Identification and mapping of disease-resistance QTLs in the eastern oyster, *Crassostrea virginica* Gmelin

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Abstract

Identification and mapping of disease-resistance QTLs (quantitative trait loci) is important for our understanding of genetic mechanisms of disease-resistance and for our ability to genetically improve cultured stocks. Disease-resistance is the most important trait for farmers of the eastern oyster (*Crassostrea virginica* Gmelin), which is affected by two major diseases: MSX and Dermo. In this study, the genome of the eastern oyster was scanned with a large number of amplified fragment length polymorphism (AFLP) markers before and after Dermo-inflicted mortalities (53% and 67%) in two reference families. Significant post-mortality shifts in genotype frequency were detected at a large number of loci. Linkage analysis revealed that most markers showing frequency shifts are closely linked to each other on the genetic map, and all markers within a cluster had frequency shifts in the same direction according to their linkage phase. This finding suggests that post-mortality shifts in genotype frequency were not random, but linked to Dermo/summer mortality-resistance QTLs. Twelve putative Dermo/summer mortality-resistance QTLs were identified on female and male maps from two families, providing candidate genome regions for further analysis.

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1. Introduction

The eastern oyster (*Crassostrea virginica* Gmelin) supports important fishery and aquaculture industries in the United States. The oyster industry is seriously affected by two major diseases: MSX (caused by the parasite *Haplosporidium nelsoni*) and Dermo (caused by the parasite *Perkinsus marinus* (Ford and Tripp, 1996). Each of the two diseases alone may kill 50--90% of the affected oysters. The two diseases, along with over-fishing and habitat destruction, are among the leading causes for the collapse of the oyster fisheries in the mid-Atlantic region (MacKenzie, 1996). They are also hindering efforts in oyster restoration and aquaculture.

Although the two diseases are extremely lethal in the eastern oyster, there is considerable evidence that some oysters are genetically resistant or tolerant to the two diseases. Resistance to MSX has been demonstrated by selective breeding, where survival is greatly improved after five generations of selection (Ford and Haskin, 1987). Moderate resistance to Dermo has also been
observed after 4–5 generations of selective breeding (Calvo et al., 2003; Guo et al., 2003). While evidence for genetic determination of disease-resistance is strong, we know little about what and how many genes are involved in determining disease-resistance in the eastern oyster, or their genomic distribution and linkage to other important traits. The identification and mapping of disease-resistance genes or quantitative trait loci (QTLs) may provide valuable information and tools for marker-assisted selection. Marker-assisted selection is particularly useful for the development of disease-resistant oysters because breeding decisions are sometimes made in the absence of disease-exposure (Guo, 2004).

One of the prerequisite for QTL mapping is the availability of a large number of genetic markers. Two types of markers, microsatellites (MS) and amplified fragment length polymorphisms (AFLP), are commonly used for linkage and QTL mapping. MS markers are excellent markers for QTL mapping because of their high levels of polymorphism and co-dominant nature. MS are also expensive to develop and use. In the eastern oyster, only about 25 MS markers are available (Brown et al., 2000; Reece et al., 2004). AFLPs are anonymous and dominant markers that are less transferable and informative than microsatellites, but they can be effectively used in backcrosses as co-dominant markers, and their poor transferability is compensated by the large number of markers that can be quickly developed without prior knowledge of DNA sequences. AFLP markers have been widely used for QTL mapping and breeding in plants (Jin et al., 1998; Goodwin et al., 2003; Bai et al., 1999; Hartl et al., 1999; Altinkut et al., 2003), as well as in aquatic animals (Jackson et al., 1998; Palti et al., 1999, 2001, 2002; Steelman and Kocher, 2002; Shirak et al., 2002; Cnaani et al., 2003). AFLPs have been shown to be effective in linkage mapping in oysters (Yu and Guo, 2003; Li and Guo, 2004).

Another requirement for QTL mapping is availability of reference families where QTLs are well defined and segregating. There is no highly inbred and disease-resistant stock available for making reference crosses in the eastern oyster. On the other hand, high levels of variability in the disease-resistant and wild stocks may provide sufficient segregation of disease-resistance QTLs. Another limitation is that resistance to some diseases (such as MSX) can only be measured by survival. Tissues from susceptible or deceased oysters are not available for genetic analysis. Disease-resistance QTLs can only be identified by markers that show significant frequency shifts after disease-inflicted mortalities. In this study, we tested the feasibility of mapping disease-resistance QTLs in the eastern oyster by screening a large number of AFLP markers before and after disease-inflicted mortalities in two heterozygous families. Our hypothesis is that shifts in frequency after disease-caused mortality are not random, but linked to disease-resistance/susceptibility QTLs on the genetic map. Here we report the identification and mapping of 12 putative disease/mortality-resistance QTLs in the eastern oyster.

