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Combining Two-Stage Testing and Interval Mapping Strategies to Detect QTL for Resistance to Bonamiosis in the European Flat Oyster *Ostrea edulis*

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Abstract:

We have identified quantitative trait loci (QTL) in the flat oyster (*Ostrea edulis*) for resistance to *Bonamia ostreae*, a parasite responsible for the dramatic reduction in the aquaculture of this species. An F_2 family from a cross between a wild oyster and an individual from a family selected for resistance to bonamiosis was cultured with wild oysters injected with the parasite, leading to 20% cumulative mortality. Selective genotyping of 92 out of a total of 550 F_2 progeny (i.e., 46 heavily infected oysters that died and 46 parasite-free oysters that survived) was performed using 20 microsatellites and 34 amplification fragment length polymorphism primer pairs. Both a two-stage testing strategy and QTL interval mapping methods were used. The two-stage detection strategy had a high power with a low rate of false positives and identified nine and six probable markers linked to genes of resistance and susceptibility, respectively. Parent-specific genetic linkage maps were built for the family, spanning ten linkage groups (n = 10) with an observed genome coverage of 69–84%. Three QTL were identified by interval mapping in the first parental map and two in the second. Good concordance was observed between the results obtained after the two-stage testing strategy and QTL mapping.

Keywords: *Ostrea edulis* - Disease resistance - QTL mapping - Survival analysis - Statistical power

1. Introduction

Genetic and genomic tools, such as QTLs and candidate genes, increasingly contribute to improve the efficiency of selective breeding programs in aquaculture species (Liu and Cordes 2004). In cultured bivalves, genetic maps have been established for the Pacific oyster *Crassostrea gigas* (Hubert and Hedgecock 2004; Li and Guo 2004), the Zhikong scallop *Chlamys farreri* (eg Li et al. 2005), the blue mussel *Mytilus edulis* (Lallias et al. 2007a) and the European flat oyster *Ostrea edulis* (Lallias et al. 2007b). However, the mapping of quantitative trait loci (QTL) onto those genetic maps has rarely been achieved in bivalves (eg in *C. virginica*, Yu and Guo 2006).

The flat oyster *O*strea edulis is a species endemic to European coasts, both Atlantic and Mediterranean. It has been introduced into USA, Canada and Japan (Ruesink et al. 2005) but most of its production is located in Europe. Its worldwide aquaculture production decreased from around 30,000 tons in the 1960's to 6,000 tons today mainly due to two parasitic diseases, marteiliosis and bonamiosis. Bonamiosis is an intrahaemocytic parasitosis due to the protist Bonamia ostreae of the phylum Cercozoa (Cavalier-Smith and Chao 2003). In Europe, it was first observed in Brittany (France) and was then reported in other European countries.

Since 1985, Ifremer (Institut français de recherche pour l'exploitation de la mer) has been undertaking a selective breeding program for resistance to bonamiosis (Naciri-Graven et al. 1998). Similarly, selective breeding was also initiated in Ireland (Culloty et al. 2004). The families selected in France showed enhanced survival and a lower prevalence of the parasite compared with control oysters in *Bonamia*-contaminated areas; the relative performances of the selected, back-crossed and control families suggested an additive genetic component for the trait (Bédier et al. 2001). However, the heritability of this trait remains to be estimated because of the technical difficulties of required experiments. In this context, the identification of QTL of resistance/susceptibility to bonamiosis would contribute to a better understanding of the genetic basis of this trait and enable an estimation of the potential of marker-assisted selection (MAS). MAS would be a valuable tool to accelerate the selective breeding process by increasing the trait response between two generations of selection.

The primary aim of our study was to identify QTL for resistance or susceptibility to bonamiosis in *O. edulis*. This relied on a 6-month trial challenge experiment in which wild oysters (injected with high concentrations of purified *B. ostreae*) were cultured with the tested oysters (F_2 segregating family) in order to transmit bonamiosis from the wild oysters to the tested oysters. Two extreme phenotypic classes were scored at microsatellite and AFLP markers: heavily infected oysters that died during the challenge experiment (susceptible to the disease) and oysters that survived and in which no parasite could be detected (non-susceptible to the disease).

A two-stage testing strategy was firstly performed (Moen et al. 2004). It is based on a Transmission Disequilibrium Test (TDT) on animals susceptible to the disease followed by a survival analysis test using all animals (susceptible and non-susceptible). This method can reduce the rate of false positives (detection of a QTL when there is none) whilst reducing the genotyping effort by genotyping only the susceptible ones. However, Moen et al. (2004) did not explore the impact of their two-stage testing strategy on statistical power of detecting a QTL. Moreover, in most settings, multiple loci are mapped and it is of interest to know the power for detection of multiple susceptibility loci, as well as the rate of false positives when carrying out multiple testing. Therefore, power and rate of false positives were computed for two-stage selection strategies in multi testing schemes aimed at mapping multiple susceptibility genes.

Finally, a regression interval mapping analysis (Haley and Knott 1992) was performed for identifying and mapping QTL of resistance or susceptibility to the disease. Both analyses (two-stage testing strategy and regression interval mapping), aim to link genotypes (different alleles of molecular markers) to phenotypes (rapidity of death, level of infection to the parasite). Results obtained with those two approaches were compared and the value of each discussed in the context of QTL mapping for disease resistance in aquaculture species.

2. Materials and methods

Segregating family

The family used in this study was initiated in 2004 by crossing a wild-type oyster (W31) and an oyster (98AC703-29) from one of the selected families that were produced by Ifremer during the selective breeding program to bonamiosis. Two full-sibs from this F_1 family were then crossed to make the F2 segregating family (OE.F2.05.04). This family was chosen, among several similar families, based on DNA polymorphism of microsatellite markers for the grand-parents and F_1 parents (410_7 and 410_8), and after parentage checking. Female flat oysters are brooding their larvae into the mantle cavity. Therefore controlled crosses can only be performed by putting two oysters in a tank, and collecting the larvae on a sieve. Therefore the female and male parents were not identified.

Bonamia ostreae challenge experiment

The experimental design consisted of 5 raceways each containing 110 oysters (8 month-old at the beginning of the experiment) from the segregating family and 44 wild oysters. A cohabitation experiment was chosen because it mimics the mode of the disease transmission in nature (Lallias et al. 2008). The 6-month cohabitation experiment was started in mid-January 2006 by injecting 1×10^6 cells of purified parasites into the heart cavity of the wild oysters (from Quiberon bay, Brittany, France). Mortality was checked daily and total shell length (from hinge to outer shell edge) of each dead oyster was measured. Heart smears were performed on the dead oysters and the level of parasitic infection was characterized: no infection ($B0^-$), low infections ($B0^+$), moderate infections ($B0^{++}$) or heavy infections ($B0^{+++}$). The challenge experiment was terminated in August 2006 when heart smears were carried out on all of the surviving oysters from the segregating family. Full details of the cohabitation challenge experiment are detailed in Lallias et al. (2008).

In addition to assessing parasitic infection levels by heart smear, PCR tests were carried out on a random subset of oysters by using the primer pairs BO/BOAS (Cochennec et al. 2000) that amplify a portion of the 18S rDNA of the parasite. Agarose gel (2%) electrophoresis was performed on PCR products alongside to a 100-bp molecular weight standard. Samples containing *B. ostreae* exhibited a 300-bp band.

Genotyping

Samples from the mapping family consisted of the 2 grand-parents (98AC703-29 and W31), the 2 F_1 parents (410_7 and 410_8) together with 46 F_2 progeny that died highly infected with the parasite (heart smear $B0^{+++}$, PCR positive) and 46 F_2 progeny that survived (heart smear $B0^-$, PCR negative).

DNA was extracted from gill tissue using a standard chloroform extraction (Sambrook et al. 1989) followed by purification with the Wizard® DNA Clean-Up System (Promega). Quality and concentration of DNA was assessed using a spectrophotometer and by running a small sample on a 2% agarose gel.

Twenty microsatellite markers selected from those developed by Naciri et al. (1995), Morgan et al. (2000), Morgan and Rogers (2001), Sobolewska et al. (2001) and Launey et al. (2002) were amplified by polymerase chain reaction (PCR) according to the authors' protocols.

AFLP analysis was performed by using a modified version of Vos et al. (1995), in which digestion and ligation were achieved in the same mix being incubated for 16 hours at 16°C. Thirty four AFLP primer pairs were genotyped in the mapping family. Electrophoresis and data collection were carried out on an ABI 3100-*Avant* (Applied Biosystems). Electrophoresis parameters were set at injection for 15 s at 15 kv, running for 25 min at 15 kv and 60°C, with POP4 polymer. Data were analysed with GeneMapper® software version 3.7 and individuals were scored for the presence [A] or absence [a] of the amplified AFLP fragment (peak). The peak-absent marker phenotype is considered to be the homozygote genotype *aa* (whereas the peak-present marker phenotype corresponds to the genotypes *Aa* or *AA*).

Distortion of segregation ratios

Segregation distortion analysis was performed using the chi-square goodness-of-fit statistical test between the F_1 parents and the F_2 progeny as detailed in Lallias et al. (2007b).

