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

Ziniu Yu [a,b], Ximing Guo [a,*]

[a] *Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, 6959 Miller Avenue, Port Norris, NJ 08349, USA*

[b] *Applied Marine Biology Laboratory, South China Sea Institute of Oceanology, Chinese Academy of Science, 164 West Xingang Road, Guangzhou 510301, People's Republic of China*

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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.

Keywords: Disease-resistance; Linkage mapping; AFLP markers; QTLs; Dermo; Summer mortality; Oyster; *Crassostrea virginica*

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

* Corresponding author. Tel.: +1 856 785 0074; fax: +1 856 785 1544.
E-mail address: xguo@hsrl.rutgers.edu (X. Guo).

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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; Streelman 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