2. Materials and methods

2.1. Mapping families and strategy

Two families with different genetic backgrounds were used in this study. The first family, NEI-1, is a pair mating between two oysters from the Rutgers disease-resistant strain NEH. NEH is a strain originated from Long Island Sound that has been selected for MSX-resistance since early 1960s and for Dermo-resistance since 1990. NEH has demonstrated strong resistance to MSX and moderate resistance to Dermo (Ford and Haskin, 1987; Guo et al., 2003). While NEH has lost some rare alleles, it has similar levels of heterozygosity as wild populations (Yu and Guo, 2005). The second family, DNE-1, was a hybrid cross between a wild female from Delaware Bay and a selected male from NEH. There have been suggestions that wild Delaware Bay oysters have developed some resistance to MSX and perhaps also to Dermo (S.E. Ford, personal communication). DNE-1 was included to cover possible QTL segregation through the wild oyster at different loci from the NEH.

The two families were produced in June, 1999 and cultured in an intertidal bag-on-rack system at the Rutgers Cape Shore Facility on Delaware Bay, New Jersey, for field exposure to MSX and Dermo. Mortalities were monitored and recorded monthly or quarterly depending on the season. Each family was sampled quarterly. At each sampling, 100 oysters from each family were randomly selected. Tissue samples from each oyster were collected and archived in a −80 °C freezer. The frequent sampling was designed to identify samples just before and after major mortality events.

To map disease-resistance or high-survival related QTLs, the before and mortality samples from two families (about 400) were first screened with about 110 AFLP markers to identify markers affected by the mortalities, and additional markers (200–300) were genotyped for half of the samples (before or after mortality) to construct linkage maps and map the affected markers. This strategy provided a balance between the need of
screening a large number of individuals to detect frequency shifts and the genotyping of a large set of markers for linkage mapping.

2.2. AFLP and microsatellite genotyping

DNA was extracted from adductor muscle tissue using a QIAamp DNA mini kit (Qiagen, CA) according to supplied protocols. AFLP analysis was conducted primarily according to PE’s (Perkin-Elmer Applied Biosystems, CA) AFLP Plant Mapping Protocol, with some modifications (Yu and Guo, 2003). Adaptors, pre-selective primers, and selective primers were ordered from MWG Biotech Inc. (Charlotte, NC), and PCR reagents were purchased from Promega (Madison, WI). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA). Genomic DNA (∼0.5 μg) was digested with restriction enzymes EcoRI and MseI, and ligated with relevant adaptors overnight at room temperature. Preselective primers complementary to the adaptor sequence only were used to amplify fragments created in the digestion–ligation step. Four microliters of diluted (20 fold) digestion–ligation product was amplified in a 20 μl reaction mixture containing 1.0 μl of each EcoRI and MseI preselective primers, and 15 μl PCR mix. Preselective PCR was run at a temperature profile of one cycle of 72 °C for 2 min, 25 cycles of 94 °C for 25 s, 56 °C for 30 s and 72 °C for 2 min, and one cycle of 60 °C for 30 min. Products from preselective PCR were diluted 20 fold with Tris/EDTA buffer and used as templates for selective amplification. The selective primers had three selective nucleotides at their 3’ ends with the EcoRI selective primer fluorescence-labeled for genotyping.

To detect post-mortality shifts in genotype frequency, six pairs of selective primers were used to screen before and after mortality samples from each family. The six selective primer pairs were EcoRI-ACT/MseI-CAA, EcoRI-ACT/MseI-CAC, EcoRI-ACT/MseI-CAG, EcoRI-ACA/MseI-CTA, EcoRI-ACA/MseI-CTC and EcoRI-ACA/MseI-CTG.

