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Mapping quantitative trait loci and identifying candidate genes affecting feed conversion ratio based onto two linkage maps in common carp (*Cyprinus carpio* L)

Cuiyun Lu [a], Muhammad Younis Laghari [b,c], Xianhu Zheng [a], Dingchen Cao [a], Xiaofeng Zhang [a], Youyi Kuang [a], Chao Li [a], Lei Cheng [a], Shahid Mahboob [d,e], Khalid A Al-Ghanim [d], Shu Wang [b], Guoliang Wang [f,g], Jing Sun [h], Yan Zhang [b,*], Xiaowen Sun [a,*]

[a] National Local Joint Engineering Laboratory for Freshwater Fish Breeding, Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin 150070, China
[b] CAFS Key Laboratory of Aquatic Genomics and Beijing Key Laboratory of Fishery Biotechnology, Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences, Beijing, 100141, China
[c] Department of Fresh Water Biology and Fisheries, University of Sindh Jamshoro, Sindh 76080, Pakistan
[d] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Zoology, Government College University, Faisalabad, Pakistan
[f] Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China
[g] University of Chinese Academy of Sciences, Beijing 100049, China
[h] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

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ABSTRACT

Feed efficiency is an economically important trait in aquaculture, which can be measured traditionally as feed conversion ratio (FCR). Because of the difficult measurement, genome-wide selection using quantitative trait loci (QTLs) affecting FCR may be an alternative for genetic improvement. In the present investigation, QTLs for FCR based on two mapping panels (mirror carp and hybrid carp panels) were found in common carp (*Cyprinus carpio* L.). After that, candidate genes were identified by comparative genomics. A total of nine QTLs, two genome-wide and seven linkage group-wide, were detected in eight linkage groups (LGs) in the mirror carp panel (FAM-A, n = 68) and nine QTLs, four genome-wide and five linkage group-wide, were detected on eight linkage groups of the hybrid carp panel (FAM-B, n = 92). Two genome-wide QTLs affecting FCR were identified in two LGs (Lg1 and Lg21) in FAM-A, which explained 32.3% and 35.6% of the phenotypic variation respectively; four genome-wide QTLs affecting FCR were detected in four LGs (LG5, LG21, LG24, and LG33) in FAM-B which explained 29.3%–33.4% of the phenotypic variation. All of eight QTL regions from FAM-A were aligned to the high-resolution linkage map with whole genomic scaffold and all genes mapped on, and 18 genes associated with growth or metabolic function were identified using the whole-genomic browser on http://www.carpbase.org/gbrowse.php. We believe that these 18 genes are valuable candidate genes affecting feed efficiency, that might be used in MAS programs to improve performance in common carp.
Statement of relevance: All animal experiments was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and it is hereby clearly indicated that such guidelines had been followed.

1. Introduction

Feed cost is a major input, often comprising 30% to 70% of the variable costs in almost an animal production system, including aquaculture (Goddard, 1996; Doupé and Lymbery, 2003). Improvements in the efficiency of feed utilization would lead to increased economic returns in the fish production system. Selection of efficient animals not only improves the producer's profitability, but can lead to significant increases in production per unit area, decrease feed cost, as well as reduced environmental impact (Basarab et al., 2003). The most commonly used measure of feed efficiency has traditionally been feed conversion ratio (FCR), which is the ratio of feed consumed to gain body weight. Selection to improve FCR has the potential to increase growth rate in young animals because the two traits (growth rate and feed conversion) are genetically correlated (Sherman et al., 2014). More parameters can be evaluated to quantity feed efficiency in domestic animals, but it is difficult for aquaculture because of difficult manipulation (Sun, 2010). So, at present, the FCR is most important in aquaculture for the description of growth as a function of feed intake.

* Corresponding authors.
E-mail addresses: zhangy@cafs.ac.cn (Y. Zhang), sunxw2002@163.com (X. Sun).

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Feed conversion efficiency is a heritable trait in fish, and very high genetic correlations are found between growth rate and feed conversion, frequently ranging from 0.80 to 0.95 (Andersen, 1977; Vangen, 1984; Crawford, 1990). Based on its heritability and substantial phenotypic variation, FCR has the potential for inclusion in selection criteria to improve feed efficiency and the profitability of fish production (Ponzoni et al., 2008; Saatchi et al., 2014; de Oliveira et al., 2014). However, individual feed intake measurements are needed for direct selection, and these are complicated to apply. This problem could be resolved if genetic markers predictive of feed intake were available. Consequently, there has been considerable recent research to develop genetic markers that can be used to select animals for improved feed efficiency.

In most aquaculture species, feed accounts for about 65–75% of the total production cost (Gjedrem and Baranski, 2009). Even in the salmon production industry, feed accounts for about 50% of the total cost (Marine Harvest, 2012). However, feed conversion rate is difficult to measure on selection candidates, although it has major effects on the productivity and profitability of many aquaculture species (Yue, 2014). Feed intake of each individual is generally difficult to measure in aquaculture species due to unequal feed intake over days and the requirement of a single tank to raise each fish in each of the reference families.

