Crossbreeding and inbreeding effects for resistance to white spot disease in Pacific white shrimp in experimental challenges and field conditions
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Summary

The objective of this study was to estimate crossbreeding and inbreeding effects in Pacific white shrimp (Penaeus vannamei) for survival time at 50% mortality in experimental challenges to white spot disease (WSD) and for survival rate to WSD in field ponds with natural infection. Data is based on crosses from a resistance line (RES) from Ecuadorian shrimp with a history of WSD resistance and a line with a high growth potential (GRO), obtained by selection over several generations in a large Mexican hatchery for a higher rate of growth and survival in the absence of specific diseases. Data was analyzed using linear models with crossbreeding effects. Results expressed in relative terms (RES = 100), based on model predictions for pure lines and F1 averages, demonstrate that RES was more resistant than GRO and F1, both in the challenges and field ponds with natural WSD outbreaks. In conclusion, RES is more resistant to WSD than GRO, particularly in field conditions. F1 was less resistant than the average of pure lines, indicating that use of crosses between the lines included in this study is not a good option to increase resistance to WSD. Inbreeding depressive effects for resistance were small in challenges or ponds without disease outbreak. Depressive effects of inbreeding on resistance in naturally-infested field ponds were greater, but changed with line and year, making general predictions on inbreeding effects difficult.

Keywords: crossbreeding, inbreeding, disease resistance challenge, disease field tests, Pacific white shrimp (Penaeus vannamei), white spot disease (WSD)

Introduction

White spot disease (WSD), produced by a virus of the genus Whisopivirus, is currently recognized as one of the most significant causes of economic losses in all areas of shrimp farming in the world (Lightner & Redman, 2010). An option for the control of this disease is the use of organisms with a greater degree of genetic resistance (Cock et al., 2009). The study of the effects of crossbreeding among different populations, including pure lines, crosses and synthetic populations derived from crosses, may help identify the most suitable populations to obtain greater resistance to this disease in specific environments (Gjedrem &
Baranski, 2010). It has been suggested that inbreeding may have a significant role in the development of this disease (Doyle, 2016). Therefore, estimating the effects of crossbreeding and inbreeding for WSD using experimental challenges and field-level data may contribute to the design of breeding strategies for the control of this disease. The objective of this study was to estimate the effects of crossbreeding and inbreeding on survival to WSD in experimental challenges and on percent survival under field conditions with and without WSD outbreaks, in Pacific white shrimp *P. vannamei*.

**Material and Methods**

Each year (2014, 2015 and 2016) animals were produced from crosses between two genetic lines; a resistance line (RES) from Ecuadorian shrimp with a history of resistance to WSD and a line with a high growth potential (GRO) of Mexican origin, obtained by selection over several generations for a higher rate of growth and survival in the absence of specific diseases. In 2014 data were analyzed from pure animals from each line and the cross of males from the RES line with females from the GRO line. In 2015 75%RES 25%GRO and 75%GRO 25%RES animals were added and in 2016 animals with 87.5%RES 12.5%GRO and 87.5%GRO 12.5%RES were added. Smaller numbers of other crosses were also studied. Families of inbred animals were produced in 2015 and 2016 mating males and females from the same family, obtaining an inbreeding level near 25%.

The experimental challenges for WSD were conducted using a virus strain isolated from a field outbreak in Sinaloa, Mexico. Muscle tissue obtained from injected diseased animals, with viral loads greater than 10⁷ copies of viral DNA/g, was cut into small pieces and homogenized and used as inoculum to distribute in small fragments in the water to be consumed *per os*. Seven 1000 L capacity tanks were used, where approximately 5 organisms per family were seeded per tank.

Assessment of survival under field conditions was conducted in 2 ponds located in Sinaloa in 2015 and 3 ponds in 2016, of which two were located in Sinaloa and one in Sonora. A known number of organisms in each pond was seeded at 65 days of age on average and upon reaching harvest age (130 days) the survivors were recovered to obtain the percent survival of each pond. The ponds are identified as Field+ and Field− based on the presence or absence of natural outbreaks of WSD each year. These outbreaks were diagnosed as WSD based on observations of diseased animals in the ponds. The number of full-sib families used in years 2014, 2015 and 2016 were 145, 173 and 125, respectively. Average family sizes used in challenges by year were between 27.4 and 34.2. Average family sizes used in ponds by year were between 40.8 and 57.5.

