement of related local species of economic interest has been observed in several occasions (Coleman, 1986; Héral, 1986; Gouilletquer and Héral, 1997). Several other reasons can be listed that make introductions of exotic species of aquacultural interest less likely in the future:

(i) Growing environmental awareness.

(ii) Numerous examples of environmental and economic nuisance due to accidental introductions of wild marine pests (Gruet *et al.*, 1976; Coleman, 1986).

(iii) The risk of introducing exotic diseases with catastrophic consequences on local cultivated shellfish species, for example the parasite *Bonamia ostreae* transferred from the New Zealand flat oyster *Tiostrea chilensis* to the European flat oyster *Ostrea edulis* via introduced stocks of *Ostrea edulis* from the USA (Hine, 1996).

(iv) The introduction of the most interesting species in terms of growth potential (*Crassostrea gigas*, *Ruditapes philippinarum*) has already been done, either intentionally or accidentally. A potential still exists however for the introduction of fast-growing *Pectinidae* species, like *Argopecten irradians* or *Patinopecten yessoensis*, into Europe.

While their introduction into new cultivation areas is unlikely in the future, shellfish species related to cultivated species may still contribute genes of interest, if they are at least partially cross-compatible. This could be exploited in the future with the genus *Crassostrea*: *C. virginica* is susceptible to MSX while *C. gigas* is not (Allen *et al.*, 1993), *C. rivularis* (= *C. ariakensis*) is susceptible to bonamiosis while *C. gigas* is not (Renault *et al.*, 1995, Cochenec *et al.*, in press).

Protocols for triploidy induction were published in the 80's, and the superiority of triploids over diploids has been experimentally and economically verified in many cases, although triploids have had little economic importance in Europe to date compared with in the USA. This can probably be attributed to the lower market share of hatchery spat compared to natural spat in Europe. But the use of triploidy in

oysters is developing progressively. Further progress should be obtained in the future with the adoption of less toxic protocols using 6-DMAP instead of Cytochalasin-B (Desrosiers *et al.*, 1993), or with the development of tetraploid strains. As it has recently been shown that triploids can be produced by crossing diploids with tetraploids (Guo *et al.*, 1996), this new method will provide 100% triploid progenies without the use of toxic chemicals.

While the use of triploidy gives improvement with a single step, further progress can only be provided by selection. This aspect of genetic improvement is the least advanced scientifically or technically in shellfish for the moment. It is clear that breeding programs have not yet reached a scale enabling proper genetic diversity management, multi-site testing or effective improvement of the commercial stocks. They have remained experimental programs aimed at evaluating the possibility of selection for traits such as growth or disease resistance, but this selection in itself has not yet started. Articles reporting experimental adaptations of cultivation (Sheridan *et al.*, 1996) and breeding practices (Newkirk, 1986) for selection are few, as are articles on selection methods.

Only with the support of the shellfish-farming community will selection programs be able to get past the experimental stage and reach an economic scale, as in the USA, "Molluscan Broodstock Program" (Hedgecock *et al.*, 1997). This is far from being the case in Europe. Public research institutes, commercial bivalve hatcheries and shellfish farmers should cooperate to adopt selective breeding programs as an integral part of the husbandry of shellfish species.

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