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Genetic improvement for disease resistance in oysters: a review

Dégremont Lionel [1, *], Garcia Céline [1], Allen Standish K. [2]

[1] IFREMER, SG2M-LGPMM, Avenue de Mus de Loup, F-17390 La Tremblade, France
[2] Aquaculture Genetics & Breeding Technology Center, Virginia Institute of Marine Science, College of William & Mary, 1208 Greate Road, Gloucester Point VA23062-1346, USA

* Corresponding author : Lionel Dégremont, email address : ldegremo@ifremer.fr

Abstract :

Oyster species suffer from numerous disease outbreaks, often causing high mortality. Because the environment cannot be controlled, genetic improvement for disease resistance to pathogens is an attractive option to reduce their impact on oyster production. We review the literature on selective breeding programs for disease resistance in oyster species, and the impact of triploidy on such resistance. Significant response to selection to improve disease resistance was observed in all studies after two to four generations of selection for Haplosporidium nelsoni and Roseovarius crassostrea in Crassostrea virginica, OsHV-1 in Crassostrea gigas, and Martelia sydneyi in Saccostrea glomerata. Clearly, resistance in these cases was heritable, but most of the studies failed to provide estimates for heritability or genetic correlations with other traits, e.g., between resistance to one disease and another. Generally, it seems breeding for higher resistance to one disease does not confer higher resistance or susceptibility to another disease. For disease resistance in triploid oysters, several studies showed that triploidy confers neither advantage nor disadvantage in survival, e.g., OsHV-1 resistance in C. gigas. Other studies showed higher disease resistance of triploids over diploid as observed in C. virginica and S. glomerata. One indirect mechanism for triploids to avoid disease was to grow faster, thus limiting the span of time when oysters might be exposed to disease.

Graphical abstract

Disease-resistant line of *Crassostrea gigas* after mortality outbreak caused by OsHV-1

Unselected group of *Crassostrea gigas* after mortality outbreak caused by OsHV-1

Highlights

► Disease related mortality affects several oyster species. ► Positive response to selection to enhance disease resistance. ► Selection for disease resistance does not confer resistance to other disease. ► Triploidy in oysters does not alter disease resistance in comparison to diploidy.

Keywords : Disease resistance, Oyster, Selection, Pathogen, Genetic, Mortality

1. Introduction

Domestication is the adaptation of a population of plants or animals to rearing by humans through unconscious selection (Price, 1984). In contrast, genetic improvement is the intentional modification of plant or animal populations by humans to better adapt them to their needs (Gallais, 1990). The main goal of selection is then to exploit the genetic variation present for desirable qualities and to accumulate the best genes among individuals, which would be inherited in following generations (Gjedrem and Baranski, 2009).

In all species, plants or animals, selection is carried out on traits of high economic importance, mainly growth rate, yield, quality of the product, food conversion efficiency, and resistance to diseases (see Gjedrem, 1983 for review in aquaculture). Selection for disease resistance began very early in crop production whereas for livestock, selective breeding intensified only over the last few decades. Although some negative trade-offs between disease resistance and other economically important traits have been shown, selective breeding continues to be a useful tool to assist in disease control so long as one or more diseases exert a significant influence on livestock production (Stear et al., 2001).

World aquaculture production of fish and shellfish (molluscs and crustaceans) has doubled every decade since the 70's, reaching 67 million tons in 2012 (FAO, 2014). During this expansion, fish and shellfish movements have increased due to higher demand. Non-indigenous species have been introduced widely. Such transfers of live fish and shellfish between countries are known for spreading serious diseases with high economic losses in the industry (Hill, 2002). Striking examples include the appearance of viral hemorrhagic septicemia in European trout (Lorenzen and Olesen, 1997), gill disease in Portuguese oyster (Grizel and Héral, 1991), OsHV-1 oyster virus throughout Europe (EFSA, 2010), and the spread of white spot disease in shrimp farms in Asia (Flegel, 2012).

The threat of disease represents an important limiting factor for the development of aquaculture. By disease, we mean a pathological condition of a part, organ, or system of an organism resulting from various causes, such as infection, genetic defect, or environmental stress, and characterized by an identifiable group of signs or symptoms. Current methods to control or eradicate disease in fish aquaculture are mostly based on medication, vaccination, and disinfection of the facilities, as well as on genetic selection for disease resistance. The last approach offers economic, environmental and ethical advantages (Van Muiswinkel et al., 1999). In molluscs, especially oysters, the main sign of a disease is generally the observation of mortality. Due to their lack of immune memory and the open environment where they are farmed, it is impossible to vaccinate or disinfect. The only available tools to safeguard production are preventing the introduction of infected animals into disease-free environments or to limit pathogen spread, which relies on the surveillance of mollusc farms. But once introduced, the only ways to fight disease are changes in rearing practices or/and selection for resistant populations (Roch, 1999).

Oysters are well suited to selective breeding because of their economic importance, the ease of controlling their biological cycle, their high fecundity, and their high genetic variability (Gosling, 2003). Significant heritability estimates have been found for a wide number of traits in oysters, but most have focused on growth rate. Disease resistance and/or survival represent less than 10% of these estimates (Dégremont, 2003). Disease resistance used to be considered a difficult trait to improve by genetic selection based on low heritability estimates for some fish species (Gjedrem, 1985). But lately, numerous studies have reported moderate to high

heritability for disease resistance and/or survival in fish species due to improvements in challenge techniques (Gjerde et al., 2009; Henryon et al., 2005; Johnson et al., 2007; Lillehammer et al., 2013; Ødegård et al., 2007; Taylor et al., 2009), indicating that selective breeding to enhance disease resistance can be successful. In contrast, such estimates for disease resistance are absent for oyster species except for OsHV-1 µvar resistance in *C. gigas* (Dégremont et al., 2015ab). Numerous observations of genetic variation for survival in oyster studies have been unaccompanied by disease screening, therefore survival cannot attributed to disease resistance *per se* (Dégremont et al., 2010a; Dégremont et al., 2007; Evans and Langdon, 2006; Usuki, 2002; Ward et al., 2005).

Oyster populations, wild and cultured, have been affected over the last century by catastrophic mass mortalities, some of these of 'unknown origin,' but numerous cases have been ascribed to epizootics caused by infections agents (Sindermann, 1975). In some cases, the epizootic has caused an oyster industry to collapse and come to a standstill, such as for bonamiosis and marteliosis in *Ostrea edulis* in France (Héral, 1989). In other examples, such as, haplosporidiosis and perkinsosis in *C. virginica* in the USA (Andrews and Wood, 1967), the industry is recovering after a period of low productivity. Response to epizootics have sometimes been met with the decision to introduce non-native species, as with the introduction of *C. gigas* in France during the 70's following massive mortality of *C. angulata* from gill disease (Grizel and Héral, 1991). An alternate way to sustain or recover oyster production, as has happened in the Chesapeake Bay, USA (Frank-Lawale et al., 2014), would be selective breeding programs to enhance survival and/or disease resistance, and judicious use of polyploids. In this paper, we review the state of the art on selection programs for disease resistance in oysters, as well as the impact of triploidy on resistance to disease.

2. **Summer mortality and Ostreid Herpesvirus type 1 (OsHV-1) resistance in *C. gigas***

The Pacific oyster, *C. gigas*, is native to the northwest Pacific Ocean and has been introduced to numerous countries worldwide (Ruesink et al., 2005). Estimated production of *C. gigas* in 2012 was about 4.2 million tons (FAO, 2014), although a good part of that may be *C. angulata* in China (Wang et al., 2010). The Pacific oyster is therefore one of the most important species in aquaculture, and mortality regularly affects it.

Summer mortality could be considered one of the main disease threats. These mortalities were reported as early as 1915 in Japan (Takeuchi et al., 1960) and again in the late 1950s in USA (Glude, 1975) and during the early 1980s in France (Maurer et al., 1986). It affects both juveniles and adults, as well as both diploids and triploids (Cheney et al., 2000). Generally, these mortalities have not been explained by a single factor but rather by the combination of several parameters including the environment, rearing techniques, physiological condition, pathogens, and genetic predisposition (Dégremont et al., 2005). Pathogens have been associated with some cases of summer mortality (Elston et al., 1987a; Friedman et al., 1991; Lipovsky and Chew, 1972; Le Roux et al., 2002; Numachi et al., 1965), but in many other cases, no clear association was found (Cheney et al., 2000; Glude, 1975; Koganezawa, 1975; Samain & al., 2007).

The first studies on selective breeding to enhance survival during summer mortality were conducted on the west coast of the USA (Beattie et al., 1980). Their breeding program, realized for three generations, was initially based on challenging adult oysters to elevated temperature (21°C) in the laboratory, which induced high mortality in adult *C. gigas* (Lipovsky and Chew, 1972). The survivors were then spawned by pair mating, and their

offspring challenged with the same laboratory challenge of elevated temperatures. Most of the selected families showed higher survival than the control, indicating that selection was effective for improving resistance to thermal stress, one factor potentially involved with summer mortality. Families were next tested in several commercial grow-out beds with a history of summer mortality problems. Survival on commercial farms was then used as the selection criterion, rather than the laboratory-induced thermal stress. Most of the families of the first and second generations had higher survival than the control (Beattie et al., 1980; Perdue et al., 1981), while all selected families of the third generation showed a significant higher survival (81% on average) in comparison to the control (38%) (Table 1) (Hershberger et al., 1984). Genetic parameters were not estimated, but improvement was evident during summer mortality outbreaks on commercial beds. Additionally, even though the etiology of summer mortality was unknown (Glude, 1975), pathogens, likely some *Vibrio* spp. were believed to play a significant role in the laboratory mortality (Grischkowsky and Liston, 1974; Lipovsky and Chew, 1972) indicating that selection to improve survival during the heat challenges could have been related to resistance to the *Vibrio* spp.

