Aquaculture 465 (2016) 134–143

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# QTL fine mapping and identification of candidate genes for growth-related traits in bighead carp (*Hypophthalmichehys nobilis*)

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ARTICLE INFO

*Article history:*
Received 2 June 2016
Received in revised form 13 August 2016
Accepted 29 August 2016
Available online 31 August 2016

*Keywords:*
Bighead carp (*Hypophthalmichthys nobilis*)
QTL
Fine mapping
Candidate genes
Growth traits

ABSTRACT

Bighead carp (*Hypophthalmichthys nobilis*) is a popular Asian aquaculture species, but its genetic improvement is still in infancy. Marker-assisted selection (MAS) can improve the selection efficiency of breeding programs in fish. In this study, we constructed a genetic linkage map in a F1 family of bighead carp using microsatellite markers. A total of 905 microsatellites were assigned onto 24 linkage groups (LGs) of a consensus map, which spanned 1631.7 cM of bighead carp genome with an average interval of 1.8 cM. Comparative genomics revealed a high level of genomic synteny between bighead carp and zebrafish. QTL mapping for growth traits was performed based on this linkage map, and three significant and 8 suggestive QTL associated with four growth traits (body length, body height, head length, body weight) were detected on LG9 and LG17, with 18.6–25.5% of phenotypic variance explained. Three candidate genes for growth were identified in or near the QTL intervals, and a SNP in one of the three genes, *TP53BP2*, was significantly associated with growth traits in different populations of bighead carp. These results of the high-density SSR genetic map and genome scan for QTL provide a basis for MAS and breeding programs for growth in bighead carp.

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## 1. Introduction

Growth is an important trait of interest in economic aquaculture animals, and it is controlled by multiple genes (quantitative trait loci, QTL) and environmental factors (Mackay, 2001; Massault et al., 2008). Faster growth rate is one of the main goals of many breeding programs for fish because it shortens rearing time and increases benefits for aquaculture industry. Traditional methods of genetic improvement have relied mainly on family and individual selection based on phenotype and pedigree information (Hulata, 2001). With the development of molecular biotechnology, marker-assisted selection (MAS) has been developed and applied in many fish genetic breeding programs. Besides its accuracy and efficiency, MAS is less labor and time consuming compared with traditional breeding methods (Sonesson and Meuwissen, 2009). The first step of MAS is to identify available genetic markers or genes associated with target traits. QTL mapping based on the phenotypic and genotypic data of mapping family provides a powerful method of founding these relationships on a genome-wide scale (Lande and Thompson, 1990; Mackay et al., 2009).

Genetic linkage map is an essential tool for QTL mapping and other genetic and genomic researches, such as comparative genomics, positional gene cloning, gene-centromere mapping and genome assembly (Lynch and Walsh, 1998; Yue, 2014). Compared with other popular molecular markers, microsatellite, or simple sequence repeat (SSR) marker is one of the best options for linkage map construction because of its many merits, such as abundance in genome, uniformly distribution, high polymorphism, co-dominant inheritance, long flanking sequences, ease of detection by PCR, and easy accession by other laboratories via published primer sequences (Liu and Cordes, 2004). Several high-density genetic linkage maps have been constructed based on microsatellites for aquaculture species, such as Asian seabass (Wang et al., 2011a), Japanese flounder (Song et al., 2012b), half-smooth tongue sole (Song et al., 2012a). QTL for disease resistance, salinity and temperature tolerance, sex determination and growth traits have been identified in various fish species based on microsatellite-based genetic maps (Laghari et al., 2014; Yue, 2014). Given the importance of production in aquaculture, QTL for growth traits have been investigated in over 20 aquaculture species, such as rainbow trout (Wringe et al., 2010), Atlantic salmon (Gutierrez et al., 2012), Arctic charr (Kuttner et al., 2011), European sea bass (Louro et al., 2016), large yellow croaker (Xiao et al., 2015), Japanese flounder (Song et al., 2012a), small abalone (Ren et al., 2016), triangle pearl mussel (Bai et al., 2015) and Chinese mitten crab (Qiu et al., 2016). A lot of candidate genes and molecular markers have been identified based on the results of QTL mapping and provide useful tools for MAS programs to improve the efficiency and precision of fish breeding (Liu et al., 2014; Wang et al., 2015b; Xia et al., 2013).

