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Breeding for QX disease resistance negatively selects one form of the defensive enzyme, phenoloxidase, in Sydney rock oysters

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Abstract

QX disease in Sydney rock oysters (*Saccostrea glomerata*) is caused by the paramyxean protozoan, *Marteilia sydneyi*. Disease outbreaks occur during summer (January to May) and can result in up to 95% mortality. The New South Wales Department of Primary Industries has been selectively breeding *S. glomerata* for resistance to QX disease since 1996. Previous work suggests that this breeding program has specifically affected the defensive phenoloxidase enzyme system of oysters. The current study more thoroughly characterises the effect of selection on the different forms of phenoloxidase found in oyster populations. Native polyacrylamide gel electrophoresis (native-PAGE) identified five discrete types of phenoloxidase in non-selected (wild type) and fourth generation QX disease resistant (QXR_4) oysters. One electrophoretically distinct form of phenoloxidase, PO^b, is significantly less frequent in resistant oysters when compared to the wild type population. The frequency of PO^b also decreased in both the wild type and QXR_4 populations over the course of a QX disease outbreak. This suggests that possession of PO^b makes oysters susceptible to QX disease and that breeding for resistance has resulted in negative selection against this form of phenoloxidase.

Keywords: *Saccostrea glomerata*; Phenoloxidase; QX disease; *Marteilia sydneyi*; Oysters

1. Introduction

Since the early 1970s, the Sydney rock oyster industry in the eastern Australian states of New South Wales (NSW) and Queensland has been seriously affected by outbreaks of QX disease [1]. QX is an infectious disease mediated by the protozoan parasite, *Marteilia sydneyi* [2]. Epizootics now affect a number of estuaries in NSW including one of the

Abbreviations: CR, native (not farmed) oysters from the Clarence River; DPI, Department of Primary Industries; FSW, filtered seawater; 4-HA, hydroquinone monomethyl ether; MAC, marine anticoagulant; MBTH, 3-methyl-2-benzothiazolinone hydrazone; native-PAGE, native polyacrylamide gel electrophoresis; NSW, New South Wales; QXR_4, fourth generation oysters bred for QX disease resistance.

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State's most productive growing areas. Mortality rates of up to 95% have been reported during some outbreaks. *M. sydneyi* infection generally occurs in mid-summer (January–February) and may be associated with heavy rainfall [3–5].

Peters and Raftos [6] have shown that *M. sydneyi* infects oysters after their immune system, notably the phenoloxidase cascade, has been suppressed. Phenoloxidase is a bifunctional enzyme that is critical to host defence and wound repair in many invertebrates [6–10]. The enzyme has both monophenolase and *o*-diphenolase activities that contribute to a cascade of biochemical reactions transforming tyrosine-based substrates into the pigment, melanin [11–13]. Melanin and a number of intermediate metabolites in the phenoloxidase pathway have immunological functions including antimicrobial activity. Peters and Raftos [6] concluded that suppression of phenoloxidase activity decreases the ability of oysters to control *M. sydneyi*, leading to the development of QX as an opportunistic disease. That conclusion is supported by recent evidence, which shows that *M. sydneyi* is present in many growing areas where QX disease outbreaks do not occur (R. Adlard, unpublished data).

There are currently no farming practices that can control QX and the disease continues to spread [6]. Hence, the development of disease resistant oysters has been made a priority by the NSW Department of Primary Industries (DPI). This government authority has been interbreeding survivors of QX disease outbreaks in the Georges River, Sydney, since 1996. Selective breeding for disease resistance reduced mortality by 22% in the second generation [14], and by almost 40% after the third generation of selection (J. Nell, unpublished data).

Similar mass selection programs have been used to generate resistance to MSX disease in the American eastern oyster, *Crassostrea virginica* [15,16] and to Bonamiasis in the European flat oyster, *Ostrea edulis* [17,18]. In *C. virginica*, strains selected for MSX resistance seem to limit infection by increasing the number of haemocytes in their haemolymph to heal lesions, remove debris and repair tissue [19]. In contrast, selectively bred *O. edulis* have decreased frequencies of hyalinocytes in their haemolymph. These cells may be a target for intracellular infection by *Bonamia ostreae*, so decreases in their frequency could make oysters less susceptible to disease [20].

Despite the success of selective breeding, the process used to obtain broodstock has significant disadvantages. Survivors of disease outbreaks are chosen for interbreeding without understanding what characteristics allowed them to survive or whether those characteristics are heritable. Individuals that remained uninfected by chance may also be included in the broodstock. To remedy these problems, we are investigating markers of QX disease resistance that can be incorporated into DPI's selective breeding program. Our data indicate that breeding for QX resistance has affected the phenoloxidase system of oysters. Newton et al. [21] found that third generation QX disease resistant (QXR_3) oysters had significantly higher phenoloxidase activities than the wild type population, and that QXR_3 and wild type oysters expressed different forms of phenoloxidase.

On the basis of these results, the authors suggested that increased disease resistance in the QXR_3 population was due to selection for different types of phenoloxidase [21]. However, the precise nature of this selection could not be resolved because of the small sample sizes tested and the limited resolution of the native-PAGE system used to identify different types of phenoloxidase. The current study more thoroughly characterises the relationship between different forms of phenoloxidase and QX disease resistance by increasing the sensitivity of the native-PAGE system and by analysing more oysters.

2. Materials and methods

2.1. Sydney rock oysters

Three populations of *S. glomerata*, designated wild type, CR and QXR_4, were used. Oysters were approximately three years old at the time of collection. Wild type oysters were collected from commercial oyster leases in Porto Bay on the Hawkesbury River, NSW (33°34′42″ S, 151°13′40″ E), where they were grown from wild caught spat. At the time of this study, the Hawkesbury River had never experienced an outbreak of QX disease and so oysters collected from this site had never undergone selection for QX disease resistance.

QXR_4 oysters were the fourth generation of oysters bred for QX disease resistance in the Georges River (34°00′ S, 151°10′ E). The Georges River has suffered annual outbreaks of QX disease since 1994. Oysters were grown in trays at Lime Kiln Bar (33°59′08″ S, 151°03′10″ E) and were transferred to Neverfail Bay (33°59′41″ S, 151°04′21″ E) during 2003.