To construct the genetic map for family DNE-1, 16 additional AFLP primer combinations and 9 MS markers (Cvi8, Cvi9, Cvi11, Cvi12 and Cvi13 from Brown et al., 2000; RU001, RU002, RU003 and RU004 from HSRL) were genotyped for post-mortality samples. Primer combinations and markers used are presented as supplemental material (Appendices A and B). The microsatellites were fluorescence-labeled with either FAM or HEX (MWG Biotech Inc., Charlotte, NC). PCR reactions were performed in a 15-μl volume containing 1× PCR buffer, 2.5 mM MgCl₂, 0.2 mM each dNTPs, 0.5 μM of each primer, ∼50 ng genomic DNA, and 0.5 U of Taq polymerase (Promega, Madison, MI), using the following temperature profile: 94 °C for 2 min, then 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 15 s.

Electrophoresis and data collection for AFLP and microsatellites were carried out on an ABI 310 Genetic Analyzer (PE). PCR products, 1.0–1.5 μl per specimen, were added to 0.2 ml sample tubes each containing 12.0 μl deionized formamide and 0.3 μl GeneScan-500 size standard (PE). Samples were denatured at 95 °C for 5 min, and then immediately cooled on ice for 5 min before being loaded onto the ABI 310 Genetic Analyzer. Electrophoresis was conducted using POP4 polymer with the following parameters: injection for 10 s at 15 kV, running for 30 min at 13 kV and 60 °C. Data were collected using the GS STR POP4 A module in the Data Collection Software (version 1.0.2), and analyzed with GeneScan Analysis software (version 3.1). Genotyper software was used to aid scoring genotypes, and electrophoretic histograms were manually examined for genotyping errors.

For NEI-1, linkage maps previously constructed with 17 AFLP primer combinations and some MS and type I markers (Yu and Guo, 2003) were used.

2.3. Data analysis

Only AFLP peaks that were present in one parent, null in the other parent and segregating in the progeny were used. Segregating AFLP loci were scored as peak (or band) present (1 for Aa) or absent (0 for aa) to create binary matrices. Also, only loci with clearly defined peaks were used for data analysis. Frequencies of the Aa genotype were determined for before and after mortality samples; and post-mortality shifts were tested with Fisher’s exact Chi-square test. Segregation ratios before and after mortality were compared with Mendelian expectations using Chi-square test. Linkage analysis for DNE-1 was conducted using Mapmaker 3.0 (Lander et al., 1987). As linkage phase was unknown, each locus was coded in both phases to detect the correct linkage phases. For NEI-1, previously constructed linkage maps from Yu and Guo (2003) were used to determine map position and linkage of affected markers.

3. Results

3.1. Mortality in mapping families

The two families had similar patterns of mortality during the studying period but differed somewhat in
magnitude (Fig. 1). According to the mortality pattern in Fig. 1, samples collected in September 2000 were chosen as the before-mortality samples, and samples collected in November 2002 were used as the after-mortality samples. There was little mortality during the first year of field deployment. Cumulative field mortality before September 2000 was 4.6% for NEI-1 and 3.1% for DNE-1. By the end of this study and at the time of our after-mortality sampling, cumulative mortality in NEI-1 and DNE-1 reached 55% and 68%, respectively. Adjusting for the initial mortalities occurred before September 2000, mortality bracketed by the before- and after-mortality samples was 53% for NEI-1 and 67% for DNE-1. Most of the mortalities occurred during the second and third summer and fall between May and November in 2001 and 2002. Pathological survey at the site during the period indicated that there was heavy infection by Dermo, and only light infection by MSX. The mortality pattern is typical of what we observe under Dermo infections.

3.2. Post-mortality shifts in genotype frequency

About 400 oysters, 100 before and 100 after mortalities from each of the two families, were analyzed with six AFLP primer combinations. DNA extraction and genotyping were successful for most of the oysters screened, and the number of genotyped oysters ranged from 81 to 95 for each sample (Table 1). The six primer combinations generated 108 and 113 segregating loci in families DNE-1 and NEI-1, respectively. For DNE-1, 56 loci segregated through the mother and 52 segregated through the father. For NEI-1, the number of loci segregated through the mother and the father was 52 and 61, respectively. The majority of the loci segregated according to the Mendelian ratio (1:1). The number of loci showing segregation distortion was 21 (19.4%) in DNE-1 and 8 (6.8%) in NEI-1 for the before mortality samples (Table 1). The number of distorted loci increased to 37 (34.6%) in DNE-1 and 15 (12.7%) in NEI-1 after the mortalities.