Power for detection of susceptibility genes in single and two-stage selection strategies

Two-stage selection strategy consists of 1) carrying out a Transmission Disequilibrium Test (TDT) on susceptible offspring to the disease and 2) testing only significant markers for susceptible and non-susceptible offspring by survival analysis. The advantage of two-stage selection strategy versus one-stage testing is in the reduced genotyping cost. In this section we investigate how power is affected by using two versus one stage selection strategies. We assume dominant markers such as AFLPs. The susceptibility locus (*S*, *s*) and the marker locus (*A*, *a*) are assumed in full linkage disequilibrium. There are three possible parents: a) homozygotes for the locus for susceptibility to the disease and banded (*SA/SA*), b) heterozygotes for the locus for susceptibility to the disease and banded (*SA/sa*), and c) homozygotes for the locus for non-susceptibility (or resistance) to the disease and not banded (*sa/sa*). This gives three possible mating types with at least one segregating parent in the full family: 1) *SA/sa x SA/SA*, 2) *SA/sa x SA/sa* and, 3) *SA/sa x sa/sa.* Matings *SA/sa x SA/SA* and *SA/sa x sa/sa* correspond to type 1:1 segregations, whereas *SA/sa x SA/sa* corresponds to type 3:1 segregation. However, the mating type *SA/sa x SA/SA* led to offspring that were all banded and therefore not segregating. Therefore, only two mating types were considered: *SA/sa x sa/sa* and *SA/sa x SA/sa*. To simplify the marker alleles are removed giving *Ss x ss* and *Ss x Ss*. We define Ψ_{SS}, Ψ_{Ss}, and Ψ_{ss} as the probabilities of developing the disease when animals have genotype *SS*, *Ss*, and *ss*, respectively. These are equivalent to "penetrance", used for hereditary diseases (Ott 1999). This parameter (Ψ) is used to account for genes other than *S/s* that may influence the disease. Table 1 illustrates the probabilities of inheritance of alleles linked to susceptibility to the disease for all three mating types.

Power and expected rate of false positives in single stage detection of susceptibility loci: In order to compute power, a χ^2 test for contingency tables was modeled Cohen 1988. Power in single stage detection of susceptibility loci was noted $P_{1,\alpha}$ (Appendix A). Power for detection of multiple susceptibility loci (n_l) under multiple testing of m markers (each marker represents one test) is given by $\boldsymbol{P^A = (P_{1,\alpha})^{n_l}}$, which assumes that probability of detection is independent for each tested locus, and therefore, susceptibility loci are not linked. The corresponding expected number of false positives is $\boldsymbol{EFP^A = \alpha(m - n_l)}$. The aim of a sound experimental design is to cover as much as possible of the genome with the highest P^A and the lowest EFP^A.

Power and expected rate of false positives in two- stage detection of susceptibility loci: Moen et al. (2004) proposed a two-stage selection strategy based on first stage detection using only TDT with susceptible animals and second stage detection using all animals (susceptible and non-susceptible) and a survival analysis. TDT (Spielman et al. 1993) compares the number of times that a marker allele is transmitted or not transmitted from a heterozygous parent to an affected offspring, and therefore only the affected offspring are considered. Their strategy also required a Mendelian segregation test to avoid markers that are not inherited in a Mendelian fashion. Use of the normal approximation to the binomial distribution was used to compute power for a transmission disequilibrium test (TDT), P_{TDT} (Appendix B). Power for detection of multiple susceptibility loci and expected number of false positives are $\boldsymbol{P^{B1} = (P_{TDT,\alpha})^{n_l}}$ and $\boldsymbol{EFP^{B1} = \alpha(m - n_l)}$.

Only significant markers will be tested in the second stage. It is assumed that tests in the two stages are fully independent of each other. Power in second stage was computed using the same formulae as for single stage selection (with performance for all animals). The overall power of detection in two-stage strategy for detection of multiple susceptibility loci is $P^B = P^{B1}P^A$. The expected number of false positives is $EFP^B = \alpha^2(m - n_l)$, which assumes that the same significant level, α, is used in either testing stage.

From the above formulae it can be observed that (1) power is always greater for single stage testing but at a higher rate of false positives; (2) TDT depends on the transmission parameters and therefore, may yield non significant results for dominant susceptibility loci. A transmission parameter (ν) is the probability of transmission of one of the parental alleles to its offspring. For example in mating type *Ss x ss*, if the probability of susceptibility to the disease is the same for carriers of either one or two copies of the S allele then ν_{Ssxss} = ½ as it is under the null hypothesis, and 3) The expected rate of false positives is always smaller for two-stage selection strategies, as proposed by Moen et al. (2004).

We computed power at significance level α=0.05 in one and two-stage QTL detection strategies and for all loci having Ψ_{SS}=0.70, Ψ_{Ss} =0.25, and Ψ_{ss} =0.05. Note that with the relative small family size, linkage disequilibrium between alleles at the eight loci would be expected for those significant markers. The number of offspring used was 92 for single-stage detection of susceptibility loci, and 46 for the transmission disequilibrium test. Additionally, we computed power for single stage detection of susceptibility loci using the Bonferroni correction so the significance level became α/#tests. The total number of markers tested was either 100 or 200 for each QTL detection strategy type.

QTL analysis

Firstly, a two-stage testing strategy was performed AFLP marker by AFLP marker to identify potential QTLs, as described in Moen et al. (2004). A transmission disequilibrium test (TDT) was applied on the 46 affected offspring (oysters that died heavily infected with the parasite) for all the AFLP markers segregating in that family. A Mendelian segregation test (MST, test of Mendelian inheritance using all offspring) was applied to the whole dataset (affected and non-affected offspring) on markers significant after the TDT. The second test was the survival analysis itself. Survival analysis is used to describe and compare the survival times of two or more groups. Only markers that were significant after the TDT and not significant after the MST were kept for the survival analysis. Survival of two groups of offspring was compared: offspring with the peak-present marker phenotype and offspring with the peak-absent marker phenotype. With the Kaplan-Meier method, survival is recalculated every time a member of the group dies. To calculate the fraction of individuals who survived on a particular day, the number alive at the end of the day is divided by the number alive at the beginning of the day. Kaplan-Meier survival curves were constructed for both groups and the hazard ratio (*h*) computed as described in Moen et al. (2004).

Secondly, a genetic linkage map was built for the F2 family with CriMap software as described in Lallias et al. (2007b). All microsatellites, all AFLPs of type 1:1 and the Mendelian 3:1 AFLPs were considered for linkage analysis. Two parental maps were constructed, one for each F_1 parent.

Finally, a QTL mapping approach was performed with the QTL express software (Seaton et al. 2002) (http://qtl.cap.ed.ac.uk). In our study, one fixed effect was tested: raceway in which the oysters were kept (5 different raceways) and one covariate: total length at the time of death (in cm). The trait analyzed was binary: the oysters that survived the challenge experiment were coded “0” and the ones that died during the challenge experiment were coded “1”. The module “Large Single Full-Sib Family Analysis (Tree)” was chosen because it is designed for the case of a family with two parents that are not assumed to come from a cross between two distinct and different genetic lines. The analysis makes a comparison between the two gametes carried by the male parent (the paternal component) and the two gametes carried by the female parent (the maternal component). The finding of QTL used a regression interval mapping approach (Haley and Knott 1992). Chromosome-wide significance threshold (which takes into account multiple testing on a specific chromosome) was estimated after performing 1000 permutations according to Churchill and Doerge (1994). Separate analyses were performed for each F_1 parent, using the parent-specific maps established with CriMap. For the parent 410_7, the “pat” model was used to fit the paternal component and to find QTL. For the parent 410_8, the “mat” model was used to fit the maternal component and to find QTL. The paternal component relating to 410_7 and the maternal component to 410_8 were arbitrary (sex of the parental oysters unknown).

Results

Power for detection of susceptibility genes in single and two-stage selection strategies

Power for detection of susceptibility genes in one and two-stage selection strategies are given in Table 2. For the offspring size of 92, power to detect multiple susceptibility loci was rather high (between 0.93 and 1) irrespectively of the mating type. Power in the two-

stage detection strategy was high, but marginally lower than power in one stage detection for all mating types.
The expected number of false positives for each type of mating is given in Table 3. As expected, single stage detection had the highest number of false positives. For one true susceptibility locus and 200 markers (tests), the number of false positives was high (~10). Bonferroni correction had the lowest number of false positives. The two-stage detection strategy had also very small expected number of false positives.

Survival analysis and the search for potential QTL of resistance/susceptibility to bonamiosis

The 34 AFLP primer pairs produced 309 markers, 201 AFLPs of type 1:1 (peak present in only one of the two parents) and 108 of type 3:1 (peak present in both parents). After the TDT, 144 markers were significant and kept for the MST: 83 of type 1:1 (22 with $p<0.05$; 20 with $p<0.01$; 41 with $p<0.001$) and 61 of type 3:1 (16 with $p<0.05$; 18 with $p<0.01$; 27 with $p<0.001$). Of the 144 markers significant after the TDT, only 26 were not significant after the MST: five markers segregated through the parent 410_7 (1:1 type), 10 through the parent 410_8 (1:1 type) and 11 through both parents (3:1 type) (Table 4).