Also on the west coast of USA, the Molluscan Breeding Program (MBP) demonstrated a significant response to selection to improve the yield of *C. gigas*, yield defined as the combined result of growth and survival (Langdon et al., 2003). Significant genetic variation for survival was observed in one-year old oysters after only 6 months deployment (Evans and Langdon, 2006). However, Evans and Langdon reported no disease data so it was impossible to know if higher survival was obtained from disease resistance. Two MBP selected lines -- for contrasting survival rates -- were then tested in Inner Tomales Bay in California. Significant differences in seed survival were observed among MBP lines with a gain of 25% for the line selected for higher survival for the spring outplant (Table 1) (Burge et al., 2007). Histological examination of oysters sampled during a mortality event in 2001 suggested herpesvirus infection. At the same site, similar results were confirmed in another study using several oyster stocks in 2003 (Burge et al., 2006). Taken together, these results suggested that survival of oysters during summer mortality episodes on the west coast of the USA was partly genetically determined and herpes-resistance may have been involved. No definitive link to the herpes virus, however, yet exists from these trials in the Pacific Northwest of the US.

In France, a large IFREMER ‘defi’ (challenge) project called “MORtality ESTival” (MOREST) was intended to better understand the summer mortality phenomenon in *C. gigas* through a multidisciplinary approach. Initiated in 2001, one of the main objectives of MOREST was to determine whether selective breeding could improve survival. Wild oysters from the Marennes-Oléron Bay were mated in a hierarchical mating design during winter of 2001, producing a first generation (G1) of full- and half-sib families. These families were tested during the summer and survival was recorded in the fall in 2001. Differences in survival among families revealed a significant and strong genetic basis for resistance in spat (Dégremont et al., 2007). To confirm these results, a second generation (G2) was produced using families showing low and high survival to summer mortality, respectively named ‘susceptible’ (S) or ‘resistant’ (R). The broodstock used to produce the G2 and subsequent generations were one year old and, more importantly, had been protected from the mortality that occurred in the field, to prevent the S oysters from obtaining resistance (Dégremont et al., 2010a). All generations (G2-G3-G4) were tested in the field, and there was a positive response to selection for increased or decreased survival of *C. gigas* spat to summer mortality in the G2, G3 and G4 generations under field conditions (Boudry et al., 2007; Dégremont et al., 2010a) (Table 1). In sites where significant summer mortality of *C. gigas* spat was observed, narrow sense heritabilities for survival ranged from 0.47 to 1.08, but without

confirmation of the etiological agent. Thus, these estimates were in agreement with those obtained on the West Coast of USA (h^2 broad sense at 6 months old = 0.49 – 0.71) (Evans and Langdon, 2006), in Japan (h^2 narrow sense at 1 year old = 0.77) (Usuki, 2002) and in Australia (h^2 at 21 months old = 0.68) (Ward et al., 2005), knowing that the broad sense heritability expresses the extent to which individuals' phenotypes are determined by the genotypes while the narrow sense heritability expresses the extent to which phenotypes are determined by the genes transmitted from the parents. The narrow sense heritability has the greatest importance in breeding programs (Falconer and Mackay, 1996).

For the work described above with tests of G2, G3 and G4 in the field, disease sampling during mortality outbreaks was absent. However, OsHV-1 was detected by PCR in moribund oysters for G1 in the field and for G2 in the nursery (unpublished data) (Dégremont, 2003). Additionally, several experiments were carried out in the laboratory using the G2 and G3. Family survival in the laboratory correlated well with family survival in the field, and OsHV-1 was detected by PCR in moribund oysters during the peak of mortality in the laboratory (Table 1)(Dégremont et al., 2010b). Altogether, the results obtained from 2001 to 2004 suggested that selective breeding on survival in *C. gigas* spat could be related to genetic variation for susceptibility to OsHV-1. Thus, pathogens could be one of the major causes of the summer mortality events in *C. gigas* with a strong implication of OsHV-1, as also identified by Burge et al. (2006), which was previously reported with another pathogen: *Vibrio splendidus* (Lacoste et al., 2001). Meanwhile, several resistant (R) and susceptible (S) families selected for their survival to the summer mortality phenomenon in G1 and G2 were also tested by experimental injection of virulent *Vibrio splendidus* strains. Low survival was observed for some families in G1 (Gay, 2004), and all families in G2 (Table 2) (unpublished data; Huvet et al., 2007), but these results did not correlate with the mortality in field or laboratory where R oysters showed much lower mortality than S oysters (Dégremont et al 2010b). This finding indicated (1) that even if *V. splendidus* can induce mortality in *C. gigas*, it was not the main cause of mortality observed in field or laboratory challenges for summer mortality and (2) resistance to summer mortality, which may be related to OsHV-1, did not confer resistance to *V. splendidus*.

Mortality related to OsHV-1 has been routinely observed in France as described in Garcia et al. (2011). Mean mortality of wild-caught spat tested in 14 sites in the Marennes-Oléron Bay was 45% between 1994 to 2007 (Mille et al., 2014). Since 2008, higher mortality rates have been reported in the Marennes-Oléron Bay, averaging 75% between 2008 and 2014 (Mille et al., 2014). Similar values were also obtained in hatchery-produced spat and wild-caught spat at other sites (Dégremont and Benabdelmouna, 2014; Pernet al., 2012). Outbreaks after 2008, associated with higher mortality, seem to be associated with a particular genotype called OsHV-1 µvar that has taken over as the predominant strain, while the OsHV-1 reference genotype has not been detected since 2008 (Martenot 2013; Ségarra et al., 2010). A recent study attested to the presence of OsHV-1 µvar on the Normandy coast (Manche, France) in 2004 and 2005, suggesting that its emergence did not occur suddenly (Martenot et al., 2012).

Many variants to OsHV-1 have been detected, some during mortality outbreaks from 1993 to 2010 (Lynch et al., 2012; Lynch et al., 2013; Martenot et al., 2012; Martenot et al., 2013; Renault et al., 2012) and some with no associated mortality (Dundon et al., 2011; Shimahara et al., 2012). However, there is a lack of data to confirm that there is a difference in virulence or ecology among OsHV-1 genotypes, or if new environmental stressors are playing a role. For example, oysters could have become more susceptible to the virus due to pollutants or shifts in ecological conditions. Indeed, mortalities associated with viruses in the marine

ecosystem are expected, in general, to arise sporadically when conditions become less favorable for the host (Suttle, 2007). Consequently, oysters selected for higher resistance 'R' to the summer mortality, for which OsHV-1 was implicated, were evaluated again in 2009.

Resistance to OsHV-1 µvar infection (now predominating) was evidenced in the G6 generation in 2009 (Dégremont, 2011). Using resistant (R), susceptible (S), and wild animals, spat were tested under field conditions and live oysters were diagnosed for the presence and intensity of three potential pathogens (OsHV-1 µvar, *Vibrio splendidus*, and *V. aestuarianus*). A mortality outbreak started two weeks post deployment and lasted one week, with significant differences in survival among the groups: 95% for R, 47% for the control, and 6% for S spawns, respectively (Fig. 1A) (Table 1). No relationship was shown between *Vibrio* spp. and survival, but high and significant negative correlations were found between the survival and the prevalence of OsHV-1 µvar, as well as between survival and OsHV-1 µvar viral load, demonstrating, for the first time, herpesvirus resistance in the R line. R oysters initially selected for higher survival to apparent OsHV-1 virus continued to show higher survival when OsHV-1 µvar was dominant. Furthermore, oysters resistant to OsHV-1 µvar infection seemed to be able to both limit the amount of the viral load in their tissues and eliminate the virus as well (Fig. 1B and C) (Dégremont, 2011). Thus, the high genetic basis of survival to the summer mortality phenomenon in *C. gigas* spat in France seems to be linked to OsHV-1 / OsHV-1 µvar resistance. Further findings continue to support inherent genetic diversity for susceptibility to OsHV-1 / OsHV-1 µvar infection (Segarra et al., 2014).

One important consideration for selective breeding programs is genotype x environment interaction (GxE), manifest by change in ranks of families across environments (Gjedrem and Baranski, 2009). We discuss this further below, but for the work on OsHV-1 µvar in France, a limited test of GxE was conducted using one unselected control spawn and one R line spawn produced in August 2010. Both spawns were deployed in 18 sites along the French coast in 2011 from the Mediterranean Sea to the English Channel. By fall 2011, mean survival across sites was 80% for the R line and 14% for the control (over all: +66% difference) (Table 1). The R line had higher survival than the control at all sites, but the difference in survival between the R line and the control was smaller in the confined environment of Thau Lagoon off the Mediterranean (+36 to +42%) and in a pond located in the Marennes-Oléron Bay (+28%). Enclosed environments had previously shown a higher relationship between OsHV-1 detection and spat mortality outbreaks between 1998-2006, suggesting that closed environments are less favorable for spat survival (Garcia et al., 2011). Generally, GxE results indicated that selection to increase OsHV-1 resistance should work across wide areas where the disease is enzootic (Dégremont et al., 2011).

