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*

ARTICLE INFO

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.

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 m³. 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 ($1 m^{s-1}$), 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).

2.4. Identification of candidate genes

All of the QTL candidate intervals in family FAM-A were selected to anchor in the high-density genetic map (Xu et al., 2014b), which included all common carp genomic data as contigs and scaffolds. To identify candidate genes, all sequences of these genomic regions (eight QTL intervals) were checked on the carpbase website (http://www.carpbase.org/index.php). All genes and candidate genes in these genomic regions were searched manually for gene function in model animals, including zebrafish (http://www.ncbi.nlm.nih.gov).

3. Results

3.1. Phenotypic trait

The phenotypic values of FCR for FAM-A ranged from 43.3% to 95.1% with an average of 65.6% ± 10.9%, and which accorded with the normal distribution ($P = 0.335$). Meanwhile, the mean FCR of the FAM-B was 37.0% ± 10.6% (range: 15.1%–65.7%, $P = 0.605$). The Pearson correlation between FCR and body weight (BW) was significant in both mapping panels, i.e., $r = 0.725$, $P < 0.01$ and $r = 0.756$, $P < 0.01$, respectively, for FAM-A and FAM-B.

3.2. Construction of linkage map of FAM-A and comparison with linkage map of FAM-B

A total of 535 markers were mapped to fifty linkage groups of FAM-A, including 101 new microsatellite markers and 434 published markers (Jin et al., 2012). The new linkage groups contained between 4 and 40 markers, with an average of 10.7 markers per group. The total length of the inherited linkage map was 2245.85 cM. The map distance between markers ranged from 1.52 cM to 160.34 cM with an average of 4.63 cM between markers.

Ninety-two shared markers were assigned in both FAM-A and FAM-B and used for similarity searches between the two linkage maps. A total of 24 matched linkage groups with more than two shared markers were identified between the two maps, providing insight into similar QTL intervals in the two families (Fig. 1). Two of these 24 matched groups, two linkage groups of FAM-B were mapped onto unique group of FAM-A, i.e., Ch 3 and 6 of linkage group FAM-B mapped onto Lg1 of FAM-A, and Ch 4 and 23 of FAM-B group compared into Lg21 of FAM-A (Fig. 1).

3.3. QTL analysis in FAM-A

QTLs related to FCR were located on various linkage groups on the linkage map of FAM-A. Nine QTLs (*qFCRM1*, *qFCRM7, qFCRM12, qFCRM16*, *qFCRM21-1*, *qFCRM21-2*, *qFCRM30*, *qFCRM33*, and *qFCRM38*) related to FCR were identified on eight LGs (Lg1, Lg7, Lg12, Lg16, Lg21, Lg30, Lg33, and Lg38), as shown in Fig. 2A. Two QTLs, *qFCRM21-1* and *qFCRM21-2*, were found on Lg21 and all other LGs had only one QTL. *qFCRM16* was found with a minimum flanking marker interval of 1.9 cM between CAFS882 and SNP0919 markers. The minimum and maximum LOD scores were recorded as 3.62 and 5.47, respectively. The phenotypic variance ranged from 17.5 to 35.6%. The minimum QTL confidence interval was found to be 1.0 cM and the maximum was 12.5 cM in this family (Table 1). Two genome-wide significant ($P < 0.01$) QTLs (*qFCRM1 and qFCRM21-2*) were identified and located on Lg1 and Lg21 which explained the largest phenotypic variance (32.3% and 35.6%), and the distances between the flanking markers were 1.0 and 5.5 cM, separately.

3.4. QTL analysis in FAM-B

In the hybrid family, nine QTLs (*qFCRH5-1*, *qFCRH5-2*, *qFCRH8*, *qFCRH19*, *qFCRH21*, *qFCRH24*, *qFCRH30*, *qFCRH33*, and *qFCRH41*) affecting the FCR trait were detected on eight LGs (Ch5, Ch8, Ch19, Ch21, Ch24, Ch30, Ch33, and Ch41), which explained 17.0% to 33.4% of the phenotypic variation. The minimum flanking marker interval of 3.1 cM between SNP1282 and SNP0995 markers was found for *qFCRH5-2*, while the maximum flanking marker interval of 28.3 cM was recorded for *qFCRH5-1* between HLJ318 and SNP1119. The minimum and maximum LOD scores were recorded as 4.23 and 5.75, respectively. In this family, four QTLs (*qFCRH5-1*, *qFCRH21*, *qFCRH24*, and, *qFCRH33*) were observed with genome-wide significance ($P < 0.01$). The QTL results are shown in Table 1 and Fig. 2B.