Of the 26 markers kept for survival analysis, 15 were significant (four with $p<0.05$, two with $p<0.01$ and nine with $p<0.001$). The peak-present marker phenotype corresponded to a resistance allele in nine cases and to a susceptible allele in six cases. Hazard ratios were in the range 0.24-0.45: inheriting the peak-present allele reduced (or increased) the mortality by ~ 24% to 45% for a resistant (or susceptible) marker (Table 5). Kaplan-Meier survival curves were constructed for the markers with $p<0.01$, these were eight AFLPs ($p<0.01$) for which the peak-present phenotype corresponded to a resistant allele (Figure 1) and three AFLPs ($p<0.01$) for which the peak-present phenotype corresponded to a susceptible allele (Figure 2).

Genetic linkage mapping

Overall, 25% of the markers were distorted, 47.4% of the microsatellites (9 out of 19) and 23.6% of the AFLPs (73 out of 309).

The 410_7 parental genetic linkage map was based on the 17 microsatellites that were informative for this parent and 157 AFLPs segregating through this parent. The AFLPs consisted of 112 markers of type 1:1 (43 from 98AC703-29, 55 from W31 and 14 from both grand-parents) and 45 markers of type 3:1 (29 from 98AC703-29 and 16 from W31). The resulting map consisted of 127 markers (73.0%), comprising 16 microsatellites (of 17: 94.1%), 94 type 1:1 AFLPs (of 112: 83.9%), 17 type 3:1 AFLPs (of 45: 37.8%). Ten linkage groups were established for the 410_7 map covering 465.6 cM (Figure 3). The estimated genome length was 553.37 cM according to method 4 of Chakravarti et al. (1991). The observed coverage was therefore 84.1 % for the 410_7 parental map. Features of the genetic linkage map are shown in Table 6. Distorted markers tended to cluster in specific linkage groups (LG3_410_7, LG4_410_7, LG7_410_7, LG8_410_7, LG9_410_7 and LG10_410_7).

The 410_8 parental genetic linkage map was based on the 18 microsatellites that were informative for this parent and 124 AFLPs segregating through this parent. The AFLPs consisted of 79 markers of type 1:1 (31 from 98AC703-29, 39 from W31 and 9 from both grand-parents) and 45 markers of type 3:1 (29 from 98AC703-29 and 16 from W31). The resulting map consisted of 98 markers (70.0%), comprising 14 microsatellites (of 18: 77.8%), 71 type 1:1 AFLPs (of 79: 89.9%), 13 type 3:1 AFLPs (of 45: 28.9%). Ten linkage groups were established for the 410_8 map covering 386.7 cM (Figure 4). The

estimated genome length was 556.91 cM. The observed coverage was therefore 69.4 % for the 410_8 map. Features of the genetic linkage map are shown in Table 7. Distorted markers towards a deficit (-) or excess (+) in *aa* homozygotes could be mapped on 6 different linkage groups: LG1_410_8, LG3_410_8, LG4_410_8, LG6_410_8, LG8_410_8 and LG10_410_8. Generally, distorted markers in the same direction and with the same level of statistical significance tended to cluster and to be mapped over a very short distance (e.g. 2 or 3 (+) markers in LG1_410_8, LG6_410_8, LG8_410_8 or LG10_410_8; 4 (-) markers in LG3_410_8).

Of the seven markers that remained significant after the survival analysis and that segregated through the parent 410_7, four could be mapped on the 410_7 map, three resistant markers and one susceptible marker: D1f203 on LG2_410_7; E3f255, E1f43 and A12f429 on LG4_410_7. The three markers on LG4_410_7 were mapped in a 14 cM area and interestingly they have the same grand-parental origin (98AC703-29), while the fourth marker (D1f203) mapped in another linkage group came from the second grand-parent (W31) (Figure 3).

For the 410_8 parent, of the 15 markers significant after the survival analysis, 12 of those could be mapped on the 410_8 map, eight resistant markers and four susceptible markers. These 12 markers were distributed on four linkage groups: D1f203 on LG2_410_8; E5f157, A3f73 and A1f150 on LG3_410_8; E1f43, E3f255 and A12f429 on LG4_410_8; D1f328, E3f169, E9f368, B12f243 and C1f99 on LG6_410_8. The three markers on LG3_410_8 mapped in a 9 cM area; the markers on LG4_410_8 clustered altogether and the five markers on LG6_410_8 mapped in a 18 cM area. Moreover, it is interesting to note that resistant markers on the same linkage group came from the same grand-parent while the susceptible markers came from the other grand-parent (LG3_410_8 and LG6_410_8) (Figure 4).

We also compared the mapping of the markers that were significant after the survival analysis in the two parental maps. The marker D1f203 mapped in the two parental maps at the same end of the linkage groups (LG2_410_8 and LG2_410_7), very close to microsatellite *Oedu*U2. Moreover, the three markers E1f43, E3f255 and A12f429 were mapped in the two parental maps, in the terminal part of the linkage groups: they were clustered at the end of group LG4_410_8, while in LG4_410_7 they were mapped in a 14 cM terminal area with two of them being clustered (E1f43 and A12f429).

QTL mapping

No significant effects were found for the fixed effect (raceway) or the covariate (length of oyster at the time of death). Consequently, they were not included in the QTL model.

For the 410_7 parent, four linkage groups exhibited a significant paternal estimate, meaning that the two different gametes carried alleles of different effect for the QTL. The best estimate of location for the QTL was 0 cM for LG2-410_7, 0 cM for LG3_410_7, 24 cM for LG4_410_7 and 8 cM for LG6_410_7. Only linkage group LG2_410_7 attained significance at the chromosome-wide 0.01 level. The two linkage groups LG4_410_7 and LG6_410_7 attained significance at the chromosome-wide 0.05 level, but only just (Table 8).

For the 410_8 parent, two linkage groups exhibited a significant maternal estimate. The best estimate of location for the QTL was 61 cM for LG3_410_8 and 17 cM for LG6_410_8. Linkage group LG3_410_8 attained significance at the chromosome-wide 0.05 level, and LG6_410_8 at the chromosome-wide 0.01 level (Table 9).

Discussion

Segregation distortion

Relatively high segregation distortion was reported in the mapping family OE.F2.05.04, averaging 25% of overall markers. The range of segregation distortion reported in this study was similar to that reported in oysters (Launey and Hedgecock 2001; Li and Guo 2004; Lallias et al. 2007b). Such a high level of segregation distortion is presumed to be due to the extremely high genetic load estimated in oysters (Bierne et al. 1998; Launey and Hedgecock 2001). Moreover, the distribution of distorted markers was not random in the genetic linkage maps produced and tended to form clusters of distorted markers that were restricted to a few linkage groups (e.g. LG3_410_7, LG8_410_7 or LG3_410_8; Figures 3 and 4). These clusters of distorted markers could therefore correspond to the location of potential deleterious genes in *O. edulis*, similarly to the mapping of potential deleterious genes in the rainbow trout (Young et al. 1998) or the Pacific oyster *C. gigas* (Li and Guo 2004).

In the second stage of the two-stage selection strategy (Moen et al. 2004), we excluded markers departing from Mendelian segregation in the first stage. Distortion may be caused by recessive lethal genes at larval stages (Bierne et al. 1998), which may include susceptibility to bonamiosis. Null alleles and other problems can also lead to such departures from Mendelian segregation. Therefore, those markers were ignored in the second stage analyses because it is very difficult with the available data to figure out the causes underlying the observed departures from Mendelian segregation.

Linkage map and genome coverage

Genome coverage was above 82% for the 410_7 parental map, and 69.4% for the 410_8 parental map. Those genome coverage estimates compared favorably with the ones established in cupped and flat oysters' species which were in the range of 70-90% depending on the study (Hubert and Hedgecock 2004; Li and Guo 2004; Lallias et al. 2007b).

Moreover, the number of linkage groups in the two parental maps matched the haploid number of 10 chromosomes in this species (Thiriot-Quiévreux and Ayraud 1982). However, more markers should be added to increase the genome coverage. Indeed, some of the linkage groups consisted of only two markers or spanned a small genetic distance (<20 cM). Therefore, these groups may in fact belong to the same chromosome and may fuse by adding more markers. Despite this, the average marker spacing (4cM) was suitable for the search of QTL (Erickson et al. 2004).

QTL mapping of resistance/susceptibility to a disease

Several studies have highlighted the potential for MAS in breeding programs in fisheries and probably shellfisheries in the future (Liu and Cordes 2004; Rothschild and Ruvinsky 2007). MAS has a huge potential in aquaculture breeding programs, especially for traits that are difficult to quantify phenotypically and would increase the response of the trait to the selection by increasing the accuracy of selection.

Although disease resistance generally seems to have a low heritability in some species it is nevertheless an ideal trait for the application of MAS, due to the economic significance of high survival in aquaculture. Traditionally, the QTL mapping approach was designed for continuously variable quantitative traits. However, it has been shown

that this analysis is robust for binary traits, such as resistance/susceptibility to a disease (death/alive trait coded as “0” or “1”) (Visscher et al. 1996). Therefore, we decided to couple the marker by marker approach with a traditional QTL mapping approach in order to compare the results obtained with these two alternative approaches. The finding of QTL was achieved by using a two-stage testing strategy (Moen et al. 2004) and a regression interval mapping approach (QTL express software, Seaton et al. 2002).