So far, the family based work on OsHV-1 resistance in France has been largely experimental, although some private French hatcheries and the CNC (Comité National de la Conchyliculture) have begun their own family based selection programs (Culture marine, 2013; Culture marine, 2014). In the meantime, at the Ifremer Genetics and Pathology laboratory, mass selection experiments on survival in *C. gigas* spat were initiated in 2009 at sites where OsHV-1 mortality is common. Over the first four generations of selection, gains in survival of 22%, 44%, 50% and 62%, respectively, were recorded over that of a wild control group (Table 1), indicating a positive response to selection to enhance OsHV-1 resistance in *C. gigas* spat (Dégremont et al., 2015a). The realized heritability for survival and OsHV-1 resistance was ranged from 0.34 to 0.63 depending of the line and the size of the oysters when facing OsHV-1 (Dégremont et al., 2015a). The genetic basis of survival and disease-resistance were confirmed through field testing and controlled experimental infections

to OsHV-1 µvar in the laboratory, with high narrow sense heritability for OsHV-1 resistance ranging from 0.49 to 0.61, strongly supporting that selective breeding program would efficiently enhance survival (Dégremont et al., 2015b).

It seems clear that selective breeding programs could improve disease resistance to OsHV-1 / OsHV-1 µvar , thereby limiting mortality from this pathogen, now reported from numerous European countries (Clegg et al., 2014; Dégremont, 2013; Domeneghetti et al.; Dundon et al., 2011; Lynch et al., 2012; Oden et al., 2011; Peeler et al., 2012; Pernet et al., 2012; Roque et al., 2012), Australia and New Zealand (Cameron and Crane, 2011; Jenkins et al., 2013; Keeling et al., 2014; Paul-Pont et al., 2013; Paul-Pont et al., 2014), the western coast of the United States (Burge and Friedman, 2012; Burge et al., 2007), Japan (Shimahara et al., 2012), and Korea (Hwang et al., 2013). OsHV-1 was also reported in China, which is by far the top producer of *C. gigas* (EFSA, 2010; Moss et al., 2007).

To date, from the several commercial projects on OsHV-1 resistance in *C. gigas* in France, from an experimental program in California, and from industry based programs in Australia and New Zealand, results have not been reported.

3. Selection for resistance to *Bonamia ostreae* in *Ostrea edulis*

One of the first selective breeding programs for disease resistance in an oyster species was for bonamiosis in the European flat oyster, *Ostrea edulis*, caused by the protozoan *Bonamia ostreae*. For natural populations, Elston et al. (1987b) demonstrated the genetic basis of resistance to *O. edulis* that had been introduced to the USA and subsequently exposed to several generations of selective disease pressure. In their study, only 1% of naïve oysters survived compared to 74% for oysters exposed to the disease over several generations (Table 1).

B. ostreae was detected in the late 70's in France and was associated with mass mortalities that led to a serious decline in flat oyster farming (Pichot et al., 1979). The pathogen probably was introduced into France with oyster spat from California (Elston et al., 1987b), subsequently spreading through other European countries with oyster transfers, becoming the most important constraint for the flat oyster industry (Da Silva et al., 2005). The first breeding program for bonamiosis resistance in France began in 1985 based on mass selection of survivors after both experimental infections by inoculation and natural infections by *B. ostreae*. Survival of the third generation was more than four times higher than the control group after 20 months of exposure in the wild, and significant reductions in parasite prevalence were recorded for the *O. edulis* line, S85 (Table 1) (Naciri-Graven et al., 1998).

In another *O. edulis* line, S89, significant gains in survival (20 to 27% over the control) were observed in the first generation regardless of the method of exposure to the parasite, which comprised natural infection in an intertidal area – more favorable to the *Bonamia* disease than subtidal areas, cohabitation with heavily infested oysters, or injection of parasites in a controlled facility (Table 1) (Martin et al., 1993). However, these gains were not observed for the second generation and this lack of effect was attributed to inbreeding (Launey et al., 2001; Naciri-Graven et al., 1998). To avoid the inbreeding problem, the French program made crosses between lines, thereby potentially exploiting heterosis (Baud et al., 1997). Selected lines and controls were tested by oyster farmers, who confirmed better survival (81% for lines vs 37% for controls) and less parasite infection (0% for lines vs 25% for controls) (Bédier et al., 2005).

In Ireland, Culloty et al. (2001) reported field and laboratory results from the Rossmore line, where survivors from natural exposure to bonamiosis were bred over two successive generations. Second generation Rossmore oysters showed about 30% higher survival and a lower parasite infection than two control groups, which used naïve parents (Table 1) (Culloty et al., 2001). A larger trial investigated the resistance of six naïve or *B. ostreae* enzootic European flat oyster populations against the Rossmore line selected for its higher resistance to the disease. All seven oyster groups were tested over two years in regions where *Bonamia* is enzootic in Ireland, France, and The Netherlands (Culloty et al., 2004). Both naïve and wild-exposed populations had higher infection levels and lower survival compared to the Rossmore line, selected for resistance to *B. ostreae*. However, survival for all groups deployed in June 1998 was low, ranging from 0 to 30% across sites (Table 1) (Culloty et al. 2004). The Culloty study used adult oysters of unspecified age and without information on previous disease history at their native sites, leading to difficulty in interpretation of the results. To date, six successive generations of the Rossmore line have been produced and survival to that point was considerable (> 75%) during the first four years of growth at Rossmore (Cork Harbour), with low prevalence of *B. ostreae* (Lynch et al., 2014).

A similar approach, comparing natural populations, was done in Spain starting in 2001. Two naïve populations – of Irish and Greek origin – and two disease affected populations from Spain were compared (Da Silva et al., 2005). For these trials, four or five families for each population were produced in the hatchery and placed in an infected area in Galicia, Spain. Survival varied among the populations after two years in the field, with the highest survival from Spanish germplasm. There was also significant variation among families within populations, ranging from 0 to 75% survival (Da Silva et al., 2005). The overall incidence of bonamiosis and cumulative mortality was significantly correlated.

All these studies reported encouraging results supporting a genetic mechanism for resistance of *O. edulis* to *B. ostreae*. No estimates of heritability for resistance were obtained.

4. Selection for resistance to *Bonamia roughleyi* and *Marteilia sydneyi* in *Saccostrea glomerata* in Australia

Winter mortality (WM) in the Sydney rock oyster, *Saccostrea glomerata,* is caused by a parasite. Its original name, *Mikrocytos roughleyi*, was later changed to *Bonamia roughleyi* (Cochennec-Laureau et al., 2003*),* but that designation was changed to *Bonamia roughleyi nomen dubium* by Carnegie et al. (2014). Nonetheless, a selective breeding program for winter mortality syndrome was first established in New South Wales (NSW), Australia in 1990 in response to a 40% decline in production between 1970 and 1997 (Nell et al., 1996). This program produced a founder population comprised of individuals from four locations in NSW and breeding was based selection of the largest individuals. A parallel set of *S. glomerata* breeding lines was also established for the Georges River site to include selection for resistance to the causal agent of WM (Nell et al., 2000). Intensity of selection was around 15% per generation, but unfortunately, the second generation experienced massive mortality in 1995 (Nell et al. 2000).

These mass mortalities were due to another protozoan parasite, *Marteilia sydneyi*, responsible for the Queensland unknown disease (QX disease) (Adlard and Ernst, 1995). In 1997, in addition to selection for fast growing oysters and resistance to the WM disease, the NSW breeding program was modified to incorporate selection for QX resistance, WM, or both QX and WM (Nell et al., 2000). The line selected for QX resistance was tested by exposure for

one year at Lime Kiln Bar. Survival increased by 22% after two generations, 57% after three and 69% after four (Table 1), indicating that selection for resistance to *M. sydneyi* was successful (Dove et al., 2013b; Nell and Hand, 2003; Nell and Perkins, 2006). After two years of exposure to *M. sydneyi* of the third and fourth generations, the gain decreased about 20% (Table 1). At the same time, oysters reached market size before significant losses occurred during the second season of exposure to *M. sydneyi*, so resistance to QX past market size was deemed unnecessary (Dove et al., 2013b; Nell and Perkins, 2006).

For selection against WM alone, test sites were located in Quibray Bay. The survival was enhanced by 23% and 29% after three and four generation of selection (Table 1), respectively, from exposure to *B. roughleyi* for two seasons. The line selected for dual resistance to QX and WM diseases at Woolooware Bay showed a significant increase in survival of about 44% (Table 1). Thus, genetic improvement was successful in enhancing resistance of *S. glomerata* to one or the other or both of these diseases. Selection for resistance to QX disease did not appear to confer resistance for WM disease and the converse, likely meaning that there is little to no overlap in the genetic factors that influence these traits (Table 2) (Dove et al., 2013b; Nell and Perkins, 2006). More recently, disease resistance was confirmed through single-pair mated families in the QX disease-resistant line compared to the unselected control (Kan et al., 2011).

The fourth generation of the dually selected lines was assessed in the Georges River, as well as other estuaries affected by the diseases, to investigate the potential G x E. Survival of the lines selected for QX resistance increased by about 60% in comparison to the control in the Hawkesbury River where QX disease was enzootic. For lines selected for WM resistance and Qx+WM resistance, only the former showed increased survival and then, only 10% higher compared to controls in Merimbula Lake where WM disease is enzootic. Survival after two seasons was higher than 72% for all the groups including the control (Dove et al., 2013a). This study by Dove et al. demonstrated that the selection to improve disease resistance will work across numerous sites affected by the disease, as observed with OsHV-1 / OsHV-1 µvar in *C. gigas* spat (Dégremont et al., 2011).

