IDENTIFICATION AND CHARACTERISATION OF QUANTITATIVE TRAIT LOCI AND GENDER SPECIFIC MARKERS IN THE KURUMA PRAWN, *Penaeus japonicus*

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SUMMARY
Following the domestication of some prawn species including *Penaeus japonicus*, there has arisen opportunity for selective breeding for improved farming of these species. A intermediate F_2 cross family (between low and high growth, HL X LH) of *P. japonicus* was developed for the identification of growth QTLs. Genetic mapping of this family was performed using a selective genetic mapping strategy with 102 animals using amplified fragment length polymorphism (AFLP) markers. The final QTL analyses identified two chromosome regions that had significant effects on the growth traits. Bulk Segregation Analysis has been performed to identify other informative markers surrounding the major growth-associated QTL, and all markers associated with QTLs have been further characterised. Gender-specific markers were also identified and assessed for their conservation across unrelated families.
Keywords: *Penaeus japonicus*, genome mapping, QTL, growth, sex determination

INTRODUCTION
Penaeid prawn farming is one of the most important aquaculture industries globally, with production of thousands of tonnes of farmed prawn throughout Asia and America. Whilst prawn farming in Australia is only a fledgling industry, factors such as vast areas of suitable coastal land and a relatively low incidence of diseases place Australia in a favourable position for future growth.

Farming of Penaeid prawns is at present reliant upon wild-caught broodstock, a practise that may be deleterious to future growth of the industry. The reliance upon wild broodstock is seen to be risky, inefficient and precludes the opportunity to enhance production through selective breeding and controlling the spread of disease. Recently the life cycle of a number of species including *P. japonicus* has been closed, opening the way for genetic improvement. Breeding objectives include improvements in growth, in-pond survival and disease resistance (Hetzel *et al*., 1997).

Prawn aquaculturalists also desire the ability to generate monosex pond populations. The present system of mixed sexes is inefficient due to a number of factors. Firstly female prawns are larger and have an improved feed conversion rate compared to their male counterparts. Therefore the ability to produce all-female ponds would generate greater profits to the farmers. Also male-associated fighting and other reproductive stresses associated with mixed sex populations have been suggested to be a major influence on poor pond performance. At present little is known about the mechanisms of sex determination in

prawn species. The identification of markers associated with gender could potentially lead to improved understanding of molecular aspects of sex determination in prawns.

Previous research by CSIRO in collaboration with industry has generated breeding populations of P. japonicus with improved growth rates through selective breeding (Hetzel *et al*., 1997). Utilising these populations in properly designed breeding programs, Moore *et al*. (1999) developed a preliminary linkage map *for P. japonicus* by generating Amplified Fragment Length Polymorphism (AFLP) DNA markers. More recently, Li *et al*. (2003) used an increased number of animals to produce a more comprehensive linkage map for detection of loci controlling commercially-important traits. The objective of the present study was to identify and validate potential growth-associated QTL and gender-specific markers, and to characterise sequence surrounding these markers.

MATERIALS AND METHODS

Animals. Three production traits were measured on prawns of the intermediate F_2 cross family (HL x LH) at the harvest age (PL155, PL156 or PL157). These include body weight (WT), total body length (TL) and carapace length (CL). One hundred and two progeny were selected from the top and bottom 8% of the size distribution. Genotyping was performed using 54 pairs of AFLP primer combinations (Li *et al.*, 2003).

DNA. DNA was extracted from pleopods using QIAGEN QIAamp Tissue Kit according to manufacturer's instructions.

QTL detection. Prior to QTL analysis, the least square analyses of variance using PROC GLM (SAS Institute, Version 8.2) were performed to the three phenotypic traits on 443 individuals in order to adjust for any significant environmental effects. The residuals from these analyses were then combined with molecular marker data for QTL analysis on the animals selected for genotyping (102 individuals). The QTL Cartographer computer package v. 1.16 (Basten *et al*., 2002) (http://statgen.ncsu.edu/pub/qtlcart/) was used to screen the genome for evidence of QTL influencing WT, TL and CL.

Bulk Segregation Analysis. Bulk Segregation Analysis (BSA) was used to identify new markers within QTL regions. Thirty-four new AFLP primer combinations were screened against two parents and 6 bulks, each bulk consisted of pooled DNA from 6 individuals with or without flanking markers. AFLP analysis was carried out essentially as described by Vos *et al*. (1995). DNA (50-100 ng) was digested with *EcoRI* and *MseI* prior to ligation of restriction site-specific adaptors. AFLP fragments were analysed on 5% polyacrylamide sequencing gels running with 1x TBE and visualised by autoradiography. Genetic screening of the full mapping population of 102 individuals was carried out using 11 AFLP primer combinations chosen from the pre-screening processor.