3.5. Similar QTL intervals related to FCR in the two families

Three QTL intervals were identified in similar linkage group regions for FAM-A and FAM-B (Fig. 2C). *qFCRM1* of FAM-A and *qFCRH5-1* of FAM-B, *qFCRM12* of FAM-A and *qFCRH24* of FAM-B, *qFCRM30* of FAM-A and *qFCRH8* of FAM-B are in similar intervals. One similar genome-wide significant QTL interval was detected in both families, *qFCRM1* of FAM-A, which explained 32.3% of the phenotypic variance, and *qFCRH5-1* of FAM-B which explained 32.1% of the phenotypic variance.

3.6. Candidate genes of FCR

To identify candidate genes of FCR, the QTL intervals of FAM-A were aligned to the high-density linkage map (Xu et al., 2014b) by shared marker (Fig. 3). To identify candidate genes for FCR, about 900 gene sequences from eight intervals were annotated by comparative genomics. The names of the genes were identified by bioinformatics using the NCBI database. About 168 genes of known function were identified in all eight QTL regions by bioinformatics, and the pathway was identified on the KEGG website (http://www.kegg.jp/). Out of 168 genes, 18 genes with known growth or metabolic function were selected as candidate genes for common carp FCR traits (Table 2).

4. Discussion

4.1. Measurement of FCR

Genetic improvement for better strains and varieties is needed for future aquaculture. About 60 fish and shellfish species have undergone selective breeding, and traits of interest have focused on growth and other phenotypes (Tong and Sun, 2015). After many years of aquaculture practice, however some species still have poor traits including the FCR trait. FCR is an economically important trait, but has received little attention from breeders, because the phenotypes for these traits are difficult to measure (Sun, 2010). Two families' QTL maps for scanning FCR traits and a high-density map for integrating linkage map and genome resource were used in this study to reduce the negative impacts of small population on finding out accuracy QTLs related to FCR.