The Australian studies also support a genetic mechanism for resistance of *S. glomerata* to QX and WM diseases, but as in *O. edulis*, heritability estimates for resistance were lacking.

5. Selection for resistance to *Roseovarius crassostrea* in *C. virginica* in USA

The bacteria *Roseovarius crassostrea* is the etiological agent of Roseovarius Oyster Disease (ROD) (formerly Juvenile Oyster Disease), in *C. virginica* in the Northeast of the USA. Since the 80's, losses exceeding 90% have occurred in enzootic sites in Maine, Massachusetts, and New York (Boettcher et al., 2000; Boettcher et al., 2005; Bricelj et al., 1992; Davis and Barber, 1994; Maloy et al., 2007). ROD generally affects oysters less than 25–30 mm shell height during the summer months. Among three cohorts tested in the Damariscotta River (Maine) in 1993, variation in mortality was negatively correlated with shell height (Davis and Barber, 1994).

Achieving refuge size to obviate disease is decidedly different than selecting for disease resistance *per se*. To test the former, Davis and Barber (1999) compared two lines of oysters selected for fast growth for two generations (Flowers and Milford) against an unselected control group during the 1994-1995 growing seasons at two sites in the Damariscotta River, an area impacted by ROD. Both lines of selected oysters outgrew their unselected counterpart

and had higher survival to ROD, presumably because they reached the refuge size sooner (Davis and Barber, 1999).

Later, in 1996, Barber et al. (1998) again tested selected lines against a control (wild oysters) for ROD resistance. This time, all oysters were well below the 25 mm refuge size during testing. Selected oysters were the result of breeding survivors of ROD mortality combined with selection for fast growth (the largest 20% of Flowers line survivors). After a mortality outbreak related to ROD, 89% of selected oysters survived compared to only 4% in the wild group (Barber et al., 1998). This approach showed, as for QX resistance in *S. glomerata*, that selection for growth and disease resistance could be complementary to limit the impact of the disease in oyster population.

Mass selection on survival of *C. virginica* to the ROD disease was also investigated on the Flowers line in New York state. Progeny selected from survivors were up to seven times more resistant to ROD as progeny from naïve broodstock after a single generation of selection (Farley et al., 1995). Positive responses to selection for enhanced survival to ROD disease were also observed in the second and third generation, averaging 50% higher survival for the selected oysters in comparison to control oysters (Table 1) (Farley et al., 1996; Farley et al., 1997). Management strategies using resistant seed have resulted in increased production to above pre-ROD levels and largely eliminated the devastating effects of this disease in many New York area farms (Farley et al., 1997).

Efforts have been made to combine disease resistance from multiple lines. In 2000, the Flowers line, resistant to ROD, was hybridized with Rutgers University's NEH line, which showed strong resistance to *Haplosporidium nelsoni* (MSX) and to a lesser extent, *Perkinsus marinus* (Dermo). The hybrids, as well as the pure Flowers and NEH lines, were deployed at four sites for 27 months. While MSX and JOD infections were low or absent at the sites, Dermo exposure was heaviest at Cape Shore (New Jersey) site. The Flowers line had low survival (18%), which was higher than the susceptible control (<1%), but lower than NEH (44%) and the hybrid (44%) (Guo et al., 2003). Resistance to one disease (i.e., ROD in the Flowers line) does not seem to confer resistance to another disease, as previously observed with *S. glomerata* for QX and WM diseases. Meanwhile, Rawson et al. (2010) suggested that the development of lines with resistance to multiple diseases and suitable for the Northeast USA are indeed possible through the cross-breeding of existing lines.

Gomez-Leon et al. (2008) also investigated the resistance to ROD of three lines under a controlled challenges in the laboratory using the NEHY (dual resistance to Dermo and MSX) and Flowers lines (resistant to ROD), and the Green Hill Pond (GHP) line derived from wild broodstock collected from an area with known moderate to heavy exposure to Dermo and MSX diseases and located in Green Hill Pond, Rhode Island USA. They found that the larvae of NEHY line had significant ($p<0.001$) higher survival (65%) than larvae of GPH line (10%) at 20°C (Table 2). Gomez-Leon et al. (2008) also showed that survival and resistance to ROD was higher for larger juvenile oysters (15-22 mm shell height) than smaller juvenile oysters for all lines (4-9 mm). Meanwhile, in the smaller size class, a significant difference in survival time ($p<0.001$) was detected between NEHY (28 days) and both Flower and GHP (9 days). For the larger size class, there was no significant difference in survival after ROD challenge, although the mean survival time was the lowest for the Flowers line (7.8 weeks) and the highest for the GHP oysters (9.7 weeks). The Gomez-Leon study also showed similar results for a *Vibrio* strain. It is interesting to note that resistance to one disease (i.e., ROD in the

Flowers line and Dermo and MSX for NEHY) does not seem to confer higher resistance or higher susceptibility to another disease (Table 2).

6. Selection for resistance to *Haplosporidium nelsoni* and *Perkinsus marinus* in *C. virginica* USA

There has been a longstanding interest in developing disease resistance for two major diseases affecting the Eastern oyster, *C. virginica*. One of the oldest and most well documented diseases affecting the Eastern oyster *C. virginica* is "Dermo" disease caused by the protozoan *Perkinsus marinus*. Dermo disease was first documented in the 1940s in the Gulf of Mexico with extensive oyster mortalities, and later, in the Chesapeake Bay (Andrews and Hewatt, 1957; Mackin et al., 1950). In addition to Dermo disease, *C. virginica* also suffers another protozoan disease caused by *Haplosporidium nelsoni*, originally called "MSX" for "Multinucleated Sphere Unknown." The parasite was first recorded during a mortality outbreak in Delaware Bay, USA in the spring of 1957, and in 1959 in the Chesapeake Bay (Andrews and Wood, 1967; Haskin et al., 1966).

In 1964, Rutgers University carried out mass selection experiments using survivors to MSX to determine if the disease resistance was heritable. For all generations, only survivors that had lived through a 33-month of exposure to MSX were used for propagation, creating a generation interval of at least three years (Haskin and Ford, 1979), but also resulting in a high selection intensity. Survival of the oysters after 33 months exposure to MSX was low for the control group (8%) but increased from the first to the fifth generation of selection from 28% to 60% (Table 1) (Ford and Haskin, 1987; Haskin and Ford, 1979). With continued exposure to *H. nelsoni* for up to 66 months, differences in survival between control and selected oysters diminished, and survival declined to 1% for the control, 7% for the F_1 generation and 14% for the F_5 (Table 1). Perhaps the high mortalities that occur later in life are an indication that the oysters are tolerant rather than resistant. Survival to five years, however, is irrelevant for aquaculture since most oysters reached the market size (75-100 mm) in two to three years at this site (Ford and Haskin, 1987). Histopathology data on *H. nelsoni* infection supported earlier findings that selected oysters became infected with *H. nelsoni* but that infections are lighter and more localized compared to those in unselected oysters.

The first investigation on MSX and Dermo resistance in *C. virginica* was conducted by the Oyster Research Laboratory (now the Haskins Shellfish Research Laboratory) at Rutgers University (New Jersey). They tested resistance to Dermo under laboratory conditions in four oysters stocks that showed an innate resistance to mortality due to MSX (Valiulis and Haskin, 1973). The comparisons between the resistance of the oysters to Dermo when injected at a low dose and their resistances to MSX under field conditions showed that: (1) the two stocks that were most resistant to MSX were also resistant to Dermo; (2) the stock most susceptible to MSX was also the one most susceptible to Dermo; (3) the stock moderately susceptible to MSX was resistant to Dermo (Table 2) (Valiulis and Haskin, 1973). In the 90's, *P. marinus* appeared in Delaware Bay and the selection program developed by Ford and Haskin (1987) integrated dual resistance to MSX and Dermo. In one report, after 27 months of evaluation, 56% of the Rutgers selected line survived versus 1% of a global susceptible control and 19% of the Delaware Bay control (Table 1) (Guo et al., 2003).

In the Chesapeake Bay, where MSX and Dermo are also enzootic, selection for disease resistance in *C. virginica* began in the late 80's at the Virginia Institute of Marine Science (VIMS). In an early experiment on this topic, Burreson (1991) tested various lines and wild

populations in the York River, Virginia where MSX and Dermo are at high prevalence. Only 1% of oysters from lines selected in Delaware Bay (for MSX resistance) survived Dermo disease, whereas 20% of oysters lived from crosses derived from Chesapeake Bay populations that had been exposed to both diseases (Table 2). Burreson (1991) concluded that resistance to MSX in *C. virginica* (as from the highly resistant Delaware Bay line) did not seem to confer general resistance to disease, but was specific to the parasite in question (Burreson, 1991). This is in agreement with other findings, i.e. QX and winter mortality in *S. glomerata*, and ROD and Dermo in *C. virginica*.