Improving the feed efficiency trait not only will decrease the farmer's stocking expenses, but also will shorten the rearing period (Laghari et al., 2014). Progress to identify FCR-related genetic markers has been made by assessments of single markers and genome scans. Although a few QTLs associated with feed efficiency traits in common carp (*Cyprinus carpio*) have been reported (Laghari et al., 2014), not all of the genetic variation in these traits has been captured because of inadequate sample size or studies limited to a single population. The extent of genetic variation for feed efficiency traits among different common carp populations remains unexplored. In recent years, various genome resources and genetic tools have been developed to facilitate genetic improvement and breeding programs, including multiple versions of linkage maps (Sun and Liang, 2004, Zhang et al., 2013b; Zheng et al., 2013), a BAC-based physical map (Xu et al., 2011; Li et al., 2011), cDNA microarrays (Williams et al., 2008), and a SNP genotyping array (Xu et al., 2014a). Moreover, the genome of Songpu mirror carp, a strain derived from the European subspecies (*C. carpio carpio*) of common carp, has been completely sequenced, providing the first reference genome for common carp genetic and genomic studies (Xu et al., 2014b). These research contents provide opportunities to identify trait-associated genetic markers and candidate genes.

In this study, QTL intervals related to the FCR trait were researched on two matched linkage groups of two small families of common carp. These two panels were constructed from full-sib families from mirror carp (FAM-A) and a hybrid, produced by crossing Heilongjiang carp and Hebao carp (FAM-B). Further, some candidate genes for FCR were predicted by comparative genomics using a high-density genetic map and a reference genome.

2. Materials and methods

2.1. Animals and phenotypic data

A total of 160 individuals from two full-sib families were used in this analysis. Out of these, 68 offspring were from FAM-A and 92 from FAM-B. FAM-A was a mirror carp panel obtained from the Songpu Aquaculture Experimental Station, Harbin, China. FAM-B, consisting of hybrids, was produced by crossing one distantly related male Heilongjiang carp (*Cyprinus carpio* var. *haematopterus*) to one female Hebao carp (*Cyprinus carpio* var. *wuyuanensis*).

The fish were stocked individually, in order to achieve accuracy of feed consumption, in a series of re-circulating aquarium systems, each with a size of 0.5 m^3. The initial average body weight (BW) was 60.27 ± 18.42 g and 82.28 ± 18.84 g for FAM-A and FAM-B, respectively. Experimental fish were fed with a local commercial feed (Tongwei Feed, The feed contain >34% crude protein and 5% crude fat, which meets aquaculture industry standard of China: the formula feed for common carp (SC/T1026-2002).) thrice a day (9:00 am, 12:00noon and 3:00 pm) of 10% BW during the experiment. All the conditions of the tanks, such as water temperature (22 °C) and water flow rate ($1m^{s-1}$), were regularly maintained throughout the experiment. Leftover feed and faeces in each tank were siphoned out daily. The water levels in the aquariums were maintained on a daily basis and a complete water change was done every week. The residue of feed was collected and dried at room temperature and deducted from the feed weight supplied to know the accurate feed consumption of fish. The BW measurements of individual fish were taken fortnightly on an electronic scale (Kern 572) for the period of three months. The FCR was calculated from the relationship of feed intake and weight gain, by the following formula:

$$FCR = m1w - m0w/mcw$$

(m1w = final mass and m0w = initial mass; mcw = amount of food consumed)

2.2. Update of FAM-A linkage map

The FAM-A and FAM-B linkage maps were separately constructed by Jin et al. (2012) and Zhang et al. (2013a, 2013b). The FAM-A linkage map covered 62 linkage groups with a total of 507 markers (186 SSRs and 321 SNPs). The FAM-B linkage map covered 51 linkage groups using a total of 307 markers (140 SSRs and 167 SNPs). Genotyping of the SNPs was performed using the Illumina Golden Gate assay on the Bead Station 500G Genotyping System (Illumina Inc., San Diego, CA), according to the manufacturer's protocol for the Golden Gate assay (Shen et al., 2005). Microsatellite markers were genotyped using the ABI 3730 DNA sequencers (Applied Biosystems, Foster City, CA).

101 SSR markers selected from the FAM-B linkage map (Zhang et al., 2013a, 2013b) and the high-density map (Xu et al., 2014b), were polymorphic and genotyped in the FAM-A family by using ABI 3730 DNA sequencers (Applied Biosystems, Foster City, CA) in order to further similarity search between FAM-A and FAM-B. These markers were used to update the FAM-A linkage map. The updated linkage map in FAM-A was constructed by JoinMap version 4.0 (Van Ooijen, 2006). There were three possible segregation patterns for parents (1:1; 1:2:1; 1:1:1:1) for performing linkage analysis with default significance levels of 3.0–8.0 LOD with a step of 1.0. The final linkage maps were constructed using LOD thresholds of 4.0. A Student's *t*-test was used to test significance of differences in the mean recombination fraction between adjacent markers. The Kosambi mapping function was used to convert recombination frequencies into map distances (centimorgan, cM). Linkage groups were graphed using Mapchart version 2.2 (Voorrips, 2002).

2.3. QTL mapping

We then conducted a QTL analysis using the marker genotype data and phenotypic data for the two families of progeny in MapQTL 6.0 (Van Ooijen, 2009). Multiple QTL model (MQM) mapping was utilized to detect any significance associated with phenotypic traits and marker loci in the data sets. Cofactors are selected by multiple regression and backward elimination. The LOD score significance thresholds were calculated by permutation tests in MapQTL 6.0, with a genome-wide significance level of $\alpha < 0.01$, $n = 1000$ for significant linkages, and with a linkage-group-wide significance level of $\alpha < 0.05$, $n = 1000$ for suggestive linkages (Churchill and Doerge, 1994; Doerge and Churchill, 1996).