Statistic power of the two-stage testing strategy and rate of false positives were computed by simulations. Power calculations were carried out assuming that multiple QTL are unlinked. If two QTL are closely linked and the alleles increasing susceptibility are on the same homologous chromosome in a parent donating meiosis then the power calculation would be similar to one QTL with increased penetrance respect to either single QTL. The opposite would occur when alleles at two QTL with high and low penetrances are on the same homologous chromosome. On the other hand, the two-stage testing strategy assumed that TDT performed on susceptible animals and the survival analyses used on the whole data set are independent tests. This assumption is not fully correct, since there may be some dependence between the variables used (affected/resistant and number of days survived). As pointed out by Moen et al. (2004), this assumption is supported by the fact that animals tested in the TDT are only a subset of the animals tested in the survival analysis.

False negatives are missing true linkages whereas false positives are false linkage claims. Mapping in genome scans should minimize both. For the two-stage strategy a total of 309 markers were tested with 9 significant results at $P<0.001$ after the survival analyses. This implies than in average less than one of the significant results is spurious (false positive). False negatives would be given by the type I error and would also be less than one for the 309 markers.

Power for detection of multiple susceptibility loci in single stage detection was the highest at the expense of a high rate of false positives. The use of a Bonferroni correction to control the false positive rate resulted in a dramatic reduction of power for some mating types when detecting multiple susceptibility loci. The two-stage detection strategy had a high power with a low rate of false positives (Tables 2, 3). The two-stage testing strategy was a powerful and robust method for identifying QTL of resistance/susceptibility to a disease and allowed us to identify 15 probable AFLP markers linked to genes of resistance (nine of them) or susceptibility (six of them) to the disease. Considering all the above, the two-stage selection strategy might be useful in aquaculture species with high family sizes and incomplete genetic maps.

The QTL mapping approach chosen was a regression interval mapping (interval mapping based on least-squares regression methods) (Haley and Knott 1992). Indeed, an interval mapping approach is based on information from two linked flanking markers and has been shown to be more powerful than a single marker analysis particularly for medium-density maps (with markers around 20-35 cM apart) and to increase the accuracy of parameters estimation (Darvasi et al. 1993). The analysis was based on a single full-sib family experimental design (and not a F_2 analysis) because the mapping family was a three-generation outbred family whose grand-parents were not issued from different genetic lines fixed for different alleles at the QTL. The analysis was interpreted in terms of paternal and maternal components, i.e. whether the two gametes of each parent carried alleles of different effect for the QTL. However, no estimation of the interaction component (and therefore dominance of the QTL) could be performed because of the lack of codominant loci (e.g. microsatellites). Furthermore, a consensus map could not be built because most markers were segregating in only one of the two parents and the estimation of the interaction component implies that the map is the same in the two parents. Moreover, because only 92 progeny were genotyped, a one-

QTL model was fitted but not a two-QTL model. The results obtained after fitting the one-QTL model should be interpreted with caution because the role of neighbouring QTL in biasing the estimation of location and gene effect of a QTL has been widely assessed (Haley and Knott 1992; Jansen 1993). The results of the one-QTL model can be misleading when there are in fact two linked QTL segregating on the same linkage group.

Despite some limitations, our study has made major progress towards the identification of genetic resistance/susceptibility to bonamiosis. Several potential markers of interest were identified and there was good concordance between the results obtained after the two-stage testing strategy, genetic mapping and QTL mapping. Identified markers tended to cluster or were restricted to a few groups: in the 410_7 map, three markers were mapped in a 15 cM area in LG4_410_7 group and a fourth marker was mapped in LG2_410_7 group (Figure 3); in the 410_8 map, three markers were mapped in a 9 cM area in LG3_410_8, five markers were mapped in a 17 cM area in LG6_410_8, three markers were clustered at the end of LG4_410_8 and one marker was mapped in LG2_410_8 (Figure 4). Moreover, the significant QTL found after the regression interval mapping approach were mapped in the same area as the markers that were significant after the survival analysis. Therefore, this study clearly demonstrates for the first time the usefulness of combining different approaches for the search of QTL in aquaculture species, associated with a high statistical power.

Hazard ratios in this study were very high, with single alleles inheritance affecting survival of the offspring bearing it by as much as 24 to 45%. We may wonder how such variation can be maintained against purifying selection. The effects may vary between families for the same alleles and their related causal genes, due to different genetic backgrounds and non-additive effects. Important G x E (Genotype x Environment) interactions might also occur. Therefore QTL analysis in a single family is limiting and QTL analysis should be extended to several families in order to test these hypotheses.
Several studies have reported the location of QTL for disease resistance in rainbow trout, based on the classical approach for QTL mapping using interval mapping, the ANOVA-based approach, or Bulk Segregant Analysis (BSA) (e.g. Ozaki et al. 2001; Rodriguez et al. 2004). The results obtained in this study, even if preliminary, are promising and represent a first step towards MAS in the flat oyster. However, before implementation of MAS in a selective breeding program, the role of epistasis and genomic background should be assessed (Danzmann et al. 1999; Perry et al. 2003). Moreover, screening natural populations for outlier levels of differentiation at QTL loci (Rogers and Bernatchez 2005) could add value in terms of robustness and universality of the QTL identified. The QTL identified could be scored in wild and selected populations. One should expect an average increase in gene frequencies of QTL markers for resistance in the selected populations and a respective decrease in the QTL for susceptibility to the disease.

The addition of codominant markers (such as microsatellites or SNPs) is critical to increase the accuracy of the genetic maps obtained, the power of detection of the QTL and the accuracy of the estimation of the QTL effects. Moreover, before implementation of MAS in *O. edulis*, fine QTL mapping should be achieved in order to restrict the region of interest to a more narrow area. A further step would be to map candidate genes involved in the resistance to the disease that were recently identified after performing a SSH (Suppression Subtractive Hybridisation) library (Morga et al. in prep). This would help to corroborate QTL with candidate genes and would represent a further step into the understanding of the genetic component of the resistance/susceptibility of *O. edulis* to *B. ostreae*.

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Appendix A Power in single stage detection of susceptibility loci

In order to compute power, a χ^2 test for contingency tables was modeled Cohen 1988. There are four types of offspring possible for each type of mating (Table 1): 1) banded and with the disease, 2) banded without the disease, 3) not banded and with the disease, and 4) not banded without the disease. Under the null hypothesis (the AFLP locus is not linked to the susceptibility locus), $\Psi_{SS}=\Psi_{Ss}=\Psi_{ss}$ =½. Let t_α be the value in a χ^2 test with 1 degree of freedom equal or higher than for expected by chance at significance level (α). Power is $\boldsymbol{P_{1,\alpha} = 1-\beta}$ with $\boldsymbol{\beta = \int_{t_\alpha}^{\infty} f(x)dx}$, being $f_x \sim \chi^2(\lambda, df)$, where λ is the non-centrality parameter of a non central χ^2 distribution with df=1 degrees of freedom. The non- central parameter of the χ^2 distribution is

$$\lambda = N\sum_{i=1}^{4}\frac{[p_0(i)-p_A(i)]^2}{p_o(i)}$$

where N is the total number of individuals in the experiment, $p_o(i)$ and $p_A(i)$ are the probabilities of each type of offspring (i=1 to 4) under the null and alternative hypothesis respectively. For the mating type *Ss x ss*: $p_o(i)$= ¼ (i=1,4); $p_A(1)$= ½ Ψ_{Ss}; $p_A(2)$= ½ (1-Ψ_{Ss}); $p_A(3)$= ½ Ψ_{ss}; and $p_A(4)$= ½ (1-Ψ_{ss}). For the mating type *Ss x Ss*: $p_o(1)$= ⅜, $p_o(2)$= ⅜, $p_o(3)$= ⅛, and $p_o(4)$= ⅛; $p_A(1)$= ¼Ψ_{SS}+ ½Ψ_{Ss}; $p_A(2)$= ¼ (3-Ψ_{SS}+2Ψ_{Ss}); $p_A(3)$= ¼Ψ_{ss}; and $p_A(4)$= ¼ (1-Ψ_{ss}).

Appendix B Power in two-stage detection of susceptibility loci

Under the null hypothesis (the AFLP is not linked to the susceptibility locus), for a sire *Ss*, the probability of transmission of the allele *S* and being banded is ½ and ¾ for segregation of mating types 1:1 and 3:1, respectively. At a significance level of α=0.05, the maximum number of offspring inheriting one of the markers alleles by chance is $Nx = \frac{n+1.96\sqrt{n}}{2}$ and $Nx = \frac{3n+1.96\sqrt{3n}}{4}$. In the above formula, n is the number of offspring in the full sib family used for TDT test, and therefore, n=N/2. This is a two sided probability to account that either marker allele could be inherited in a distorted fashion. Under the alternative hypothesis, we assume that the distorted frequency of segregation of marker alleles is the transmission disequilibrium parameter, ν Gomez-Raya 2001. This parameter has different values for the different mating types:

$$\nu_{SsxSs} = \frac{\frac{1}{4}\psi_{SS} + \frac{1}{2}\psi_{Ss}}{\frac{1}{4}\psi_{SS} + \frac{1}{2}\psi_{Ss} + \frac{1}{4}\psi_{ss}},$$

$$\nu_{Ssxss} = \frac{\frac{1}{2}\psi_{Ss}}{\frac{1}{2}\psi_{Ss} + \frac{1}{2}\psi_{ss}}$$

Transmission parameter ν_{Ssxss} corresponds to segregation 1:1, whereas transmission parameter ν_{SsxSs} corresponds to segregation 3:1. The probability of Nx or more individuals inheriting the given marker allele under ν is the power of the test:

$P_{TDT} = \int_{-z}^{\infty} f(x)dx$, where *f(x)* is the normal density, with $z = \frac{\frac{n + 1.96\sqrt{n}}{2} - n\nu}{\sqrt{n\nu(1-\nu)}}$ and $z = \frac{\frac{3n + 1.96\sqrt{3n}}{4} - n\nu}{\sqrt{n\nu(1-\nu)}}$ for mating with segregation types 1:1, and 3:1, respectively.