VIMS began its selective breeding program by creating a line from native Delaware Bay oysters – named DEBY – largely because these were the only population that survived in sufficient numbers after the first generation of exposure to MSX. Survivors of 2-5 year disease exposures were propagated. The performances of the third and fourth generation DEBY were evaluated from 1993 to 1995 and from 1997 to 1999, respectively, and compared to other selected lines and controls. In the presence of high infection pressure from both MSX and Dermo in the York River, the F_3-DEBY line showed significantly higher survival (47%) than the other lines (17% and 25%) at the termination of the study. Similar results were found for the F_4-DEBY line with a final mean cumulative survival of 79% in the Great Wicomico River, 48% in the York River and 64% in Burton Bay, whereas survival of other lines was below 18% at all three sites (Table 1) (Ragone-Calvo et al., 2003). In addition, the DEBY line also had the lowest infection rate for MSX, with intermediate infections of Dermo. Ragone-Calvo et al. (2003) maintained that these results demonstrated that selection can enhance resistance for both diseases. Up to this time, it had been amply demonstrated that resistance to MSX was highly heritable; the same information about Dermo was lacking. Largely unanswered in the Ragone-Calvo work is the independent estimate of Dermo resistance, in the absence of MSX resistance. That is, it is quite likely that for the oyster, attaining resistance to MSX provides a healthier baseline to avoid Dermo disease, which was supported by the findings observed in Valiulis and Haskin (1973).

The DEBY line was also evaluated in the Patuxent River (MD) from 2003 to 2006 compared to a regional wild control cross under pressure from Dermo (MSX was non-existent in this estuary). Cumulative survival was the highest for the DEBY line with 83% in comparison to the two other lines (67% and 72%) (Table 1), and their value in aquaculture applications was evident (Abbe et al., 2010).

The DEBY line, as well as other lines selected for disease resistance, were tested from 2004 to 2006 by the Aquaculture Genetics and Breeding Technology Center (ABC) of VIMS in four sites in Virginia that were characterized by different salinity regimes and, therefore, varying disease pressure. By the end of deployment, selected lines had, in general, a greater survival than the wild Mobjack Bay control cross (Table 1) (Frank-Lawale et al., 2014). In this study, the DEBY line had been under selection for two additional generations (F_6) and was tested in two sites where the F_4 was evaluated by Ragone-Calvo et al (2003). The F_6 seemingly performed worse than the F_4. Survival of the DEBY line after 30 months in the York River (moderate salinity, high disease pressure for both MSX and Dermo) was 47% for the F_4 and 21% for the F_6; survival in Wachapreague (high salinity, intermittent disease pressure for both MSX and Dermo) was 64% for the F_4 and 38% for the F_6 (Frank-Lawale et al., 2014). Comparing the histological results between the two trials, possible because they were both done by the VIMS Shellfish Pathology Laboratory, prevalence and intensity of heavy infections by Dermo were much greater in the Frank-Lawale et al. (2014) study than in Ragone Calvo et al. (2003) one. Thus, only 7% and 13% of the oysters screened had heavy

and moderate infections by Dermo after two years of disease exposure in the York River and in Burtons Bay near Wachapreague for the F_4-DEBY line while it was 65% for both sites for the F_6-DEBY line). This result highlights one of the largest complications with breeding oysters under natural conditions that change temporally and spatially (Frank-Lawale et al., 2014).

Lack of control over the environment means that repeatable disease challenges are almost impossible because disease pressure can change from one generation to the next, possibly making cumulative gains for disease resistance difficult. The Frank-Lawale et al. (2014) study also found that Louisiana oysters, incorporated into the breeding program because of their higher innate resistance to Dermo infection, but naïve against MSX, had dramatic mortality due to MSX (up to 100%), reinforcing that resistance of *C. virginica* to one disease does not confer resistance to another disease (Table 2). Among the lines in the Frank-Lawale study, those selected for their higher resistance to both diseases were clearly highly resistant to MSX, but to a much lesser extent resistant to Dermo. Meanwhile, survival of those same lines after 30 months of exposure reached 40%, which was much higher than the 1% survival for the two MSX-resistant lines reported in Burreson (1991). It is also worth noting that some oysters in the Frank-Lawale study reached market size after 18 months, clearly providing another avenue for avoiding disease, especially to Dermo.

Finally, there was clearly a significant G x E for survival among lines, mostly due to different disease pressures, which has significant consequences for a selective breeding program (Frank-Lawale et al., 2014). First, the best genotype in one environment is not necessarily the best in another. Breeding oysters from a single environment to improve performance in dissimilar environments seems pointless. Second, it is clear that the advantage of years of selection for improved survival is unnecessary in an environment where the trait is not required. Where disease is not an issue (i.e., in the low-salinity zones), new production traits can be explored that influence the profitability of production, traits, such as, growth rate and harvest traits (e.g., meat weight), whereas, in areas where disease is an issue, the focus can be shifted to fast growth in addition to disease resistance (Frank-Lawale et al., 2014).

ABC also began producing family crosses in 2003 to begin estimating the genetic parameters needed for a family based selection program. Cohorts of about 50 families were produced by single pair mating in 2004, 2005, and 2006 and all families were tested for about 30 months in the York River, where MSX and Dermo are enzootic, and in the Yeocomico River, where Dermo is present but sporadic. The genetic parameters of survival and other traits will be reported elsewhere, but results on disease effects are given for the first cohort produced in 2004 and then tested in 2005 and 2006 in Table 2. The 2004 cohort of families were produced from individuals taken from various selected lines developed at ABC. These families were tested at the same time in the York River as those lines described in Frank-Lawale et al. (2014). MSX had limited impact on the families (Table 2) although, judging by its toll on the Louisiana lines (Frank-Lawale et al., 2014), MSX was very active. In the first year, mean survival of the 50 families was 65%, ranging from 28 to 90% (Table 2). Dermo prevalence was high for all families, but the Mackin score, an index of the infection intensity (Dungan and Bushek, this volume), was the lowest for the families showing the highest survival, and the highest for the families showing the lowest survival. These relationships were confirmed the following year, with survival of 64% for the five best families, 35% over all the families, and 5% for the five worst families (Table 1) (Table 2) and Dermo intensity ranking similarly. For one family (#42), 84% of the oysters had survived after 30 months of exposure to Dermo and MSX. Given the families were founded from disease resistant lines and also resisted

Dermo disease, the data comprise the first confirmed report of dual resistance in *C. virginica* in an environment where both diseases were very active.

7. Ploidy investigation for disease resistance in oyster species

Triploids oysters are of interest for a number of reasons, not all applicable in every location: generally they grow faster than diploids as a result of their partial sterility; they are genetically more heterozygous; they have different energy allocations for growth and gametogenesis than do diploids; or some combination thereof (Allen and Downing, 1986; Garnier-Géré et al., 2002; Hand and Nell, 1999; Hawkins et al., 1994; Hawkins et al., 2000; Normand et al., 2008). Theoretically, if triploid oysters could reach market size earlier, they could avoid disease mortality (Barber and Mann, 1991), resulting in increased yields even if oysters had limited disease resistance. Nevertheless, such an advantage would be useless for the diseases that affect larvae, spat and juvenile stages, such as, MSX and JOD in *C. virginica* or OsHV-1 in *C. gigas*.

The first investigation on the effect of ploidy on disease resistance in oysters was performed with *C. gigas* and *C. virginica* in 1989 by examining their susceptibility to laboratory challenges to Dermo (Meyers et al., 1991) and field challenges for *C. virginica* only (Barber and Mann, 1991). Both studies showed that triploidy provided no advantage for disease resistance to Dermo in either species. For laboratory trials, survival under Dermo challenge was 0% in diploid and triploid *C. virginica.* In *C. gigas,* survival was higher, 75% for diploids and 66% for triploids (Table 4), although the mortality did not appear to be disease related (Meyers et al., 1991). All triploids in these studies were chemically induced using cytochalasin-B (CB). For parentage, the *C. gigas* shared the same parents with the diploid control, but this was not the case for *C. virginica* either for the laboratory or field studies. Therefore, interpretation of the results was difficult because ploidy and founder effects were confounded.

After two growing seasons in MSX enzootic waters, diploid and chemical triploid *C. virginica*, derived from two different spawns using the same parent stock – seventh generation MSX resistant line described in Ford and Haskin (1987) – showed that triploids had a limited survival advantage (+7%) over the diploids (Table 4), despite a higher prevalence of MSX infection in the triploid (Matthiessen and Davis, 1992). Once again, interpretation of such results could be biased due to the low number of parents (six males and three females for the triploid, two males and two females for the diploid) used to produce the spawns.

More recently, Dégremont et al. (2012) tested diploid and triploid *C. virginica* in three Chesapeake Bay sites characterized by low (<15‰) or moderate (15-25‰) salinity regimes (Dégremont et al., 2012). Each ploidy level was replicated three times by producing separate spawns from one unselected broodstock and several disease-resistant broodstocks but with different level of resistance. Triploids were produced by mating diploid females to tetraploid males in order to obtain all-triploid progenies as described in Guo et al. (1996). By the end of the study in October 2007, ploidy had a consistent effect on the performance of *C. virginica* at all three test sites with higher survival (+13% and +20% greater than diploids in low and moderate salinity regime, respectively) (Table 4) and much faster growth for triploids. There was no difference in prevalence or infection intensity for Dermo or MSX during the first year. While growth was similar among the three diploid crosses and similar among the triploids, there was high variation for survival for each ploidy. Thus, there was no significant difference in survival between ploidy (Table 4), even if triploid *C. virginica* exhibited a clear growth

advantage over diploids when both were disease resistant. Also clear is that the progress in selection for disease-resistant lines is heritable through both the diploid and tetraploid lines and appeared to be additive (Dégremont et al., 2012). Thus, selective breeding programs to reduce mortality related to Dermo and MSX, coupled with triploid production to increase growth, can further optimize yield.