4.2. Utilization of high density map and reference genome

The markers linked to candidate genes for FCR cannot be chosen directly from the investigation panel owing to the small numbers of markers (Fig. 1). By employing a high-density map (Xu et al., 2014b), 900 gene sequences in eight QTL intervals were obtained, and then eighteen candidate genes were identified with growth or metabolic function according to the reference genome of common carp (www.carpbase.org) and the zebrafish model organism databases (http://www.ncbi.nlm.nih.gov). The key to success is that many similar markers used in this research panels as well as previous high-density map (Xu et al., 2014b). Whole-genome data including all known
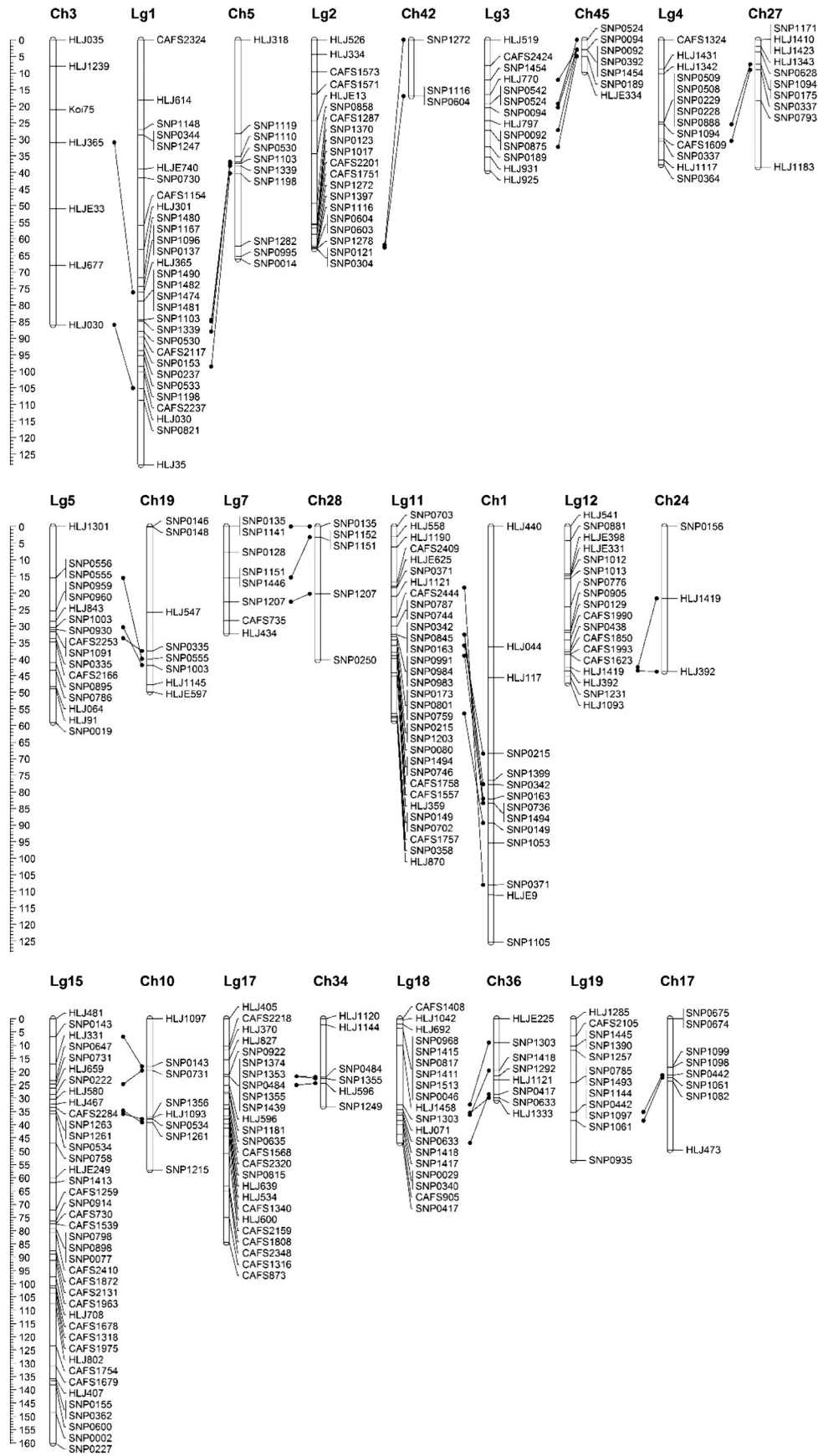
Fig. 1. Comparative linkage map of *Cyprinus carpio* between FAM-A and FAM-B. Comparisons between the two maps are identified by similar markers. Linkage groups of FAM-A are represented by Lg, and linkage groups of FAM-B are represented by Ch. A total of 24 matched linkage groups with more than two shared markers were identified between two maps. Of these paired groups, Ch3 and Ch6 of linkage group FAM-B compared to Lg1 of FAM-A; Ch4 and Ch23 of FAM-B group compared to Lg21 of FAM-A.
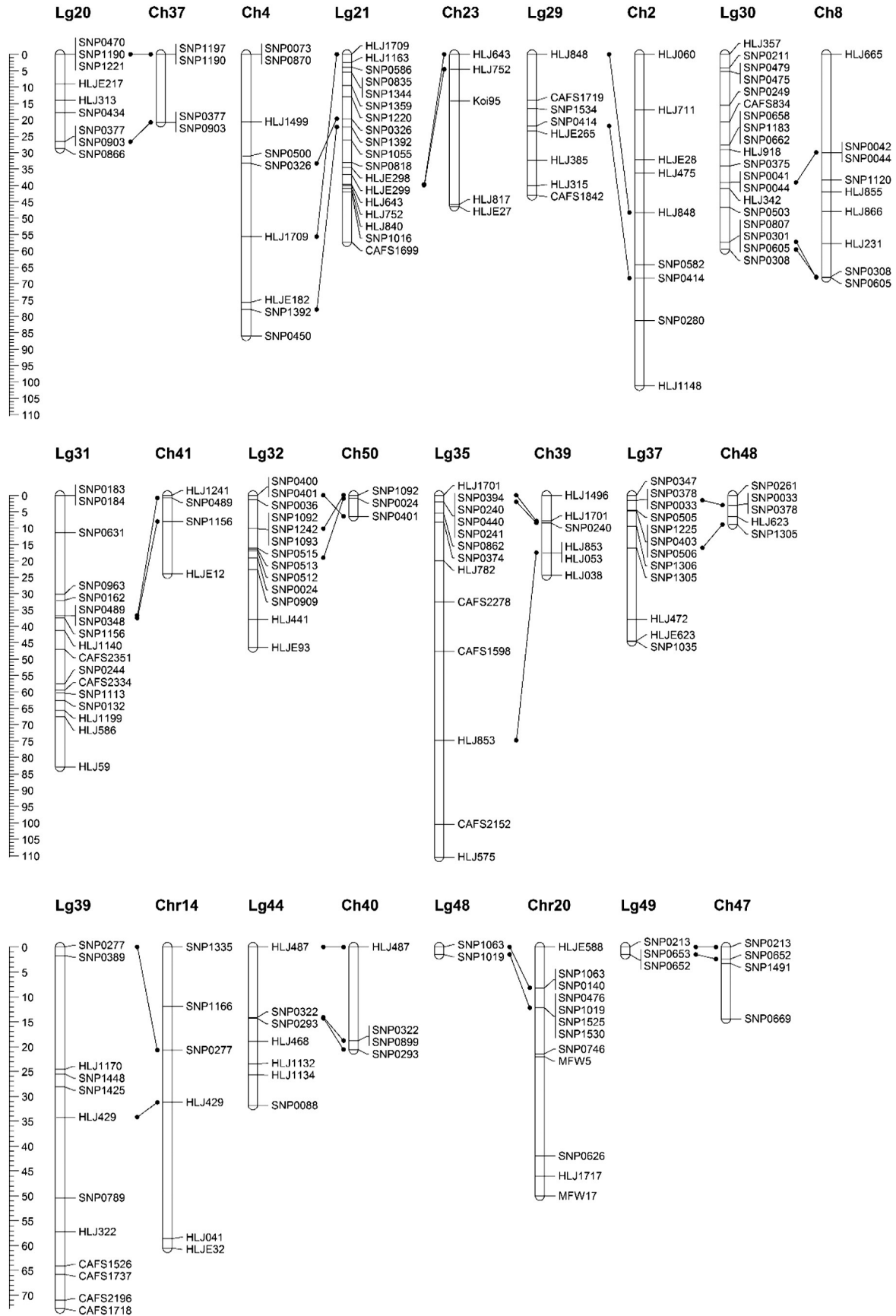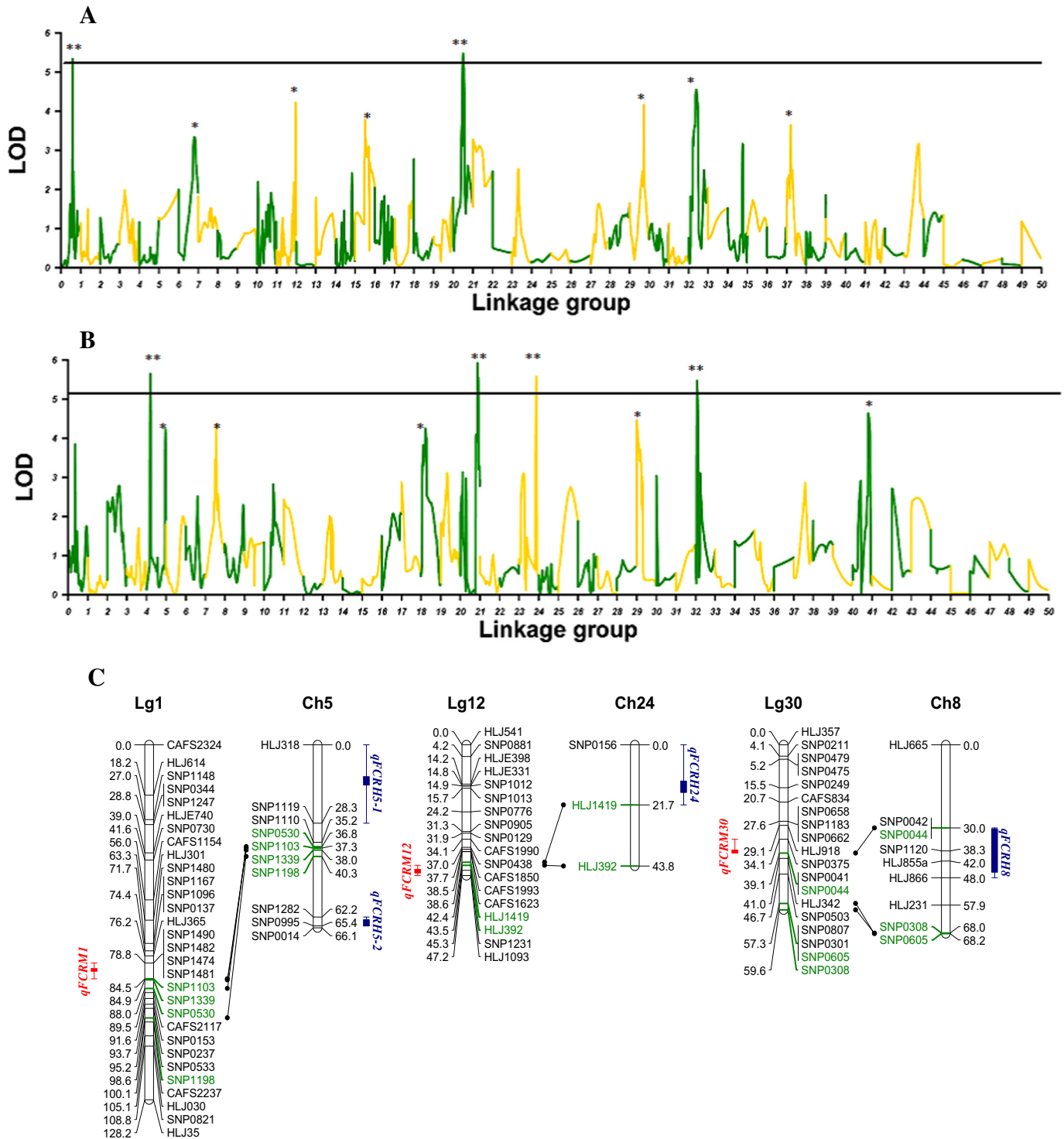
Fig. 1 (continued).