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Tables

Table 1 Probabilities of susceptible and non susceptible offspring with different genotypes for different types of mating Ss x SS, Ss x Ss, and Ss x ss in a full sib family. Ψ_{SS}, Ψ_{Ss}, and Ψ_{ss} are the probabilities of developing the disease when animals have genotype *SS*, *Ss*, and *ss*, respectively.

Mating	Offspring Genotype	Probability	
		Susceptible	Non Susceptible
Ss x Ss	SS	$¼\Psi_{SS}$	$¼ (1-\Psi_{SS})$
	Ss	$¼\Psi_{Ss}$	$¼ (1-\Psi_{Ss})$
	sS	$¼\Psi_{Ss}$	$¼ (1-\Psi_{Ss})$
	ss	$¼\Psi_{ss}$	$¼ (1-\Psi_{ss})$
Ss x ss	Ss	$½\Psi_{Ss}$	$½(1-\Psi_{Ss})$
	ss	$½\Psi_{ss}$	$½(1-\Psi_{ss})$

Table 2 Power to detect multiple susceptibility loci in one or two-stage testing strategies. TRUE SL: number of true QTL segregating in the population ; α= significance level. Ψ_{SS}=0.70; Ψ_{Ss}=0.25; Ψ_{ss}=0.05.

A)

TRUE SL	Power. Mating type Ss x Ss		
	Single stage α=0.05	Single stage α=0.05/100	Two-stage α=0.05
8	0.9696	0.3396	0.9692
7	0.9734	0.3887	0.9730
6	0.9771	0.4449	0.9768
5	0.9809	0.5092	0.9806
4	0.9847	0.5828	0.9845
3	0.9885	0.6670	0.9883
2	0.9923	0.7634	0.9922
1	0.9962	0.8737	0.9961

B)

TRUE SL	Power. Mating type Ss x ss		
	Single stage α=0.05	Single stage α=0.05/100	Two-stage α=0.05
8	1.0000	0.9982	0.9976
7	1.0000	0.9984	0.9979
6	1.0000	0.9986	0.9982
5	1.0000	0.9988	0.9985
4	1.0000	0.9991	0.9988
3	1.0000	0.9993	0.9991
2	1.0000	0.9995	0.9994
1	1.0000	0.9998	0.9997

Table 3 Expected number of false positives detected in one or two-stage QTL detection strategies. TRUE SL: number of true QTL segregating in the population; # Tests: number of tests; α= significance level. Ψ_{SS}=0.70; Ψ_{Ss}=0.25; Ψ_{ss}=0.05.

TRUE SL	Single stage α=0.05	Single stage α=0.05/100	Two-stage α=0.05
	# Tests	# Tests	# Tests

	100	200	100	200	100	200
8	4.60	9.60	0.05	0.10	0.23	0.48
7	4.65	9.65	0.05	0.10	0.23	0.48
6	4.70	9.70	0.05	0.10	0.24	0.49
5	4.75	9.75	0.05	0.10	0.24	0.49
4	4.80	9.80	0.05	0.10	0.24	0.49
3	4.85	9.85	0.05	0.10	0.24	0.49
2	4.90	9.90	0.05	0.10	0.25	0.50
1	4.95	9.95	0.05	0.10	0.25	0.50

Table 4 TDT and MST for the 26 markers that were kept for survival analysis (significant after TDT among susceptible progeny and non significant after MST in the whole progeny). present: band-present phenotype (Aa or A?), absent: band-absent phenotype (aa); TDT: transmission disequilibrium test; MST: Mendelian segregation test; *: p<0.05, **: p<0.01, ***: p<0.001.

Marker	Parental genotypes		No. of susceptible offspring (dead)		No. of resistant offspring (surviving)		TDT	MST
	410-7	410-8	present	absent	present	absent		
A1f150	aa	Aa	33	12	21	25	9.8**	3.1
E1f43	Aa	Aa	25	21	38	8	9.2**	2.1
A3f73	aa	Aa	12	34	25	21	10.5**	3.5
A3f165	Aa	Aa	42	4	35	11	8.1**	3.7
E3f169	aa	Aa	10	36	27	19	14.7***	3.5
E3f255	Aa	Aa	25	21	38	8	9.2**	2.1
E4f291	Aa	aa	16	30	23	23	4.3*	2.1
A5f225	aa	Aa	16	30	21	25	4.3*	3.5
E5f126	Aa	Aa	40	6	33	13	4.0*	0.9
E5f157	aa	Aa	13	33	24	22	8.7**	3.5
B8f234	Aa	Aa	43	3	32	14	10.9***	2.1
E9f147	Aa	Aa	40	6	37	9	4.0*	3.7
E9f368	aa	Aa	9	37	28	18	17.0***	3.5
E9f371	Aa	aa	31	15	22	24	5.6*	2.1
A12f288	Aa	Aa	43	3	33	13	10.9***	2.8
A12f429	Aa	Aa	25	21	38	8	9.2**	2.1
B12f52	aa	Aa	16	30	21	25	4.3*	3.5
B12f243	aa	Aa	37	9	17	29	17.0***	2.8
B12f478	Aa	aa	31	15	22	24	5.6*	2.1
C1f99	aa	Aa	37	9	18	28	17.0***	3.5
D1f129	Aa	Aa	42	4	33	13	8.1**	2.1
D1f162	Aa	aa	31	15	23	23	5.6*	2.8
D1f203	Aa	Aa	22	24	40	6	15.5***	2.8
D1f328	aa	Aa	12	34	30	16	10.5**	0.7
C5f112	Aa	aa	31	15	24	22	5.6*	3.5
D5f203	Aa	Aa	40	6	37	9	4.0*	3.7

Table 5 Survival analysis results, hazard ratio and LRANK. R: resistance marker, S: susceptible marker; O_a: total number of dead offspring in the band-absent marker

phenotype and E_a its relative expected count; O_p: total number of dead offspring in the band-present marker phenotype and E_p its relative expected count. *: p<0.05, **: p<0.01, ***: p<0.001.

Marker	Origin	R/S	O_a	E_a	O_p	E_p	Hazard ratio (h)	LRANK
A1f150	W31, 410-8	S	12	21.8	33	24.2	0.40	7.7**
E1f43	703-29, 410-7, 410-8	R	21	10.0	25	36.0	0.33	15.5***
A3f73	703-29, 410-8	R	34	24.2	12	21.8	0.39	8.4**
E3f169	W31, 410-8	R	36	24.1	10	21.9	0.31	12.3***
E3f255	703-29, 410-7, 410-8	R	21	9.5	25	36.5	0.31	17.6***
E5f157	703-29, 410-8	R	33	24.5	13	21.5	0.45	6.4*
B8f234	703-29, 410-7, 410-8	S	3	10.1	43	35.9	0.25	6.4*
E9f368	W31, 410-8	R	37	23.6	9	22.4	0.26	15.6***
A12f288	703-29, 410-7, 410-8	S	3	9.5	43	36.5	0.27	5.5*
A12f429	703-29, 410-7, 410-8	R	21	10.0	25	36.0	0.33	15.5***
B12f243	703-29, 410-8	S	9	23.1	37	22.9	0.24	17.3***
C1f99	703-29, 410-8	S	9	22.4	37	23.6	0.26	15.6***
D1f129	703-29, 410-7, 410-8	S	4	9.7	42	36.3	0.36	4.3*
D1f203	W31, 410-7, 410-8	R	24	12.6	22	33.4	0.34	14.3***
D1f328	W31, 410-8	R	34	21.5	12	24.5	0.31	13.7***

Table 6 Length, number of markers, average spacing and largest interval between markers in linkage groups of the 410_7 parental map in *O. edulis* established with CriMap.

Linkage group	Length (cM)	No. of markers	Average spacing (cM)	Largest interval (cM)
LG1_410_7	83.9	11	8.39	38.9
LG2_410_7	70.2	10	7.8	33.3
LG3_410_7	70.1	25	2.92	16.7
LG4_410_7	59.6	23	2.71	17.0
LG5_410_7	49.1	10	5.46	14.5
LG6_410_7	45.2	12	4.11	19.4
LG7_410_7	33.0	9	4.13	10.8
LG8_410_7	19.9	11	1.99	12.2
LG9_410_7	19.5	5	4.88	12.0
LG10_410_7	15.1	11	1.51	8.6
Total	465.6	127		

Table 7 Length, number of markers, average spacing and largest interval between markers in linkage groups of the 410_8 parental map in *O. edulis* established with CriMap.