The effect of ploidy on disease resistance was also investigated in Sydney rock oysters, *S. glomerata*. All triploids were chemically induced. Diploid and triploid siblings of *S. glomerata* were tested for seven months in Woolooware Bay in New South Wales (NSW) where oysters regularly suffer winter mortality (WM) from the parasite *B. roughleyi*. No differences in survival between diploid and triploid groups occurred (Table 4) (Nell et al., 1994). A larger trial tested diploid and triploid sibling oysters at seven sites in New South Wales for 25-28 months from 1994 to 1996 (Hand et al., 1998a). At the end of the trial, diploids had lower survival (65%) than triploids (88%) (Table 4), and the difference in survival was much higher in the site heavily impacted by WM: 23% in diploids versus 83% in triploids (Hand et al., 1998b). Another study, using the same two groups as the Hand study, showed that both ploidies had infestation with *B. roughleyi*, with high survival for both (Table 4) (Smith et al., 2000).

Additional studies comparing diploid and triploid siblings also showed similar results with high survival overall, but disease levels were not reported (Hand et al., 2004; Troup et al., 2005). Nevertheless, all studies on the effect of ploidy on *S. glomerata* consistently demonstrated superior growth for triploids after a 30 month grow-out period, corresponding to a reduction of approximately 6 months to market size. Altogether, even if further research is required to investigate disease resistance of triploid *S. glomerata* during outbreaks of WM or QX disease, triploidy would provide the Sydney rock oyster industry in NSW with significant improvements in profitability (Hand et al., 1998b; Hand et al., 2004).

Triploids have become important in French oyster production of *C. gigas*, which is based on two types of spat, wild-caught and hatchery-produced. For the latter, the number of hatchery-produced seed increases regularly each year, reaching approximately three billion in 2012, primarily from the production of all-triploids (diploid x tetraploid mating) (Dégremont et al., 2014). The first investigation on the effect of ploidy on resistance to summer mortality in *C. gigas* spat was carried out in 2003, which was characterized by an intense heat wave resulting in exceptionally high mortality of spat and juveniles compared to previous years (Dégremont et al., 2010a; Mille et al., 2014). Three cohorts of oysters were produced from a selected diploid resistant line (2nR – selected for higher survival after summer mortality), a susceptible line (2nS – from low surviving families) and an unselected diploid control (2nC). Sperm from unselected tetraploid oysters was used to fertilize about half the eggs from all three groups: R eggs to make 3nRCC, S eggs to make 3nSCC, and control eggs to make 3nCCC triploids. All groups of oysters were then tested in a raceway at Ifremer facilities in La Tremblade (Atlantic coast, southwest France) and in the field during the summer 2003. Mortality outbreaks were reported in both sites, and OsHV-1 was detected in moribund oysters during mortality that occurred in the raceway (disease sampling was not done in the field due to limited access). At the end of summer, survival observed for all lines in the raceway were highly correlated to those in the field and significantly different between lines ($p<0.0001$) (Dégremont et al., 2010b). The mean survival in the field and in the laboratory was 71% for 2nR, 51% for 2nC and 38% for 2nS lines, while in the triploids, survival was 62% for 3nR, 52% for 3nC, and 48% for 3nS (Table 4) (Boudry et al., 2007; Dégremont et al., 2010b). These results suggest triploidy confers no survival advantage or disadvantage over diploidy at the juvenile stage in

C. gigas for summer mortality. The caveat to this conclusion, however, is that the genotypes of diploids and triploids varied quite a bit. For the diploid R, S and control, the genotypes would have been RR, SS, and CC; for the triploid R, S, and control – RCC, SCC, and CCC, the first set of genes coming from the diploid female and the two others from the unselected tetraploid male (Table 4). So, although the survival was the about the same for diploid and triploid R crosses, the triploid did it with only one dose of R. In that light, it makes sense that survival of the 2nR was slightly higher than the 3nR and that the survival of 2nS was lower than the 3nS. Attesting to the equivalence of diploids and triploids is the control, where CC survived the same as CCC (p=0.96) (Dégremont et al., 2003).

Since 2008, mortality outbreaks related to OsHV-1 are now routine in France, especially in spat and juveniles (Dégremont, 2013; EFSA, 2010; Pernet et al., 2012). Similar questions about the effect of ploidy were directed at OsHV-1 and selection for OsHV-1 resistance. The first study used diploid and triploid siblings, the latter produced by CB treatment, of both unselected C oysters and selected R oysters, so that diploids and triploids were truly comparable, *i.e.*, RR vs RRR and CC vs CCC. These four groups were produced and tested in five sites heavily impacted by OsHV-1 µvar in 2011. By the end of the trial, mean survival rates across all the sites were not significantly different (p=0.58) between diploid (38%) and for triploids (41%). Resistant crosses, both diploid and triploid siblings, survived better than unselected diploid and triploid siblings: OsHV-1 resistant diploid (RR) – 67%, triploid (RRR) – 68%; unselected diploid (CC) – 9%, triploid (CCC) – 15% (Table 4) (Dégremont et al., 2014). Similar results were reported from other crosses involving broodstock selected for four generations by mass selection on survival of *C. gigas* spat during mortality outbreaks caused by OsHV-1. For selected diploids and triploid (chemically induced by CB), survival was 70% and 79%, respectively, compared to 7% for unselected control (Table 4) (Dégremont et al., 2014).

The second study concerning the effect of ploidy on OsHV-1 µvar resistance was done in 2011 by making selected and unselected tetraploids to be mated with selected and unselected diploids. Triploids made in this manner are quite different than those made by chemical induction. The work proceeded in two steps: (1) transmit the genetic gain for OsHV-1 µvar resistance in one generation from the diploid to the tetraploid broodstock as described in Benabdelmouna and Ledu (2007), and (2) produce all-triploid oysters from the selected and unselected broodstocks. The resulting genotypes for diploids were CC and RR; the resulting genotypes for triploids were CCC, RCC, CRR, and RRR, again, the first set of genes coming from the diploid females and the two others from the tetraploid males. For triploids, as the proportion of the genome that comprised resistance genes (R) increased, so too did the survival to OsHV-1 µvar exposure of spat (Table 4) (Benabdelmouna et al., 2014). Yet diploid RR (100% R) and triploid RRR (100% R) had nearly equivalent survival with 56% and 54%, respectively (Table 4). These findings clearly demonstrated that OsHV-1 µvar resistance was not substantially altered by triploidization and also indicated that disease resistance can be transmitted by the tetraploid (Benabdelmouna et al., 2014).

De Decker et al. (2011) investigated resistance of *C. gigas* to *Vibrio aestuarianus* in diploid and triploid siblings in France. Oysters were injected with the bacteria at two different doses, and at different times ranging from one year old to 18 months old, but no consistent trend was observed. The authors concluded inconsistent results could, in part, be explained in relation to different patterns of energy allocation in diploid and triploid Pacific oysters at different times of the year (Table 4) (De Decker et al., 2011).

8. Conclusions

There have been many studies on disease resistance in oysters, as reviewed above. All share a common rationale for the work: increasing productivity for oyster farming. The various labs reporting this work comprise everything from curious investigators quenching their thirst about the effects of disease (e.g., H. Haskin and S. Ford in the early MSX studies) to continuous breeding programs directed specifically to industry productivity (e.g., ABC at VIMS). In between these extremes are numerous research labs trying to string generations together with sporadic, often grant funded projects. Breeding programs that are cobbled together like this do not reflect badly on the investigators *per se*, but highlight the difficulty in obtaining long term funding for shellfish breeding. In fact, without core institutional support for breeding programs, arguably breeding programs are impossible to maintain. This is because breeding is not research, or at least, does not necessarily have to have a strong research component. Proposing to a competitive granting agency for yet another generation of breeding work in competition with other cutting edge research proposals borders on hopeless. Rather, what is needed are predicable resources – a hatchery and a person/ people to run it, field holdings and people to run them, and genetic expertise and people to use it.

That such programs could be a worthy investment is indicated by the results reviewed here. Selective breeding for disease resistance in oyster species has shown positive results in most cases, with high gain in survival after only two to four generations of selection. As yet, there are few heritability estimates for disease resistance, although it is clear that useful genetic variation exists. There is also no convincing evidence that selection for one disease confers resistance to another, although it seems possible to select for two diseases simultaneously. Heritabilities and genetic correlations are essential for driving breeding programs, especially if they are focusing on multiple traits. Several studies found that triploidy in oysters does not confer survival advantage or disadvantage for disease resistance. These studies, however, are a mix of chemical triploid comparisons versus mated (diploid x tetraploid) comparisons, the two types of triploid clearly having different genetic architectures. Furthermore, mated triploids themselves can have different architectures when derived from multiple tetraploid lines. Aside from resistance to disease *per se*, triploid oysters consistently grow faster compared to same-parent diploids, corresponding to a reduction in the time of exposure to diseases. Triploids then can be a genetic tool to manage around disease.

Finally, other diseases are reported in oyster species for which any breeding program has been initiated yet, such as *Martelia refringens* in *O. edulis*, *H. costale* in *C. virginica* or *V. aestuarianus* in *C. gigas*. The encouraging results found in this review could suggest that disease-resistance could also be developed successfully.