Genotype frequencies before and after mortality for both families are represented in Fig. 2. Overall,
frequencies in DNE-1 were more variable and showed larger post-mortality shifts than NEI-1. Chi-square test indicated that genotype frequency shifts were significant at 53 loci (49.1%) in DNE-1: 47 at the significance level of \( P < 0.05 \) and 6 at 0.05 \( < P < 0.10 \) (Table 1). Among the loci showing significant shifts, the frequency of the \( Aa \) genotype increased at 28 loci and decreased at 25 loci. In NEI-1, 28 loci (24.8%) showed significant shifts in genotype frequency: 22 at \( P < 0.05 \) and 6 at 0.05 \( < P < 0.10 \). The frequency of the \( Aa \) genotype increased at 9 loci and decreased at 19 loci (Table 1).

3.3. Mapping of affected loci

For mapping analysis in DNE-1, the 22 AFLP primer combinations produced 394 markers. Among the 9 MS used, 7 segregated in the female, 6 in the male and 4 in both parents. Female and male linkage maps were constructed for DNE-1 using 208 and 199 segregating markers, respectively. The female framework map consisted of 145 markers in 12 linkage groups (LGs), spanning 820.8 cM (Fig. 3A). The average inter-marker spacing was 5.7 cM. Sixty-three markers that were linked to the framework map but with uncertain positions were not presented. The male map consisted of 149 markers in 10 LGs with a total genetic length of 464.6 cM (Fig. 3B). The average inter-marker distance was 3.1 cM. Fifty markers whose map position was uncertain were not presented as part of the framework map.

In DNE-1, 46 of the 53 affected loci (or 86.8%) were initially mapped. However, sometimes two markers with different fragment lengths from the same primer combination were completely (or very tightly, \(< 1 \) cM) linked, and we believe they should belong to the same locus. They are likely caused by length polymorphism between the primer binding sites due to microsatellites or short interspersed nuclear elements (SINEs) in between, either in cis or trans. Subsequently, nine such markers were not included in the linkage maps, and 37 of affected loci were presented: 24 to the female and 13 to the male maps. The distribution of the affected markers was clearly not random, and the majority of the affected markers (27 of 37 or 73%) were found in clusters or pairs with intervals less than 20 cM. Furthermore, all affected markers in each cluster or pair had genotype frequencies shifted in the same direction, a strong indication that the association is not accidental. In female LG4, for example, four markers that showed significant \( (P < 0.02 \) or 0.0001) increases in \( Aa \) genotypes were closely linked to each other in a region of about 11 cM (Table 2; Fig. 3A). Similarly, three markers that had significant increases in \( Aa \) genotypes were closely linked to each other in a 3.3 cM region in LG12. In what appeared as an exception, markers \( C6f\text{140} \) and \( F1f\text{090} \) were 8.5 cM apart in LG2 and showed frequency shifts in opposite directions—the \( Aa \) frequency decreased at \( C6f\text{140} \) but increased at \( F1f\text{090} \). However, a close examination indicated that
the two loci were linked in the opposite phase, i.e., the \( a \) allele at \( C6f140 \) is linked to the \( A \) allele at \( F1f090 \), providing a perfect explanation for the opposing shifts in genotype frequency. A total of seven clusters of 2–4 affected markers were found on the female map within a distance of 20 cM, and all markers within each cluster showed frequency shifts in the same direction according to linkage phase (Table 2).

The same pattern of distribution was evident for the male map. Among 13 affected markers that were

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Markers</th>
<th>Female Map</th>
<th>Male Map</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A6f1222, F41101, F8f126, A6f1208, F31145, A71002</td>
<td>C71157, A77348</td>
<td>M7f193, A31006</td>
</tr>
<tr>
<td>2</td>
<td>C8f243, A21076, RU004, A27199</td>
<td>F11124+</td>
<td>Cvi8</td>
</tr>
<tr>
<td>3</td>
<td>C6f140, C71153, M71270, F21232, F11201</td>
<td>C6f146, A11348</td>
<td>C5f1457+</td>
</tr>
<tr>
<td>4</td>
<td>C71140, F11090+, F11207</td>
<td>C5f1278</td>
<td>Cvi7</td>
</tr>
<tr>
<td>5</td>
<td>H3f195, A4f1251, C71194-</td>
<td>C7f104-</td>
<td>A1f324, A2f184</td>
</tr>
<tr>
<td>6</td>
<td>C8f274, A21282, A5f070, F5f1192</td>
<td>C7f191</td>
<td>C7f192</td>
</tr>
<tr>
<td>7</td>
<td>A5f1178, A21276</td>
<td>A6f132, A5f1178, A21276</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>A71186, A21135</td>
<td>C6f121+, A81105</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>A5f1102, A81092, A81104</td>
<td>C6f1068</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>C71135, C18924, A21184, RU002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>A5f1102, A81092, A81104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>C71135, C18924, A21184, RU002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Female (A) and male (B) genetic linkage maps constructed with Family DNE-1 using AFLP and microsatellite markers, showing close linkage among markers with significant post-mortality shifts in genotype frequency. Markers showing significant increase and decrease in the \( Aa \) genotype are suffixed with “+” and “−”, respectively.
mapped, 9 were founded in three clusters of two or more markers (Fig. 3B). Without exception, all affected markers within a cluster had genotype frequency shifts in the same direction (Table 2). As an example, four markers in LG1 (C6f144, C5f480, F1f221 and F1f240) that showed significant increases in Aa genotype frequency were closely linked to each other within a 6.2 cM region.