Linkage group	Length (cM)	No. of markers	Average spacing (cM)	Largest interval (cM)
LG1_410_8	68.1	19	3.78	13.1

LG2_410_8	66.1	10	7.34	40.9
LG3_410_8	61.5	13	5.12	20.4
LG4_410_8	54.1	15	3.86	14.7
LG5_410_8	51.7	4	17.2	35.0
LG6_410_8	46.1	17	2.88	8.6
LG7_410_8	45.0	6	9.0	24.0
LG8_410_8	24.0	6	4.8	22.9
LG9_410_8	13.2	4	4.4	9.9
LG10_410_8	11.0	4	3.67	11.0
Total	98	386.7		

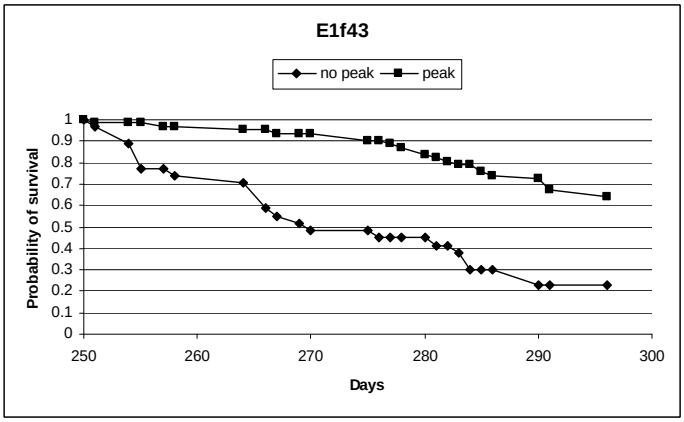
Table 8 Results from fitting a single QTL for the parent 410_7 (QTL express software). Threshold p 0.05 and threshold p 0.01 correspond to chromosome-wide significance thresholds at α=5% and 1% after performing 1000 permutations.

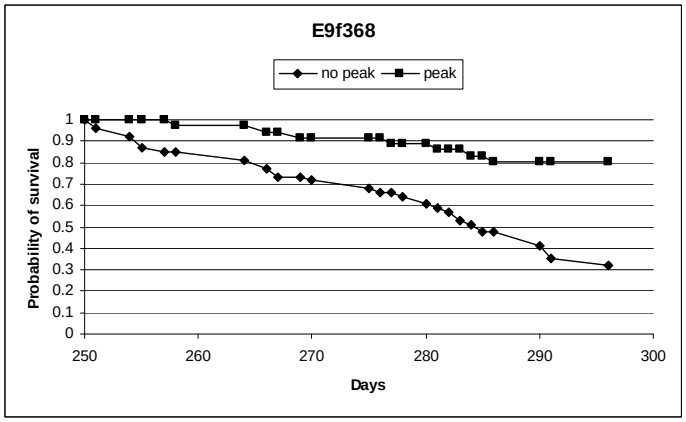
Linkage group	F ratios			Location (cM)	Paternal estimate (standard error)
	Threshold p 0.05	Threshold p 0.01	Observed		
LG2_410_7	6.78	9.87	83.65	0	0.3574 (0.039)
LG3_410_7	7.97	12.14	5.57	0	-0.134 (0.057)
LG4_410_7	6.63	11.72	6.73	24	-0.1601 (0.062)
LG6_410_7	6.65	10.65	6.65	8	-0.1329 (0.051)

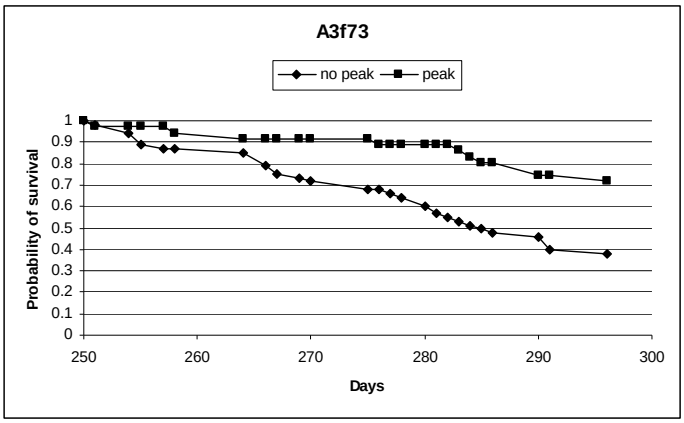
Table 9 Results from fitting a single QTL for the parent 410_8 (QTL express software). Threshold p 0.05 and threshold p 0.01 correspond to chromosome-wide significance thresholds at α=5% and 1% after performing 1000 permutations.

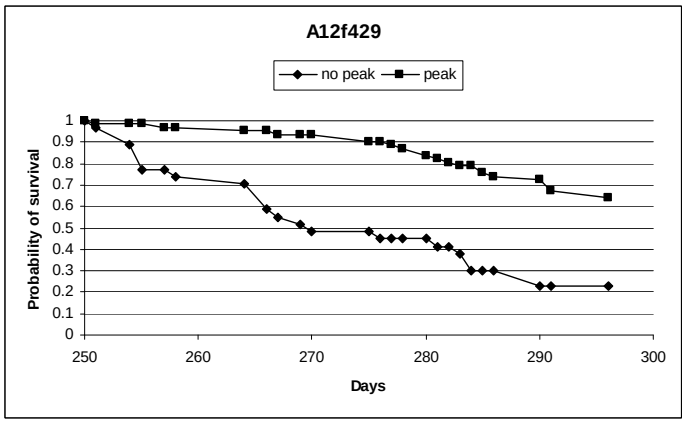
Linkage group	F ratios			Location (cM)	Maternal estimate (standard error)
	Threshold p 0.05	Threshold p 0.01	Observed		
LG3_410_8	7.22	12.08	8.17	61	-0.149 (0.052)
LG6_410_8	7.96	12.14	22.19	17	0.229 (0.049)