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Table 1

Disease / mortality	Species	Country	Method	Stage	Number of generation[a]	Method of evaluation for resistance	Year	Survival		Statistical difference/Note[b]	Reference
								control	selected		
Summer mortality	*Crassostrea . gigas*	USA	Family selection using survivors of elevated temperature challenge	adult	2	Laboratory – temperature at 21°C	1977-1978	50%	46-94%	NA / 90% of the selected families had higher survival than control	Beattie et al., 1980
						Field in Rocky Bay	1977-1978	52-67%	14-95%		
				spat	2	Field in three sites in Puget Sound	1979	51%	5-89%	NA / 68% of the selected families had higher survival than control	Perdue et al., 1981
				NA	3	NA	1982	38%	81%	NA / All selected families had higher survival than control	Hershberger et al., 1984
Summer mortality / OsHV-1 suspected	*C. gigas*	USA	Family selection on yield (survival x growth)	spat	NA	Field in Inner Tomales Bay/Spring outplant	2000/2001	0%	25%	P < 0.0001	Burge et al., 2007
Summer mortality / OsHV-1 detected	*C. gigas*	France	Family selection	spat	1/3[a]	Field in South Brittany	2003	53%	74%	P < 0.0001	Dégremont et al., 2010b
					1/3[a]	Laboratory (temperature > 16°C)	2003	56%	81%	P < 0.0001	
OsHV-1 µvar	*C. gigas*	France	Family selection	spat	1/6[a]	Field in Marennes-Oléron Bay	2009	47%	95%	P < 0.0001	Dégremont 2011
			Family selection		3/8[b]	Field in 18 sites	2011	14%	80%	P < 0.0001	Dégremont et al., 2011
			Mass selection	spat	4	Field in Marennes-Oléron Bay	2013	7%	69%	h^2 = 0.34-0.63	Dégremont et al., 2015a
			Family selection	spat	0	Field in Marennes-Oléron Bay	2011	29-100%		h^2 = 0.49-0.60	Dégremont et al., 2015b
						Injection	2011	8-100%		h^2 = 0.61	
Bonamia ostrea	*Ostrea edulis*	USA	Mass selection	NA	4 to 10	Cohabitation	NA	1%	74%	NA	Elston et al., 1987b
		France	Mass selection	adult	3	Field in South Brittany	1993-1995	14%	59%	P < 0.001	Naciri-Graven et al., 1998
				adult	1	Field in South Brittany	1989-1991	66%	93%	P < 0.01	Martin et al., 1993
						Injection	1991	70%	90%	P < 0.01	
						Cohabitation	1991	48%	72%	P < 0.01	
				juvenile / adult	2	Field in Cork Harbour	1996-1998	0-10%	32-36%	NA	Culloty et al., 2001
				adult	NA (4-6)	Field in three sites (Ireland/France/The Netherland)	1998-2000	0-8%	14-23%	NA	Culloty et al., 2004
B. roughleyi (WM)	*Saccostrea glomerata*	Australia	Mass selection	adult	4	Field in Lime Klin Bar	2005-2007	3%	72%	P < 0.05	Dove et al., 2013b
							2005-2008	2%	54%	P < 0.05	
				adult	4	Field in Merimbula lake	2005-2008	72%	82%	P < 0.05	Dove et al., 2013a
Marteilia sydneyi (QX)	*S. glomerata*	Australia	Mass selection	adult	4	Field in Quibray Bay	2005-2007	78%	92%	P < 0.05	Dove et al., 2013b
							2005-2008	48%	77%	P < 0.05	
				adult	4	Field in Hawkesbury River	2005-2008	20%	78%	P < 0.05	Dove et al., 2013a

B. roughleyi & M. sydneyi (WM+QX)	*S. glomerata*	Australia	Mass selection	adult	4	Field in Woolooware Bay	2005-2007	66%	83%	P < 0.05	Dove et al., 2013b
							2005-2008	14%	58%		
Roseovarius crassostrea (ROD)	*C. virginica*	USA	Mass selection	juvenile	2	Field in Maine	1996	4%	89%	P < 0.0001	Barber et al., 1998
				juvenile	3	Field in five sites in Long Island	1996	16%-45%	74%-100%	NA	Farley et al., 1997
Haplosporidium nelsoni (MSX)	*C. virginica*	USA	Mass selection	adult	5	Field in Delaware Bay	1981-1983	8%	68%	NA	Ford and Haskin, 1987
							1981-1985	1%	15%	NA	
H. nelsoni and *Perkinsus marinus* (Dermo)	*C. virginica*	USA	Mass selection	adult	NA	Field in Cape shore (Delaware Bay)	2000-2003	1-19%	56%	NA	Guo et al., 2003
			Mass selection	adult	4	*High salinity site (>28‰) (Burton Bay)*	1997-1999	>82%	64%	P < 0.005	Ragone-Calvo et al., 2003
						Medium salinity sites (10-25‰)	1997-1999	>82%	48-79%	P < 0.005	
			Mass selection	adult	5	*Field in three sites in Patuxent River (low salinity 9-13‰)*	2003-2007	72%	83%	NA	Abbe et al., 2010
			Mass selection	adult	0-6	*Low salinity site (5‰-14‰)*	2004-2006	65%	45%-85%	P < 0.001 50% of the selected lines had higher survival than control	Lawale et al., 2014
						Medium salinity sites (15‰-23‰)	2004-2006	15%	0%-35%	P < 0.001 70% of the selected lines had higher survival than control	
						High salinity sites (20‰-30‰)	2004-2006	25%	0-75%	P < 0.001 50% of the selected lines had higher survival than control	

[a] the first number indicates the number of generation under selection, while the second number, if available, indicates the number of generation of reproduction (see Dégremont et al., 2010a for further details: Selection was done between Generation 1 and Generation 2, while Generation 3 was simply propagations of the batches produced in Generation 2 in the absence of additional selection, and so on),

[b] h^2: heritability,

NA: not available

Table 2

Disease resistance	Species	Country	Other disease tested	Line/stage	Year/	Survival		Statistical difference/Note	Reference
						Control	Selected		
Summer mortality/OsHV-1 detected	*Crassostrea. gigas*	France	*Vibrio splendidus*	6 R and 6 S families G1	2002	S 30-90%	R 43%-97%	NA	Gay, 2004
				3 R and 3 S families G2	2003	28%	20%	NA	Huvet et al., 2007
Bonamia roughleyi (WM)	*Saccostrea glomerata*	Australia	*Marteilia sydneyi (QX)*	Quibray Bay	2008	2%	15%	P < 0.05	Dove et al., 2013b
M.sydneyi (QX)	*S. glomerata*	Australia	*B. roughleyi* (WM)	Kiln Bat	2008	48%	49%	P > 0.05	Dove et al., 2013b
Roseovarius crassostrea (ROD)	*C. virginica*	USA	*Perkinsus marinus* (Dermo)	Flowers	2000-2003	1%	15%	NA	Guo et al., 2003
			Vibrio spp	Flowers (15-22mm)	NA	5%	25%	P > 0.05	Gomez-Leon et al., 2008
Haplosporidium nelsoni and *P. marinus* (Dermo)	*C. virginica*	USA	*Vibrio* spp RE101	NEHY (larvae at 20°C)	NA	20%	65%	P < 0.001	Gomez-Leon et al., 2008
			Vibrio spp RE22	NEHY (larvae at 20°C)	NA	3%	10%	P > 0.05	
			R. crassostrea (ROD)	NEHY (larvae at 20°C)	NA	10%	65%	P < 0.001	
			R. crassostrea (ROD)	NEHY (15-22mm)	NA	15%	30%	P > 0.05	
			Vibrio spp RE22	NEHY (15-22mm)	NA	5%	20%	P > 0.05	
H. nelsoni (MSX)	*C. virginica*	USA	*P. marinus* (Dermo)	Unknown	NA	NA	NA	Dual resistance observed	Valiulis and Haskin, 1973
			P. marinus (Dermo)	F6 selected by Ford and Haskin, 1987	1987-1990	20%	1%	P < 0.01	Burreson, 1991
P. marinus (Dermo)	*C. virginica*	USA	*H nelsoni* (MSX)	LSU line	2004-2006	20-30%	0%	P < 0.001	Lawale et al., 2014

		Year 1					Year 2				
			Dermo		MSX			Dermo		MSX	
	Family name	Survival (%)	Prevalence (%)	Mackin Score	Prevalence (%)	Mackin Score	Survival (%)	Prevalence (%)	Mackin Score	Prevalence (%)	Mackin Score
Best	42	90	100	1.1	0	0	84	80	0.8	0	0
	55	89	80	1.1	0	0	64	100	1.4	13	0.1
	54	88	73	1.0	0	0	58	93	1.9	13	0.1
	27	82	100	2.6	7	0.1	56	100	2.3	7	0.1
	19	85	100	2.3	7	0.1	55	93	1.4	13	0.4
Worst	24	40	100	3.1	7	0.1	7	100	3.7	13	0.1
	49	28	100	2.3	7	0.1	6	100	2.5	0	0
	15	49	100	4.5	7	0.1	4	100	4.5	0	0
	31	41	100	4.2	20	0.5	3	100	2.5	40	0.8
	12	37	100	4.6	13	0.7	3	100	3.5	47	1.9
mean		65	97	2.7	7	0.1	35	97	2.5	15	0.3