For NEI-1, the existing linkage map (Yu and Guo, 2003), which included the 113 markers screened before and after mortality in this study, was examined for the distribution of affected markers. In this family, 17 of the 28 affected markers were found on the linkage maps: 11 on the male map and 6 on the female map. Probably because of the small number of affected markers mapped, only two clusters of affected markers were found, one on the male map and the other on the female map (maps not shown, see Yu and Guo, 2003). On the female map, three markers, F1f227, F1f216 and F3f384, showed significant increases in Aa genotype and closely

Fig. 3 (continued).
linked in a 13.8 cM region in LG9 (Table 2). On the male map, three markers, F1f327, F3f082 and F1f119 had significant decreases in Aa genotype and were closely linked to each other within a 14.4 cM region in LG8.

4. Discussion

4.1. Mapping of putative dermo-resistance QTLs

As far as we can determine, this study represents the first genome scan targeting disease-resistance genes in a mollusc. The number of markers used and the maps developed are adequate for genome wide scan in the eastern oyster. The eastern oyster has an estimated genetic length of 500–650 cM based on chiasmata data (Guo et al., unpublished). The number of markers used for before and after mortality screening, 108 in DNE-1 and 113 in NEI-1, has an expected inter-marker spacing of about 6 cM. The male map has 10 linkage groups, which is in agreement with the haploid chromosome number-10 (Longwell and Stiles, 1967; Wang et al., 2005). The female map has 12 LGs including two that are very small (<5 cM) and likely belong to other linkage groups. The genetic maps have moderately high marker densities of 3.1–5.7 cM, which are sufficient for mapping of QTLs and affected markers.

The most significant finding from this study is that post-mortality shifts in genotype frequency are clearly not random, but caused by selective mortality of certain genotypes at specific genome regions. The fact that the majority (73%) of the markers showing significant post-mortality shifts are linked to each other suggests the presence of QTLs influencing disease resistance.

### Table 2

Putative disease-resistance or survival related QTL regions in the eastern oyster identified by linkage of AFLP markers showing significant post-mortality shifts in the “Aa” genotype

<table>
<thead>
<tr>
<th>Family</th>
<th>QTL Linkage group</th>
<th>Marker</th>
<th>Distance (cM)</th>
<th>Coverage (cM)</th>
<th>Genotype frequency Before</th>
<th>Genotype frequency After</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNE-1, female 1</td>
<td>LG2</td>
<td>C6f140</td>
<td>8.5</td>
<td>8.5</td>
<td>0.553</td>
<td>0.436</td>
<td>0.0245</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1f090</td>
<td>14.0</td>
<td>14.0</td>
<td>0.457</td>
<td>0.691</td>
<td>0.0000</td>
</tr>
<tr>
<td>DNE-1, female 3</td>
<td>LG4</td>
<td>C7f340</td>
<td>1.1</td>
<td>11.1</td>
<td>0.489</td>
<td>0.688</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1f176</td>
<td>14.0</td>
<td>14.0</td>
<td>0.484</td>
<td>0.681</td>
<td>0.0001</td>
</tr>
<tr>
<td>DNE-1, male 4</td>
<td>LG1</td>
<td>F1f240</td>
<td>2.2</td>
<td>2.2</td>
<td>0.430</td>
<td>0.585</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C5f457</td>
<td>15.3</td>
<td>15.3</td>
<td>0.495</td>
<td>0.372</td>
<td>0.0166</td>
</tr>
<tr>
<td>NEI-1, female 11</td>
<td>LG9</td>
<td>F1f227</td>
<td>1.1</td>
<td>1.1</td>
<td>0.543</td>
<td>0.372</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1f216</td>
<td>12.7</td>
<td>12.7</td>
<td>0.519</td>
<td>0.404</td>
<td>0.0304</td>
</tr>
<tr>
<td>NEI-1, male  12</td>
<td>LG8</td>
<td>F1f327</td>
<td>0.0</td>
<td>0.0</td>
<td>0.580</td>
<td>0.478</td>
<td>0.0489</td>
</tr>
</tbody>
</table>