Data were analyzed by means of cross-sectional linear models (Ødegård et al., 2011) with JMP software (JMP® Version 12.1.0., 2015). The response variable used for the challenges was survival time to WSD in hours at 50% mortality (ST50), since it maximized the observed differences between genetic groups compared to survival time at other mortality rates and final survival rate. The response variable for the field ponds was survival as a binary variable (0, 1). Models used included effects of crossbreeding (direct, heterosis and maternal depending on their estimability). Additionally, the linear effect of the inbreeding coefficient was estimated. Other effects considered in the models to analyze ST50 were the initial age and random tank effects. Predictions of the variables studied for GRO lines, RES lines and F1 crosses were obtained from the results of the analysis of the models, as well as estimates of the effects of inbreeding, expressed as the percent change in trait mean per 10% increase in the inbreeding coefficient (DF10).
Results and Discussion

Expressing the results in relative terms (RES line = 100), the RES line was more resistant than the GRO line and the F1, both in the challenges and field ponds with natural WSD outbreaks (Table 1). The differences between lines in the challenges are similar across years, although they are smaller than those found in the field for Field+ ponds. In the absence of disease in the field, the differences between lines were not significant, which coincides with the results of the control tanks in the challenges (data not shown). The most consistent crossbreeding effects across years (P < 0.05) were direct and heterosis effects.

In the challenges, F1 animals had resistance levels similar to those of the GRO line, indicating a fully recessive mechanism. However, in Field+ ponds, although F1 were below the average of the two pure lines, they were higher than the GRO line.

Table 1. Average relative ST50 in challenges (Chall) or survival in outbreak (Field+) or without outbreak (Field–) field ponds by genetic group based on the predicted values from the model. (Resistance line =100).

<table>
<thead>
<tr>
<th>Year</th>
<th>Genetic group</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chall</td>
<td>Chall</td>
<td>Field+</td>
<td>Field–</td>
</tr>
<tr>
<td>Resistance</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>F1</td>
<td>87.6</td>
<td>97.1</td>
<td>40.7</td>
<td>101.0</td>
</tr>
<tr>
<td>Growth</td>
<td>90.2</td>
<td>91.8</td>
<td>3.3</td>
<td>89.6</td>
</tr>
</tbody>
</table>

1 Average of two ponds

Results from the effects of inbreeding within the GRO and RES lines measured with DF10 were different for each line and each year (Table 2). In the challenges, the effects were relatively small in both lines in the years studied. Under field conditions, the results are different according to the line and the year. In 2015, inbreeding reduced resistance only in the RES line, and in 2016, only in GRO line, indicating that the effects of inbreeding are sensitive to line and environmental effects. Another possible explanation may be some confounding between crossbreeding and inbreeding effects.

Table 2. Average inbreeding effects (DF10) for ST50 in challenges (Chall), or survival with outbreak (Field+) or without outbreak (Field–) ponds by year and genetic group.

<table>
<thead>
<tr>
<th>Year</th>
<th>Genetic group</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chall</td>
<td>Field+</td>
<td>Field–</td>
</tr>
<tr>
<td>Resistance</td>
<td>-2.3</td>
<td>-13.7</td>
<td>-0.6</td>
</tr>
<tr>
<td>Growth</td>
<td>5.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1 Average of two ponds

2 A value too high and positive was replaced by zero

Conclusions
The RES line is more resistant to WSD than the GRO line, particularly in field conditions. F1 was less resistant than the average of pure lines, indicating that use of crosses between the lines included in this study is not in principle a good option to maximize resistance to WSD. Inbreeding depressive effects for resistance were small in challenges or ponds without disease outbreak. Depressive effects of inbreeding on resistance in naturally-infested field ponds were greater, but changed with line and year, making general predictions on inbreeding effects difficult.

**List of References**