Sequence Analysis. The AFLP bands associated with QTL regions or gender-specific were isolated and sequenced, and confirmed by Southern hybridisation to be the mapped markers. Sequence surrounding

these AFLP clones was initially characterised by inverse PCR. Polymorphisms were confirmed by PCR in a number of animals. Once polymorphisms were confirmed genome walking strategies including Universal Genome Walker Library generation (Clontech) and library screening were employed to characterise the genomic regions upstream and downstream of these clones.

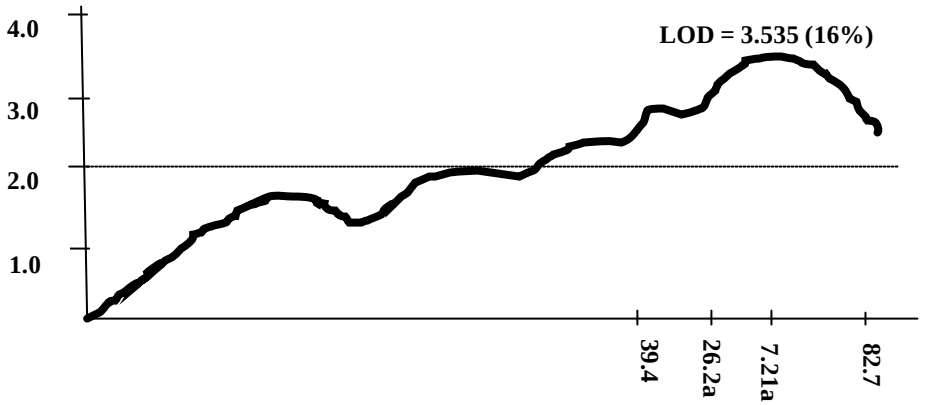
RESULTS AND DISCUSSION

QTL identification. The linkage analyses with 1:1 segregation AFLP markers resulted in a paternal map consisting of 43 linkage groups (217 markers) with the genome coverage of 1780 cM, and a maternal map of 33 linkage groups (125 markers) covering 1026 cM. The average density of markers was approximately 1 per 10 cM. (Li *et al*. 2003). Based on the map information, the QTL analysis using the interval mapping and composite interval mapping methods identified two putative QTL regions on a paternal map having pleiotropic effects on WT, TL and CL. These QTLs each explained between 7 and 14% of the phenotypic variation of the traits. This is the first time that the QTL regions have been identified for penaid species. BSA analysis and sequence characterisation was performed on the QTL region contributing 14% phenotypic variation.

BSA Analysis. BSA was used as a means of generating new markers within region. For the second and more influential of the two regions the LOD peak was identified at the end of the chromosome (Marker C on Figure 1), and so it was determined that flanking markers to the right of the peak were needed to accurately access this region. Of the 34 new primer combinations assessed, 11 primer combinations produced bands with the predicted banding pattern. Genetic screening of the full mapping population of 102 individuals was therefore carried out using these 11 AFLP primer combinations. Thirty seven new polymorphic markers were identified, 19 markers were ordered into 15 linkage groups of the paternal map and 11 markers in 10 linkage groups (8 existing ones and 2 new ones) of the maternal map. The new genome coverage was 2032 cM for the male map and 1106 cM for the female map. BSA analysis proved fruitful in that one marker was successfully identified to the right of the major QTL peak (Marker D on Figure 1)

Characterisation of QTL sequence. Four AFLP markers (A-D Figure 1)) were isolated, cloned and sequenced. Confirmation that sequenced clones corresponded to the AFLP marker of interest was obtained by repeating the AFLP reactions using known positive and negative samples, followed by Southern blotting of the products and hybridisation using the appropriate AFLP clone as probe. Attempts are currently underway to identify relevant polymorphisms and characterise sequences surrounding these markers.

Figure 1. Mapping of the major growth-associated QTL with characterised markers



Sex markers. Two AFLP markers were identified to be only present in females. Both markers were mapped to a single locus on one chromosome of the maternal map. These are particular of interest as they may suggest that the female is the heterogametic sex in this shrimp species. This would also explain why the male map contains more markers than the female map, as is suggested by the Haldane rule (Haldane, 1922). These gender-specific markers were characterised in a similar manner to that used for QTL-associated markers. One marker was found to be highly repetitive throughout the prawn genome making it impossible to further characterise. However flanking sequence of the second marker has been characterised and a deletion resulting in the loss of an *EcoR1* cleavage site identified in males. Early results suggest that this polymorphism is not confined to family 107. Attempts are currently being undertaken to assess this marker in other non-related specimens.

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