* P-value=0.0000 means P<0.0001.

b Frequencies of “aa” genotype are presented due to linkage in opposite phase.
mortality frequency shifts are closely linked in clusters of less than 20 cM on the genetic maps suggests that these markers are located in genome regions that are preferentially affected by the mortalities. The linkage among the affected markers was not accidental, as all affected markers closely linked to each other had genotype frequency shifts in the same direction for all 12 clusters or pairs identified in this study, without a single exception. Therefore, we have good reasons to believe that the 12 clusters or pairs of affected markers represent genome regions where survival-related genes or QTLs reside. This study demonstrates that not only post-mortality shifts in genotype frequency are detectable and non-random, but also they can be used to identify and map disease-resistance QTLs when coupled with linkage analysis.

Mapping disease-resistance genes is a challenge in many organisms, because often disease-resistance cannot be quantified for a given genotype. In the eastern oyster, disease-resistance can only be measured by survival. Susceptible genotypes are not recognized before the oysters die and tissues are rotten. Although intensity of Dermo-infection can be quantified, it may not be a true measure of resistance, as disease-resistance in oysters is often due to increased tolerance of infections (Ford and Tripp, 1996). Therefore, it is difficult to measure and map disease-resistance traits using traditional QTL mapping protocols such as interval mapping (Kao et al., 1999; Robison et al., 2001). The before- and after-mortality analysis is similar to the association-based linkage analysis or linkage disequilibrium mapping that is widely used for QTL mapping (Trongnitz et al., 2002; Wu et al., 2002; Kraakman et al., 2004; Tsuang et al., 2005). The addition of linkage analysis provides not only further confirmation of the marker–trait association, but also the map position of the QTL.

The observed mortalities occurred during a 2-year period. Many factors may have contributed to the mortalities. However, the primary cause for the observed mortalities in this study is undoubtedly Dermo, a lethal disease caused by the parasite P. marinus. Although we did not have infection data on the oysters sampled, disease monitoring at the same site and during the same period found heavy Dermo infections and only light MSX infections (Guo et al., 2003). The MSX infection intensity was too low to cause any significant mortality. Most of mortality occurred between May and November in Year 2 and 3, a pattern that is consistent with Dermo-caused mortalities. Secondly, stresses caused by high summer temperature and reproduction may be responsible for some of the mortalities. Summer mortalities have been reported in several species of molluscs, where stresses from high temperature, low oxygen, reproduction and perhaps opportunistic pathogens are the primary causes (Cheney et al., 2000; Xiao et al., this issue). Although stress-caused summer mortalities are possible, they have not been reported in the eastern oyster. Mortalities in adult eastern oysters are usually caused by MSX or Dermo (Ford and Tripp, 1996). Non-disease caused summer mortality is probably not a significant component of the mortalities observed in this study. We can eliminate predation as a cause because oysters were deployed in cages and no longer vulnerable to predation beyond the first year.

Since Dermo is likely the main cause of the observed mortalities, we argue that most of the QTL regions are likely related to Dermo-resistance. It is possible that some of the 12 QTLs are unrelated to Dermo-resistance or any diseases, but represent genes that affect the survival of oysters under summer conditions. Further studies using lab-based Dermo-challenges are needed to determine which QTLs are specific for Dermo-resistance. Regardless of the nature of the mortalities, all 12 QTLs identified here are important to the survival of the eastern oyster under our culture conditions and therefore potentially important for marker-assisted selection.

Before initiating this study, we had expected to map MSX-resistance genes because of the strong MSX-resistance demonstrated by the Rutgers strains (Ford and Haskin, 1987). Unfortunately, there was little or no MSX infection during this study and therefore, we do not expect that any of the 12 QTLs are specific for MSX-resistance. It is possible that some of the QTLs may represent genes that are important for the general resistance to parasitic infections.

