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Mapping quantitative trait loci for flesh fat content in common carp (*Cyprinus carpio*)

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ABSTRACT

Flesh fat content is considered to play an important role in flesh quality in fish. Therefore, improving the fat content of fish fillets is one of the breeding goals in aquaculture. To provide data for genetic improvement and pave the way for marker-assisted selection (MAS) of flesh fat content in common carp (*Cyprinus carpio*), a genome-wide scan for quantitative trait loci (QTL) affecting flesh fat content was conducted on eight full-sib families containing a total of 522 progenies. All families were genotyped using 250 informative microsatellite markers and then a dual QTL mapping strategy was employed. Initially, the QTL analyses were conducted using a sib-pair model to take advantage of the full-sib pedigree structure. A half-sib model was then used to account for the large differences between the male and female recombination rates in common carp. Using these strategies, a genome-wide significant QTL was detected at 14 cM on LG31. This QTL explained 36.2% of the phenotypic variance. In addition, three QTL were identified on LG3, LG42, and LG45 at the chromosome-wide significant level with phenotypic variance explained that had a range of 15.3–19.5%. The identified QTL can potentially be applied in MAS programs, and can be explored further to help understand the biology of fat deposition in common carp.

1. Introduction

For more than half a century, the aim of common carp (*Cyprinus carpio*) breeding programs have been focused mainly on growth and feed conversion efficiency, to reduce the cost of production. With the development of the aquaculture industry, flesh quality traits (e.g., carcass and fillet yields, fat content, fatty acid profiles, flesh color, and muscle fiber) have also become important to the aquaculture industry and consumer market because they are related directly to human health and nutrition (Alsted et al., 1995; Gjedrem, 1997). Although flesh quality is influenced by a large number of factors including muscle characteristics, nutrition, and environmental conditions, genetic effects play a particularly important role in establishing meat quality (Nguyen et al., 2010).

Studies have shown that muscle fat content is the most important attribute of flesh quality because it is associated with flesh texture, flavor, and juiciness (Johansson et al., 2000). Fat content in fish muscle is generally influenced by the availability of dietary energy, growth rate, temperature, exercise, and steroid supplementation (Fauconneau et al., 1995). However, genetic origin is an important factor that affects fat content because it can influence fat content from feeding (Fauconneau et al., 1991). Heritability is an extremely important parameter in quantitative genetics. In general, improvements in phenotypic traits through selection are dependent on the heritability and selection intensity (Fletcher et al., 2012). In fish, the estimate of the heritability for fat percentage is fairly high. Kocour et al. (2007) found that the heritability for mean muscle percent fat within Hungarian synthetic mirror carp was 0.58, indicating the prospect of achieving a rapid genetic gain in the improvement of fat content by selection in common carp. In Atlantic salmon, the heritability for fat percentage was reported to be from 0.19 to 0.38 (Rye and Gjerde, 1996; Quinton et al., 2005; Vieira et al., 2007; Powell et al., 2008). In rainbow trout, a medium to high heritability for muscle fat (0.25–0.40) has been estimated (Quillet et al., 2005; Tobin et al., 2006). These moderate estimates of heritabilities for muscle fat content confirm that there is a considerable amount of genetic variation available for genetic improvement through selective breeding programs or in combination with marker-assisted selection (MAS).

Although the genetic parameters of fish fat traits have been the focus of many studies, the genetic architecture of those traits is still not understood. The genome-wide scan strategy can be used to detect markers linked to a chromosomal region harboring a quantitative trait locus (QTL), and identification of such markers can help in the implementation of marker-assisted breeding schemes. For fat percentage, a preliminary genome-wide QTL scan carried out in five full-sib Atlantic salmon

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families has been reported (Derayat et al., 2007). In the study, a QTL at the genome-wide significance level was detected on linkage group LNS16, and a microsatellite marker (Ssa0016NVH) tightly linked to the QTL was found. To date, only one genome-wide association scan for fillet fat content has been reported in Atlantic salmon in which five high-scoring, single-nucleotide polymorphisms (SNPs) for fat content trait were detected on chromosomes 9 and 10 (Sodeland et al., 2013). QTL for traits related to flesh quality in fish, such as flesh color (Baranski et al., 2010), muscle fiber (Zhang et al., 2011), and fatty acid (Xia et al., 2014), have been reported in the recent years.

The main aim of the present study was to identify QTL that control fat content in the flesh of the common carp using eight full-sib families. One significant and three suggestive QTL for fat content in fish fillets were detected. To our knowledge, this is the first report of QTL mapping of flesh fat content in common carp.

2. Materials and methods

2.1. Source of fish and phenotypic measurements

A commercial broodstock of 60 brooders, including 40 female and 20 male fish, was selected based on the prior records of good performance indicating high genetic value. In May 2009, 30 full-sib families were constructed by artificial combination and propagation. The larvae were cultured in hatchery cages for 50 days post hatch (dph), and then approximately 2000 fish fry were selected randomly to feed in the same pond. After rearing the fish for two years, we determined the genetic diversity and paternity relationships among 30 families contained 991 fish using 25 microsatellite loci. Eight families containing 522 individuals were selected from 30 families for genotyping and QTL analysis. Total lipids were extracted from the dorsal muscles of each fish and determined in duplicate according to Folch et al. (1957). The mean results from the two samples were used in the statistical analyses. All animal procedures were approved by the Animal Care and Use Committee of Heilongjiang River Fisheries Research Institute prior to this study.