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http://dx.doi.org/10.1016/j.aquaculture.2016.08.039
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Bighead carp is one of the most important commercial aquaculture fish in China, and it is not only consumed as edible fish but also used to control water quality in many other countries. The global aquaculture production of this species was 3.25 million tons and its economic value reached 4.19 billion dollars in 2014 (FAO). However, natural populations of bighead carp have seriously declined during recent decades due to ongoing human activities such as overfishing and pollution. Worse still, unscientific artificial releasing and frequent flooding may lead to a mixture of natural and cultured individuals, which has resulted in in-breeding depression and reduction of growth performance (Liu et al., 1997; Zhu et al., 2014). Furthermore, the traditional selective breeding program is inefficient in bighead carp because of its relatively long generation interval (about 4–5 years). Hence, it's necessary to deploy MAS program and accelerate the breeding program for bighead carp. Although some genetic and genomic resources have been developed in bighead carp, such as microsatellite markers (Feng et al., 2014; Guo et al., 2013a; Zhu et al., 2013a), the first and second generation genetic linkage map (Liao et al., 2007; Zhu et al., 2014), microsatellite-centromere mapping (Zhu et al., 2013b) and comparative genomics (Zhu et al., 2015), the genetic architecture of economic traits remains unknown.

The objectives of this study include: (i) construction of a high-density SSR-based linkage map; (ii) QTL fine mapping for growth traits; (iii) identification of candidate genes associated with growth traits. The high-density linkage map will build a foundation for fine QTL mapping and detection of candidate genes that may affect growth traits in bighead carp, a large domestic cyprinid fish with significance to China's and world aquaculture industry.

## 2. Materials and methods

### 2.1. Mapping family

Adult male and female bighead carp were collected from different sections of the Yangtze River and raised in muddy ponds at the Zhangdu Lake Fish Farm (Wuhan, China). In May 2011, genetic distances among 50 matured bighead carp were evaluated by using 10 polymorphic microsatellite markers. 5 dams and 5 sires with relatively higher genetic distance (0.21–0.30) were selected to produce a multiple-family population by mass crossing in artificial propagation. About 2000 fry were raised in an about 0.6 ha (60 $hm^2$) muddy pond. In December 2011, 840 progenies were randomly collected and five growth-related phenotypic traits were measured, i.e. body length (BL), body height (BH), head length (HL) and body weight (BW). Fin tissues were sampled from both parents and progenies and preserved in anhydrous ethanol at 4 °C. Genomic DNA was extracted from fin tissues following a standard phenol-chloroform protocol (Sambrook and Russell, 2001). All parents and progenies were genotyped by 8 highly polymorphic microsatellites to verify parent-offspring relationships. Parentage assignment for the mixed families was estimated using the likelihood-based approach with the program Cervus 3.0 (Kalinowski et al., 2007), and each progeny was correctly assigned to a single parental pair (100% success). Finally, a F1 full-sib family with 90 progenies exhibiting high variation in growth traits was selected as the mapping population for genetic map and QTL in this study. The correlation analysis among four growth traits and normal distribution test for four growth traits were implemented using a software package SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

To verify our QTL results in different population and different environment, in May 2012, 6 dams and 6 sires were randomly selected to produce multiply-family population by mass crossing in artificial propagation. A total of 36 families were produced and then raised in the same muddy pond. In December 2012, 414 progenies were randomly collected and four growth-related phenotypic traits (BL, BH, HL and BW) were measured. Two extreme groups (large size group: 41 fishes, and small size group: 41 fishes) for growth traits were selected from the verification population and used for association analysis.

