Aquaculture 434 (2014) 476–485

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Quantitative trait loci and genetic association analysis reveals insights into complex pearl quality traits in donor silver-lipped pearl oysters

David B. Jones [a,b,*], Dean R. Jerry [a,b], Mehar S. Khatkar [c], Gerhard Moser [c,d], Herman W. Raadsma [c], Joseph J. Taylor [e], Kyall R. Zenger [a,b]

[a] Centre for Sustainable Tropical Fisheries & Aquaculture, James Cook University, Townsville, QLD, Australia
[b] School of Marine and Tropical Biology, James Cook University, Townsville, QLD, Australia
[c] Reprogen — Animal Bioscience, Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia
[d] Dairy Futures Cooperative Research Centre (CRC), Bundoora, Victoria, Australia
[e] Atlas Pearls and Perfumes, Pertokoan Sanur Raya 18-19, Jalan Bypass Ngurah Rai Sanur, Denpasar Selatan, 80227 Bali, Indonesia

ARTICLE INFO

Article history:
Received 7 June 2014
Received in revised form 21 August 2014
Accepted 26 August 2014
Available online 4 September 2014

Keywords:
Pearl oysters
Pinctada maxima
Pearl quality
Quantitative trait loci
Genome-wide association study

ABSTRACT

Pearl oysters are commercially farmed for their gemstone quality pearls worldwide and are an important animal model for understanding bivalve biology. However, despite their economic and scientific significance, limited quantitative genetic studies have been undertaken to identify genes that regulate important pearl quality traits and unique biological characteristics (i.e. biomineralisation). Over the last decade, pearling industries worldwide have shown strong interest in genetic stock improvement aiming to increase the production of high quality 'South Sea' pearls. However, before genetic breeding programmes can be initiated, the genetic architecture of such traits needs to be elucidated. This study investigates the genetic architecture of complex pearl quality traits (pearl size, weight, surface complexion and colour) and presents the first putative quantitative trait loci (QTL) and genetic associations to these commercially important pearl quality traits.

To identify QTL and genetic associations to pearl quality traits, a total of 2114 pearl grading records were recorded over 342 pearl oysters. Utilising these phenotypic records, this study provides strong evidence that pearl quality traits have a low to moderate additive genetic component (h^2 from 0.14 to 0.34) and supports previous quantitative genetic studies that these traits are polygenic in nature. A total of nine putative QTL and 25 marker associations for pearl colour, one QTL for pearl surface complexion and three genetic associations to pearl size and weight were identified using 11 half-sib families. The majority of QTL and genetic associations were detected for pearl colour whereby the most prominent QTL were located within a 2 cM interval on LG10. QTL in this region were mapped for four out of five sub-categories of pearl colour and explained from 32% to 46.1% of the phenotypic variation observed in pearl colour. Segregation in multiple families provides further support that genes localised to this region have significant effects on pearl colour.

The segregation of these preliminary QTL and detection of genetic associations provide insights into the genetic architecture of pearl quality traits and will direct further research into the establishment of genetic breeding programmes for pearl quality within the *Pinctada maxima* pearling industry.

1. Introduction

The highly sought-after white, silver, and gold 'South Sea' pearls produced by the silver-lipped pearl oyster, *Pinctada maxima*, are the leading cultured pearl products worldwide (Torrey and Sheung, 2008). In view of their commercial importance, traditional genetic evaluations of commercially valuable pearl oyster traits (i.e. oyster shell growth and pearl quality) have been undertaken aiming to further improve pearl farming profitability (Wada and Jerry, 2008). However, previous genetic improvement programmes have been limited by their sole reliance upon phenotypic records and the genetic complexity of pearl production traits which stems from the nature of the pearl production process. For example, commercial pearl production involves pearl seeding, a procedure where tissue is cut from a donor oyster (termed the 'saibo' tissue) and surgically implanted along with a nucleus seed into a host oyster (see Taylor and Strack, 2008). Since both the donor and host oyster tissues are known to be expressed during the production of a pearl (Arnaud-Haond et al., 2007), this procedure obscures selection since

* Corresponding author. Tel.: +61 456 195 836.
E-mail addresses: david.jones051986@gmail.com (D.B. Jones), dean.jerry@jcu.edu.au (D.R. Jerry), mehar.khatkar@sydney.edu.au (M.S. Khatkar), gerhard.moser@bigpond.com (G. Moser), herman.raadsma@sydney.edu.au (H.W. Raadsma), joseph@atlaspearlsandperfumes.com.au (J.J. Taylor), kyall.zenger@jcu.edu.au (K.R. Zenger).

http://dx.doi.org/10.1016/j.aquaculture.2014.08.038
0044-8486/

the production of one pearl potentially involves the genomes of two animals (host and donor oysters). Recent studies have begun to dissect the contribution that host and donor oysters have on various pearl quality traits (McGinty et al., 2011, 2012).

In addition to the complications that arise from host and donor contributions, quantitative genetic studies have revealed that pearl quality traits, which drive the value of a pearl, are influenced by a multitude of genetic and environmental factors (e.g. genetic stock and environment dependent growth patterns), and have low to moderate heritabilities (from 0.06 to 0.25, Jerry et al., 2012). Nevertheless, the reported heritabilities, albeit low to moderate, suggest that these pearl quality traits can be improved through genetic selection programmes and it has been suggested that the incorporation of molecular tools [i.e. marker assisted selection (MAS)] would vastly improve selection accuracy compared to conventional breeding programmes (Wada and Jerry, 2008).

The genetic dissection and improvement of complex traits with low heritabilities has been one of the most challenging tasks in animal production (Goddard and Hayes, 2009; Hayes et al., 2009). Recent advances in molecular genetics and genome technologies (i.e. rapid, cost-effective genomic sequencing and high-throughput genotyping) have opened up new possibilities in this field by facilitating the development of genomic resources necessary for unravelling the genetic architecture of complex traits (Liu, 2011; Siu et al., 2011).