2.2. Genotype data and linkage map

DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK) following manufacturer's protocol. To achieve wide coverage within and across the linkage groups (LGs), microsatellite markers were chosen for the genome scan based on their locations in the consensus linkage map of the common carp (Zhang et al., 2013). A total of 250 informative microsatellites (Table S1) from 50 groups were selected to genotype the eight full-sib families. Genotyping was performed on an ABI 3130xl Genetic Analyzer (Applied Biosystems) after PCR amplification using fluorescently-labeled primers as described in our previous study (Zhang et al., 2013).

The linkage between all the markers was evaluated initially using the "two-point" option in Crimap version 2.4 (Green et al., 1990). A LOD (logarithm of the odds) score of >3.0 was considered as significant linkage between markers. Markers showing significant linkage were then grouped and, for LGs with more than two markers, the most likely marker order was evaluated using the "build" and "flipsn" options in Crimap. The genetic distance between the markers was then calculated using the in Crimap "fixed" option.

2.3. QTL analysis

QTL data were analyzed using a regression-interval mapping method (Knott et al., 1996), as implemented in the web-based software package GridQTL (Seaton et al., 2006). Marker information contents were also obtained using the software. QTL analyses were carried out using a dual mapping strategy. In the first method, analyses were conducted using a sib-pair model to take advantage of the full-sib pedigree structure and the genotypes of the parents. Using body weight as a covariate, across-family analyses were carried out for each LG to test for evidence of QTL segregation in all families for which information was available. This was followed by within-family analyses to identify specific families segregating for putative QTL.

Take into account the disparity in female and male recombination rate (female:male ratio was 4.2:1) (Zhang et al., 2013). QTL scans were also carried out using half-sib analyses. Briefly, the detection of QTL in the initial genome scan was based on the sire analysis because the low recombination rate gives greater power to detect QTL using low resolution with few markers per LG (Hayes et al., 2006). Subsequently, dam analyses were carried out for the LGs that showed evidence of QTL segregation in the male map, because the larger female map has greater resolving capacity to locate putative QTL. The proportion of within-family phenotypic variance explained (PVE) by a QTL was calculated according to the method described by (Knott et al., 1996). In the sire-based analysis, the formula $h^2_{QTL} = 4[1 - (MSE_{full} / MSE_{reduced})]$ was used, where MSE_{full} is the mean squared error of the model including the QTL, and $MSE_{reduced}$ is the mean squared error of the model fitting only a family mean. Accordingly, the PVE by a putative QTL was calculated from the combined sire and dam half-sib analyses according to the formula $h^2_{QTL} = 2\{[1 - (MSE_{full} / MSE_{reduced})^{sire}] + [1 - (MSE_{full} / MSE_{reduced})]^{dam}\}$.

To identify the most likely position of a QTL, *F*-statistic values were generated at 1-cM interval on each LG for each analytical approach. Chromosome-wide significance thresholds were determined empirically by a permutation analysis (Churchill and Doerge, 1994) with 10,000 permutations. Genome-wide thresholds were calculated by applying a Bonferroni correction, $P_{genome\text{-}wide} = 1 - (1 - P_{chromosome\text{-}wide})^n$, where n represents the number of chromosomes (Knott et al., 1998). QTL that exceeded a chromosome-wide *F*-statistic threshold of $P < 0.05$ were reported as suggestive QTL, whereas QTL that exceeded a chromosome-wide *F*-statistic threshold of $P < 0.01$ were considered to be empirical evidence for significant QTL. The confidence intervals (CIs) were calculated using a bootstrapping approach (Visscher et al., 1996).

3. Results

3.1. Phenotypic variation

The eight full-sib families (F234, F275, F4039, F171, F217, F373, F336, and F259) were chosen for QTL analyses on the basis of them having larger number of progenies (45–107), with an average family size of 65 (Zheng, 2012). The flesh fat content (FC) and body weight (BW) for the eight families, as well as the number of individuals, mean value, standard deviation, and maximum and minimum values, are shown in Table 1. The mean, standard deviation for FC and BW within each family were: F234 (FC = 1.56 ± 0.74, BW = 612.44 ± 159.10); F275 (FC = 1.54 ± 0.69, BW = 603.23 ± 181.59); F4039 (FC = 1.54 ± 0.74, BW = 527.19 ± 204.79); F171 (FC = 1.66 ± 0.85,

Table 1
Description statistics of fat content and body weight for the eight full-sib families of the common carp.

Family	*n*	Fat content			Body weight		
		Mean(SD)	Max	Min	Mean(SD)	Max	Min
F234	107	1.56(0.74)	4.11	0.60	612.44(159.10)	951.10	250.80
F275	70	1.54(0.69)	4.13	0.59	603.23(181.59)	991.70	234.80
F4039	70	1.54(0.74)	3.72	0.62	527.19(204.79)	907.40	225.30
F171	69	1.66(0.85)	4.00	0.67	598.96(181.26)	1064.50	280.10
F217	65	1.71(0.95)	4.30	0.48	475.87(186.74)	109.80	239.20
F373	50	1.51(0.68)	3.42	0.47	589.76(167.53)	985.30	287.60
F336	46	1.57(0.77)	3.44	0.51	555.55(165.86)	893.40	333.10
F259	45	1.37(0.47)	2.83	0.46	722.85(236.05)	1086.10	274.20

n, number of individuals; Mean, average value; SD, standard deviation; Min and Max, minimum and maximum values, respectively.