### 2.2. SSR markers and genotyping

To test the feasibility of PCR application and genetic segregation in the mapping panel, a total of 4325 microsatellite markers were used in the initial screening. These microsatellite markers were recruited from four sources: (1) 800 bighead carp markers (Arsd) and 1912 silver carp markers (Hysd) were developed from genome sequencing (Guo et al., 2013b; Zhu et al., 2014), (2) 406 markers (HysdE) were developed through silver carp transcriptome sequences (Feng et al., 2014), (3) 775 markers were developed from three microsatellite-enriched genomic libraries, with 559 from bighead carp assigned with the prefixes 'ArGA' and 'ArGT' (Guo et al., 2013b; Zhu et al., 2014), 216 from silver carp assigned with the prefixes 'HyGT' (Guo et al., 2013b; Zhu et al., 2014), (4) 432 microsatellites which were developed and used in silver carp genetic map with the prefixes 'Hym' (Zhang et al., 2010) were also applied in this study.

All microsatellite markers mentioned above were applied to initial polymorphism screening by genotyping two parents and four progenies. Those segregated markers were subsequently genotyped in the whole mapping population to generate genotype data for the construction of a genetic linkage map in bighead carp. PCR reactions were carried out in a ABI Veriti 96 well thermal cycler with a total volume of 12.5 μl, containing 30–50 ng of template DNA, 1.25 μl of 10× reaction buffer, 1 U of Taq polymerase (TaKaRa, Japan), 0.5 μl of dNTP (2.5 mmol/l), 0.5 μl of forward and reverse primer mixture (2.5 μmol/l each) and water to the final volume. The PCR programs were as follows: 94 °C for 4 min, 35 cycles at 94 °C for 35 s, optimal annealing temperature (Additional File 1) for 35 s, 72 °C for 40 s, and a final extension of 72 °C for 10 min. The PCR products were separated on 8% polyacrylamide gels (PAGE) and visualized by the JS-780 gel imaging system (Peiqing, China) after staining by ethidium bromide.

### 2.3. Map construction and estimation of genome size

The linkage analysis was implemented by JoinMap 4.0 program (Van Ooijen, 2006) using the CP (cross pollination) population type, and five CP separation types (nn × np, lm × ll, hk × hk, ef × eg, ab × cd) were presented in two parents and 90 progenies. Markers with >10% missing data were eliminated from further analysis. Chi-square tests were performed to determine the fitness of marker segregation data to the expected 1:1 ratio. Distorted markers were also used for linkage analysis, and they were indicated with asterisks on LGs. The sex-averaged linkage map was constructed using the function of "Create Population Nodes" in the JoinMap 4.0 program. A logarithm of odd (LOD) threshold of 5.0 was set for clustering markers into linkage groups (LGs). Graphical representations of linkage groups were created using the software MapChart 2.2 (Voorrips, 2002). LGs were named according to the chromosome assignments corresponding to homologous groups of the previous linkage map for bighead carp (Zhu et al., 2014).

The estimated genome lengths for consensus map were calculated based on two different approaches: (1) Ge1 was calculated as the method described (Fishman et al., 2001), (2) Ge2 was calculated by a different method (Chakravarti et al., 1991). The estimated genome lengths (Ge) are the averages of the lengths calculated by these two methods.

### 2.4. Comparative genomics with zebrafish genome

Flanking sequences of the SSR markers assigned on the genetic map of bighead carp were used to search against *Danio rerio* genome (GRCz10) using NCBI-BLAST-2.2.31+ (BLASTN) with the expect value (e-value) less than $1e^{-10}$. If a single marker sequence was aligned to the *D. rerio* genome with multiple positions, only the alignment with the lowest e-value was reserved. The genomic synteny between bighead carp and zebrafish was visualized using the software Circos v0.67 (Krzywinski et al., 2009).

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