4.2. Family differences

The two families produced considerably different results, possibly due to their differences in genetic background. The most striking difference is that 10 of the 12 putative QTLs are mapped in family DNE-1, while only two are mapped in NEI-1. Both parents of NEI-1 were from the Rutgers disease-resistance strain (NEH), while DNE-1 is a cross between a wild female and a selected NEH male. Family DNE-1 experienced heavier mortalities (67% vs. 53%) than NEI-1, possibly because NEI-1 has stronger resistance to Dermo than DNE-1. DNE-1 had significantly more loci (53) showing significant shifts in genotype frequency than NEI-1 (28). The small number of affected loci in NEI-1 may have made it difficult to find linkage among these markers. It is also possible that most Dermo-resistance
genes in NEI-1 are fixed due to selective breeding, and more resistance QTLs were heterozygous and segregating in the wild maternal parent of DNE-1. If so, the seven resistance QTLs maternally segregating in DNE-1 (out of 10) represent genes with additive effects, where homozygous genotypes for resistance survived better than heterozygous genotypes.

The fact that several QTLs segregated through the wild parent indicates that the wild population contains considerable genetic variability in Dermo-resistance. It has been suggested that wild Delaware Bay oysters have acquired some natural resistance to MSX and perhaps also Dermo (S.E. Ford, personal communication). This study demonstrates the need of using multiple families for QTL mapping in the eastern oyster.

The detection of 12 QTLs confirms that Dermo/mortality-resistance is a multigenic trait. Because the 12 QTL-regions were identified on four different genetic maps, it is possible and even likely that not all of them are independent of each other. On the other hand, the identification and mapping of seven QTLs on one genetic map (female map of DNE-1) suggest that Dermo/summer mortality-resistance is under the control of at least seven QTLs. Because of the nature of AFLP markers, markers segregating through the female cannot be mapped to the male map for comparison and consolidation. Also, the two families shared only a small number of segregating markers. This study shows the power and efficiency of AFLP markers as well as their limitations. The number of co-dominant MS markers used in this study was small and did not allow much cross-family comparison. The addition of more microsatellite and type I markers is needed to facilitate cross-family comparisons, consolidate genetic maps and possibly validate the QTLs identified here in other populations.

4.3. Opposing linkage between larval and adult fitness

For most affected markers studied here, genotype frequencies were in Mendelian ratios before the mortalities and became distorted afterward. However, genotype frequencies of two clusters of markers (QTL7 in DNE-1 female LG12 and QTL12 in NEI-1 male LG8) were distorted before the mortalities, but restored to Mendelian ratios after the mortalities (Table 2). At QTL7, the three affected markers showed significant deficiency of $Aa$ before the mortalities, and at QTL12, the three affected markers had a significant excess of $Aa$ before the mortalities. Markers in both QTL regions returned to the 1:1 Mendelian segregation ratio after the Dermo/summer-caused mortalities. Segregation distortion before Dermo/summer-caused mortalities was likely caused by mortalities during larval culture, because there was no notable mortality from settlement to 1-year of age. Recessive lethal genes have been shown to cause larval mortality and segregation distortion in molluscs (Launey and Hedgecock, 2001; Yu and Guo, 2003; Li and Guo, 2004). Therefore, we argue that these two QTL regions (QTL7 and 12) contain genes that affect larval and adult survival differently or in opposite directions. It can be explained either by a gene that have opposite effects on larval and adult fitness, or the close linkage of two genes, one affecting larval survival and the other affecting Dermo-resistance at adult stage. If the opposing linkage hypothesis is true and widespread, it provides a mechanism for balanced selection and may explain why disease-resistance has been slow to evolve in the eastern oyster. It can also help to explain why it is difficult to purge recessive lethal genes and hence the high genetic load in oysters (Launey and Hedgecock, 2001).

In summary, this study provides the identification and mapping of putative Dermo-resistance or survival-related QTLs for the first time in the eastern oyster. This study demonstrates that the association-based linkage analysis is effective in identifying and mapping disease-resistance genes in the eastern oyster. Although the nature of the 12 QTLs is not known at this time, the association between markers and the mortality is strong, and their linkage to each other is unambiguous. The 12 putative QTLs identified in this study provide candidate genome regions for further analysis and confirmation.

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Appendix A. Supplementary materials

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.aquaculture.2005.10.016.

References