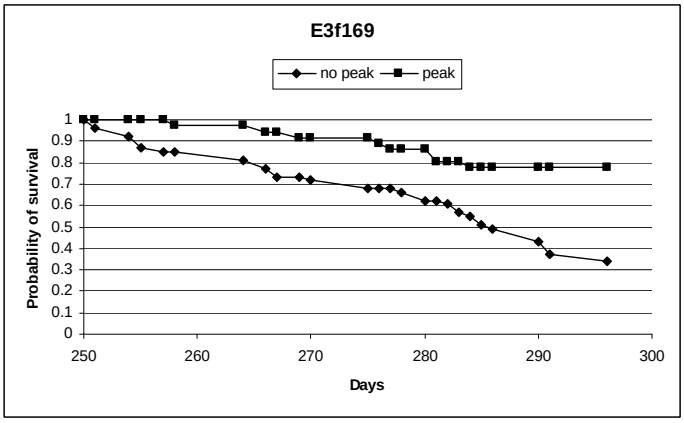
Figures

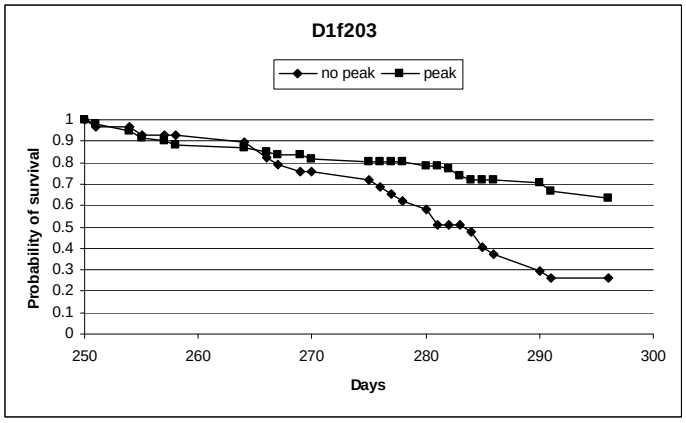

E1f43
no peak
peak
Probability of survival
Days

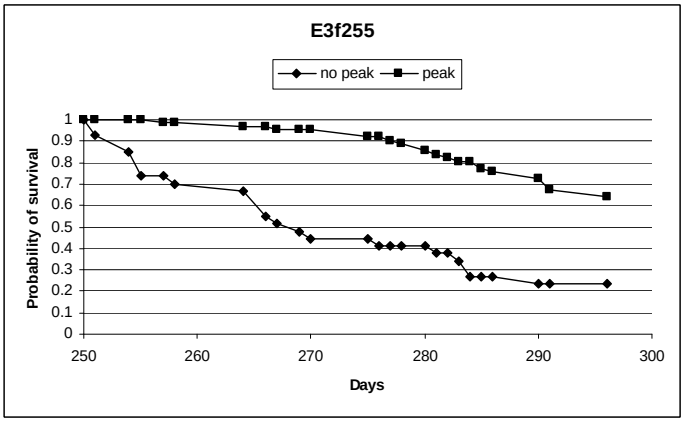

E9f368
no peak
peak
Probability of survival
Days


A3f73
no peak
peak
Probability of survival
Days


A12f429
no peak
peak
Probability of survival
Days


E3f169
no peak
peak
Probability of survival
Days


D1f203
no peak
peak
Probability of survival
Days


E3f255
no peak
peak
Probability of survival
Days

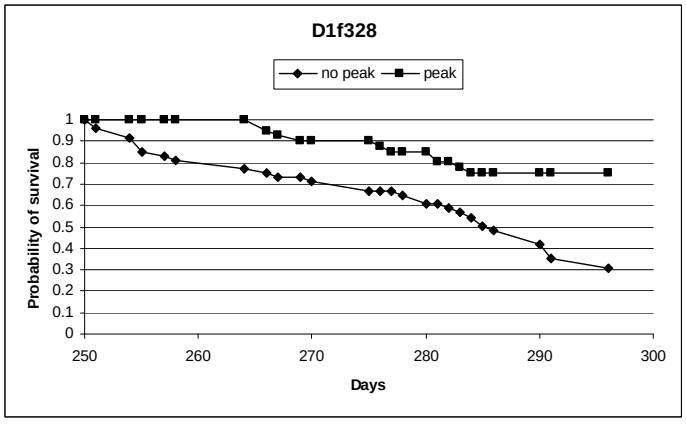

D1f328
no peak
peak
Probability of survival
Days

Figure 1

Figure 1. Kaplan-Meier survival curves for eight AFLP markers (p<0.01) for which the peak-present phenotype corresponded to a resistant allele. Days: number of days after the beginning of the challenge experiment.

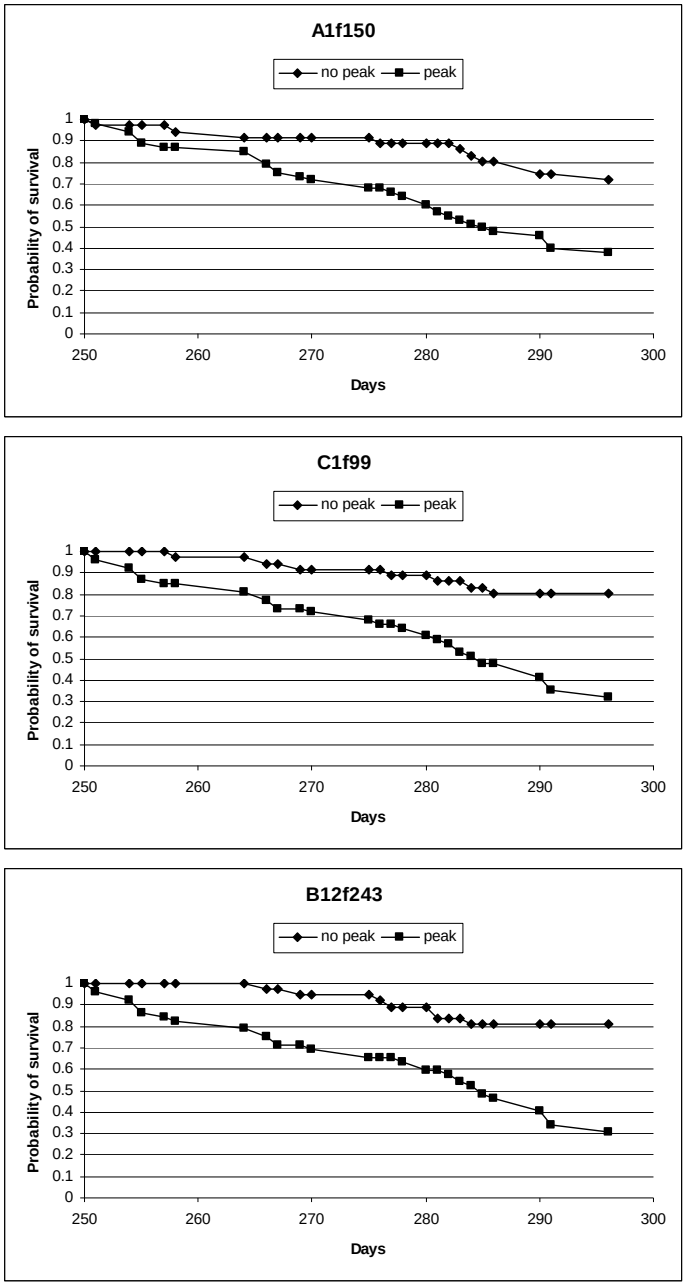
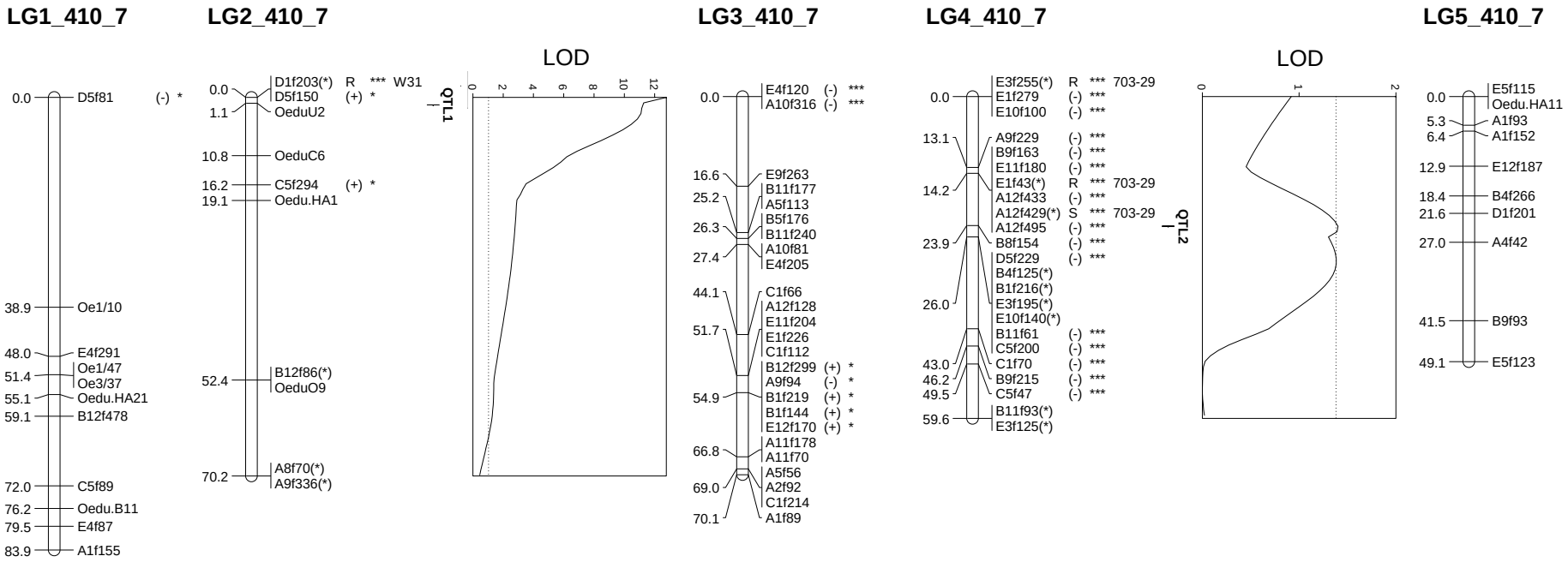


Figure 2

Figure 2. Kaplan-Meier survival curves for three AFLP markers (p<0.01) for which the peak-present phenotype corresponded to a susceptible allele. Days: number of days after the beginning of the challenge experiment.


LG1_410_7
LG2_410_7
LG3_410_7
LG4_410_7
LG5_410_7
LOD
QTL1
QTL2
0.0 D5f81 (-) *
38.9 Oe1/10
48.0 E4f291
51.4 Oe1/47
Oe3/37
55.1 Oedu.HA21
59.1 B12f478
72.0 C5f89
76.2 Oedu.B11
79.5 E4f87
83.9 A1f155
0.0 D1f203(*) R *** W31
1.1 D5f150 (+) *
OeduU2
10.8 OeduC6
16.2 C5f294 (+) *
19.1 Oedu.HA1
52.4 B12f86(*)
OeduO9
70.2 A8f70(*)
A9f336(*)
0 2 4 6 8 10 12
0.0 E4f120 (-) ***
A10f316 (-) ***
16.6 E9f263
25.2 B11f177
A5f113
26.3 B5f176
B11f240
27.4 A10f81
E4f205
44.1 C1f66
A12f128
51.7 E11f204
E1f226
C1f112
B12f299 (+) *
A9f94 (-) *
54.9 B1f219 (+) *
B1f144 (+) *
E12f170 (+) *
66.8 A11f178
A11f70
A5f56
69.0 A2f92
C1f214
70.1 A1f89
0.0 E3f255(*) R *** 703-29
E1f279 (-) ***
E10f100 (-) ***
13.1 A9f229 (-) ***
B9f163 (-) ***
E11f180 (-) ***
14.2 E1f43(*) R *** 703-29
A12f433 (-) ***
A12f429(*) S *** 703-29
A12f495 (-) ***
23.9 B8f154 (-) ***
D5f229 (-) ***
B4f125(*)
B1f216(*)
E3f195(*)
26.0 E10f140(*)
B11f61 (-) ***
C5f200 (-) ***
43.0 C1f70 (-) ***
46.2 B9f215 (-) ***
49.5 C5f47 (-) ***
59.6 B11f93(*)
E3f125(*)
0 1 2
0.0 E5f115
Oedu.HA11
5.3 A1f93
6.4 A1f152
12.9 E12f187
18.4 B4f266
21.6 D1f201
27.0 A4f42
41.5 B9f93
49.1 E5f123