Pearl quality traits such as pearl size, weight, colour, lustre, and surface complexion define the commercial value of a pearl. Numerous studies investigating the variability of shell (and pearl) nacre colouration and pearl weight have been undertaken for *Pinctada fucata* (Wada, 1984, 1985, 1986a, 1986b, 1990; Wada and Komaru, 1994, 1996). These studies have shown that both traits are potentially correlated (i.e. pearls with yellow nacre were significantly heavier than those with non-yellow nacre; Wada, 1986a). In addition, recent quantitative genetic studies in *P. maxima* have confirmed their heritability and reported high correlations between pearl quality trait such as size, surface complexion, shape and colour (Jerry et al., 2012; Ky et al., 2014). To harness this underlying additive genetic potential for these pearl quality traits within MAS breeding programmes, genetic associations to phenotypic variants of commercial interest need to be identified. The characterisation of many pearl quality quantitative trait loci (QTL) and marker associations may lead to more efficient breeding programmes using MAS programmes and will contribute to a better understanding of shell and pearl biomineralisation.

The recent development and validation of comprehensive genome-wide SNPs (Jones et al., 2013b) and high-density genetic linkage map (Jones et al., 2013a) for *P. maxima*, along with the acquisition of phenotypic data for traits of commercial interest (Jerry et al., 2012), now make it possible to identify QTL and genetic associations to pearl quality traits, a feat not yet attempted in pearl oysters. This study aims to conduct a first pass quantitative analysis of pearl quality traits by utilising QTL analysis and genome-wide association studies (GWAS) to identify genomic regions harbouring genes of major effect and markers that are correlated with pearl quality traits. Such information not only provides insights into pearl trait architecture, but hopefully will be eventually useful within selective breeding programmes for *P. maxima* to preselect candidate oysters prior to pearl quality testing, thus shortening the generation interval and increasing genetic gains.

2. Materials and methods

2.1. Experimental animals, initial oyster grow-out and pearl seeding

The collection and rearing of the experimental donor oysters, production of pedigrees for QTL analysis, tissue collection and DNA extraction methods utilised within this study are described in a companion study published in Jones et al. (2014-in this issue). Oyster grow-out and pearl nuclei seeding was undertaken as part of a previous research project and are described in Jerry et al. (2012). Briefly, oysters were bred from wild caught broodstock and families were grown-out under commercial conditions. Once oysters were large enough for pearl seeding (18–22 months, dorsal ventral shell measurement ≥120 mm; Gervis and Sims, 1992), a total of 585 oysters chosen at random from families were designated as donors to provide saibo mantle tissue for seeding. A thin layer of saibo tissue from the mantle of these donor oysters was cut into multiple 3×3 mm^2 pieces before being implanted into the gonads of the 9810 remaining host oysters along with a pearl nucleus. Host and donor oyster pairs were only made between oysters originating from the same parental broodstock population to limit levels of environmental variation between individuals. To reduce nuclei rejection rates after implantation, oysters were conditioned so that they were in an active stage of gametogenesis prior to seeding. Numerous environmental and technical factors can influence variation observed in pearl quality traits, such as the surgical seeding operation, grafting techniques, and seeding technician. Considering this, variation across these factors were kept to a minimum (i.e. the same five seeding technicians at the Bali culture site were used throughout the experiment) and were recorded so they can be incorporated as fixed effects in downstream quantitative analysis. After seeding, oysters were transferred to a single long line for a ten week recovery period before being split again between two grow-out locations, Bali and Lombok, Indonesia, for the pearl grow-out period.

2.2. SNP genotyping and half-sib families

A total of 1147 informative single nucleotide polymorphisms (SNPs) that have undergone thorough data integrity (described in Jones et al., 2013b) were utilised in the current study (average SNP call rate per individual was 99.5%). All family and pedigree relationships were verified using SNP genotypic data produced from the iSelect arrays in Cervus version 3.0 (Kalinowski et al., 2007), as described in Jones et al (2014-in this issue). A total of 11 maternal half-sib families consisting of 342 individuals (average of 31 individuals per family) with known phenotypic records and successful SNP genotypes were utilised for QTL analysis. The pedigree structures of these families are presented in Jones et al. (2014-in this issue).

2.3. Phenotypic evaluation of pearl quality traits

Phenotypic data was gathered for pearl quality at harvest (multiple records per oyster) from 342 donor oysters with pedigree and genotypic information as described in Jerry et al. (2012). For oysters that produced pearls at harvest (18–22 months after seeding), the pearls were individually collected, cleaned and graded for pearl size (mm), weight (g), shape, lustre, surface complexion, and colour. All pearls were graded by professional pearl graders following commercial pearl grading systems to maintain precision and consistency in pearl quality data. Since saibo tissue from donor oysters were seeded multiple times into many hosts, multiple pearl grading records were collected for each donor oyster. From all 9810 pearl nuclei seedings, a total of 2114 pearl grading records were documented from the 342 donor oysters with pedigree and genotypic information. As the genetic contribution to pearl biomineralisation is predominantly driven by the donor oyster (McGinty et al., 2011, 2012), pearls produced from the seeding of saibo tissue from the same donor oyster were considered replicates for their respective donor oyster. The number of replicates per donor oyster ranged from 1 to 19 with an average of 6.4 (Fig. 1).

2.4. Statistical data analysis: genetic parameters and estimated breeding values (EBVs)

Pearl size and weight were analysed as continuous normally distributed traits. For pearl surface complexion and pearl colour, raw phenotypic data was re-categorised into ordered semi-quantitative categories best reflecting discrete categories as follows: pearl surface