Table 3

Table 4

Disease / mortality	Species	Country	Broodstock	2n and 3n sibling [a]	Triploidy induction method [b]	Method of evaluation for resistance	Year	Survival		Statistical difference/Note	Reference
								2n	3n		
Perkinsus marinus (Dermo)	*Crassostrea virginica*	USA	Unselected	No	CB	Field in medium salinity site in the Chesapeake Bay	1989-1990	NA	NA	NA / Same susceptibility to *P. marinus*	Barber and Mann, 1991
			Unselected	No	CB	Laboratory	1989-1991	0%	0%	P > 0.05 / 2n died faster than 3n	Meyers et al., 1991
			Unselected and MSX and/or Dermo resistant	No, but 3 replicate spawns within ploidy	♀ 2n x ♂ 4n	Field in low salinity site (<15‰) in Yeocomico River	2005-2007	69%	82%	P > 0.05	Dégremont et al., 2012
	C. gigas	USA	Unselected	Yes	CB	Laboratory	1989-1991	75%	66%	P < 0.01	Meyers et al., 1991
Haplosporidium nelson (MSX)	*Crassostrea virginica*	USA	MSX resistant strain	No	CB	Field in Cotuit Bay in the northeastern USA	1990-1991	31-34%	37-43%	NA	Matthiessen and Davis, 1992
H. nelsoni & *P. marinus*	*Crassostrea virginica*	USA	Unselected and MSX and/or Dermo resistant	No, but 3 replicate spawns within ploidy	♀ 2n x ♂ 4n	Field in moderate salinity sites (15-25‰)	2005-2007	52-66%	74-83%	P > 0.05	Dégremont et al., 2012
Bonamia roughleyi (WM)	*S. glomerata*	Australie	Unselected	Yes	CB M2	Field in Woolooware Bay	1990-1992	59%	54%	P > 0.05	Nell et al., 1994
			Unselected	Yes	CB M2	Field in 7 oyster farms in New South Wales (NSW)	1994-1997	65%	88%	P < 0.01 for 6 farms	Hand et al., 1998b
			Unselected	Yes	CB M2	Field in Merimbula	1994-1996	88%	93%	P > 0.05	Smith et al., 2000
Summer mortality/OsHV-1 detected	*Crassostrea gigas*	France	Unselected	Partially	♀ 2nC x ♂ 4nC	Field and laboratory	2003	CC[c] 51%	CCC[c] 52%	P > 0.05	Dégremont et al., 2003,2010b
			Selected resistant 2nR	Partially	♀ 2nR x ♂ 4nC	Field and laboratory	2003	RR[c] 71%	RCC[c] 62%	P < 0.05	
			Selected susceptible 2nS	Partially	♀ 2nS x ♂ 4nC	Field and laboratory	2003	SS[c] 38%	SCC[c] 48%	P < 0.05	
OsHV-1 µvar	*C. gigas*	France	Unselected	Yes	CB M2	Field in 5 sites along the Atlantic coasts	2011	CC[c] 9%	CCC[c] 15%	P > 0.05	Dégremont et al., 2014
			Summer mortality resistant	Yes	CB M2	Field in 5 sites along the Atlantic coasts	2011	RR[c] 67%	RRR[c] 68%	P > 0.05	

			2nR OsHV-1 resistant	Yes	CB M2	Field in the Marennes-Oléron Bay	2013	RR[c] 70%	RRR[c] 79%	P > 0.05	
			Unselected	Partially	♀ 2nC x ♂ 4nC	Cohabitation	2011	CC[c] 13%	CCC[c] 15%	NA	Benabdelmouna et al., 2014 and unpublished data
			Selected 2n	Partially	♀ 2nR x ♂ 4nC	Cohabitation	2011	RR[c] 56%	RCC[c] 25%		
			Selected 4n	Partially	♀ 2nC x ♂ 4nR	Cohabitation	2011		CRR[c] 37%		
			Selected 2n and4n	Partially	♀ 2nR x ♂ 4nR	Cohabitation	2011		RRR[c] 54%		
Vibrio aestuarianus	*C. gigas*	France	Unselected	Yes	CB M2	Injection	2006-2007	15-65%	10-80%	2n = 3n, 2n>3n and 2n<3n	De Decker et al., 2011

NA: not available

[a] yes indicates that the same oysters were used as parents to produce both 2n and 3n

[b] when reported, which polar body was blocked to make triploid. M2 = second polar body. CB = cytochalasin B

[c] CC, RR, SS were the genotypes for control, selected resistant and selected susceptible diploids, while CCC, RCC, CRR, SCC and RRR were the genotypes from ♀ 2nC x ♂ 4nC , ♀ 2nR x ♂ 4nC, ♀ 2nC x ♂ 4nR, ♀ 2nS x ♂ 4nC,♀ 2nR x ♂ 4nR, respectively (the first set of genes coming from the diploid female and the two others from the tetraploid male). For CCC and RRR produced by CB (without 4n), were the genotypes from ♀ 2nC x ♂ 2nC and ♀ 2nR x ♂ 2nR, respectively (the two first sets of genes coming from the diploid female, and the last from the diploid male)

Legend

Table 1

Summary table for studies comparing disease resistance in control and selected oyster species.

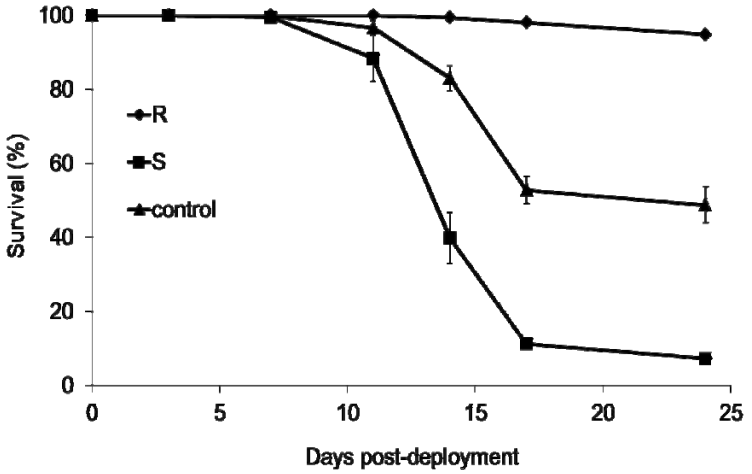
Table 2

Summary table for studies comparing the resistance to a second disease of disease-resistant lines.

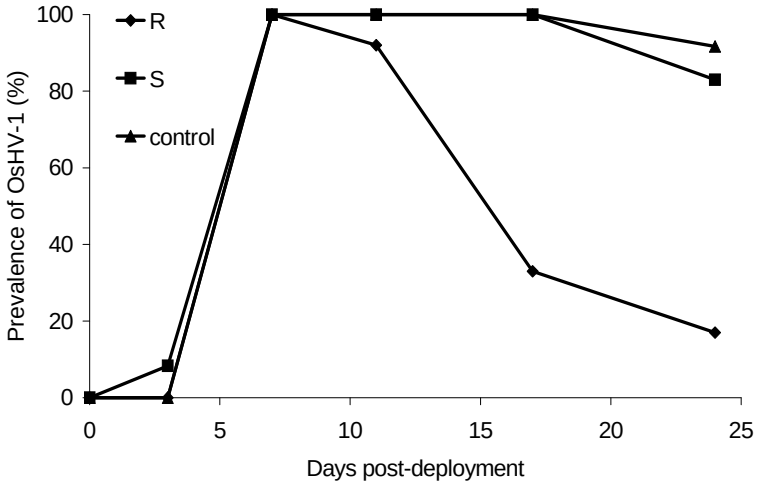
Table 3
Survival of the five best and the five worst families of *C. virginica* from a cohort of 50 families produced at the ABC-VIMS in 2004 and then tested in the York River in 2005 (Year 1) and 2006 (Year 2), and the corresponding prevalence (percent infected) and Mackin Score (intensity: 0-5) for Dermo and MSX.

Table 4
Summary table for studies comparing disease resistance in diploid and triploid oyster species.

(A)



(B)



(C)

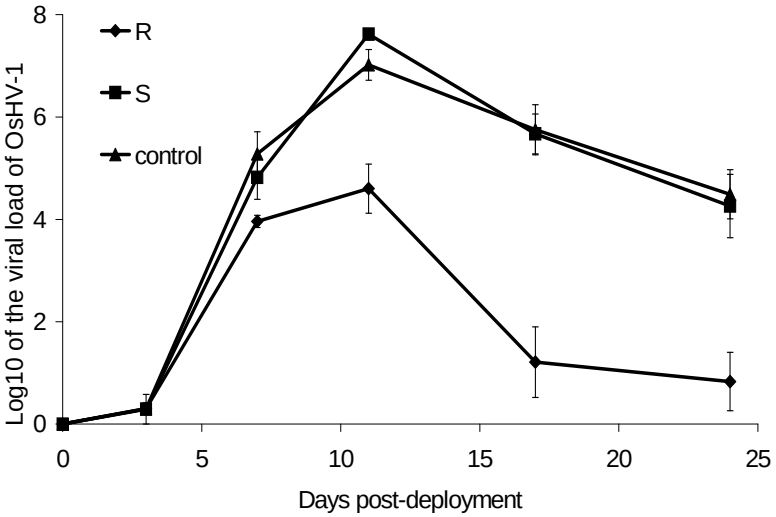


Fig. 1.

a) Cumulative survival for the R, S and control crosses of *C. gigas* (bars represent the standard error among the two bags). R and S are selected oyster groups, 'resistant' and 'susceptible' to summer mortality, respectively, and control used wild oysters as parents.

b) Kinetics of the prevalence of OsHV-1 for live oysters of the R, S and control crosses of *C. gigas* (bars represent the standard error).

c) Kinetics of the viral load (number of DNA copies per mg of fresh tissue) for live oysters of the R, S and control crosses of *C. gigas*

From Dégremont (2011)