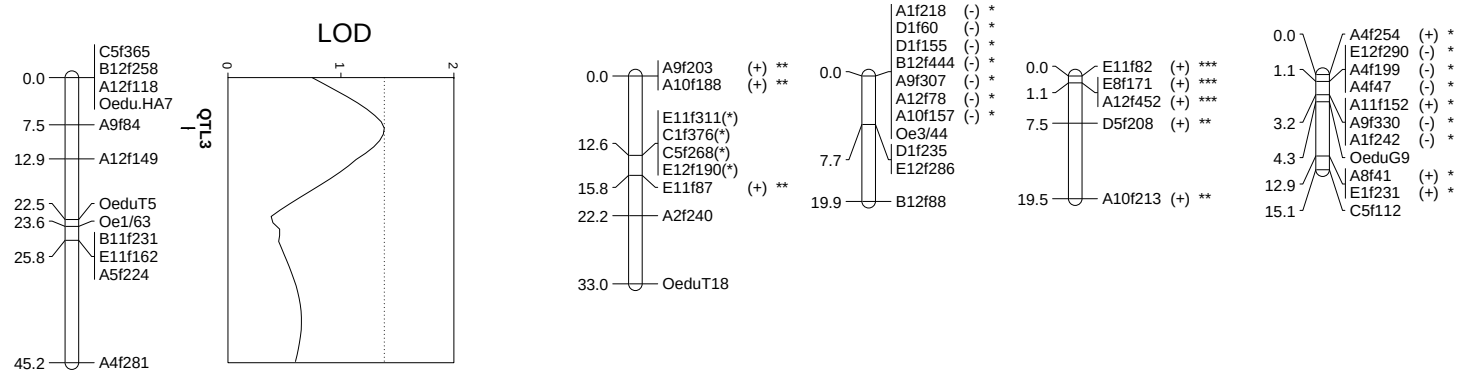

LG6_410_7
LG7_410_7
LG8_410_7
LG9_410_7
LG10_410_7
LOD
QTL3
0.0 C5f365
B12f258
A12f118
Oedu.HA7
7.5 A9f84
12.9 A12f149
22.5 OeduT5
23.6 Oe1/63
B11f231
25.8 E11f162
A5f224
45.2 A4f281
0 1 2
0.0 A9f203 (+) **
A10f188 (+) **
12.6 E11f311(*)
C1f376(*)
C5f268(*)
E12f190(*)
15.8 E11f87 (+) **
22.2 A2f240
33.0 OeduT18
0.0 A1f218 (-) *
D1f60 (-) *
D1f155 (-) *
B12f444 (-) *
A9f307 (-) *
A12f78 (-) *
A10f157 (-) *
Oe3/44
7.7 D1f235
E12f286
19.9 B12f88
0.0 E11f82 (+) ***
E8f171 (+) ***
1.1 A12f452 (+) ***
7.5 D5f208 (+) **
19.5 A10f213 (+) **
0.0 A4f254 (+) *
E12f290 (-) *
1.1 A4f199 (-) *
A4f47 (-) *
A11f152 (+) *
3.2 A9f330 (-) *
A1f242 (-) *
4.3 OeduG9
A8f41 (+) *
12.9 E1f231 (+) *
15.1 C5f112

Figure 3

Figure 3. Microsatellite and AFLP-based linkage map of the flat oyster *O. edulis* in the mapping family OE.F2.05.04: 410_7 parental map obtained with CriMap, 127 markers, 466 cM. AFLP markers are labelled with the primer pair name followed by the letter “f” (for fragment) and a 3-digit fragment size in base pairs. Markers are indicated on the right; and absolute positions on the left (in Kosambi cM). On the right of AFLP locus name are specified the direction of the segregation distortion: towards a deficit (-) or excess (+) of null homozygotes with the statistical significance (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$). R: resistant marker, S: susceptible marker (detected by the approach described in Moen et al., 2004), with the statistical significance and grand-parental origin. Locus name immediately followed by an asterisk (*) correspond to 3:1 type AFLP. Interval mapping results for bonamiosis resistance/susceptibility are shown for linkage groups G2_410_7, G4_410_7 and G6_410_7: the LOD score is plotted against the position (cM) along the linkage group, with the dotted line representing the chromosome-wide significance threshold.

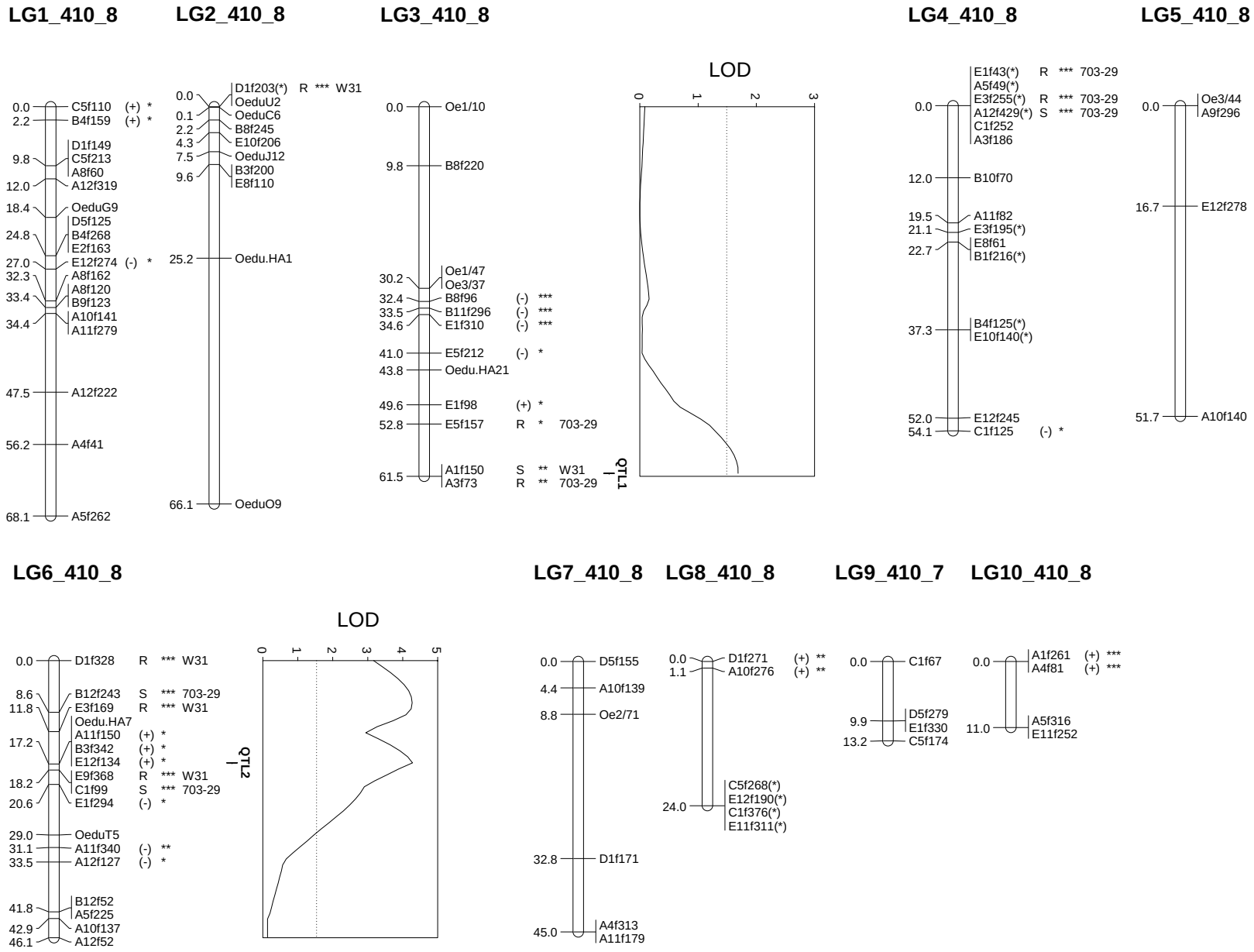

LG1_410_8
LG2_410_8
LG3_410_8
LG4_410_8
LG5_410_8
LOD
QTL1
LG6_410_8
LG7_410_8
LG8_410_8
LG9_410_7
LG10_410_8
LOD
QTL2

Figure 4

Figure 4. Microsatellite and AFLP-based linkage map of the flat oyster *O. edulis* in the mapping family OE.F2.05.04: 410_8 parental map obtained with CriMap, 98 markers, 387 cM. Interval mapping results for bonamiosis resistance/susceptibility are shown for linkage groups G3_410_8 and G6_410_8: the LOD score is plotted against the position (cM) along the linkage group, with the dotted line representing the chromosome-wide significance threshold. See Figure 3 for abbreviations.