Fig. 2. Putative QTL effects and LOD scores for the common carp genome estimated by QTL mapping. LOD threshold for FCR trait at genome-wide LOD significance ($P < 0.01$) is shown. A. QTL mapping with QTL effects and LOD scores for FAM-A with genome threshold LOD value 5.4; two genome-wide QTLs affecting FCR were identified on Lg1 and Lg21 (**). In addition, seven linkage group-wide QTLs were detected on Lg7, Lg12, Lg16, Lg21, Lg30, Lg33, and Lg38 with different threshold ($P < 0.05$) (*), respectively. B. QTL mapping with QTL effects and LOD scores for FAM-B with genome threshold LOD value 5.5; four genome-wide QTLs affecting FCR were detected on Ch5, Ch21, Ch24, and Ch33 (**). Five linkage group-wide QTLs were identified on five LGs (Ch5, Ch8, Ch19, Ch30, and Ch41) (*). C. Locations of QTL intervals related to the feed conversion ratio trait on matched linkage groups of the two families. Three QTL intervals were identified in similar linkage-group regions for FAM-A and FAM-B. *qFCRM1* of FAM-A and *qFCRH5* of FAM-B, *qFCRM12* of FAM-A and *qFCRH24* of FAM-B, *qFCRM30* of FAM-A and *qFCRH8* of FAM-B is the similar interval.

genes were aligned with the high-density map, so that candidate genes could be searched for within genomic regions in these putative QTL-bearing intervals. We believe that the strategy of alignment a high density map and searching for genes in the reference genome could reduce the sample size and workload. This is especially useful for FCR traits those require a lot of labor and instrument.

Table 1
Location of quantitative loci (QTLs) for feed conversion rate (FCR) in *Cyprinus carpio* L.

Panel	QTL name	G	95% confidence interval (cM)	Flanking markers	Flanking marker interval (cM)	Maximum LOD	Linkage group-wide threshold	Genome-wide threshold	VE (%)
Mirror carp	qFCRM1	1[a]	80.8–81.8	SNP1481-SNP1103	5.7	5.33	3.9	5.1	32.3
	qFCRM7	7	24.7–27.2	SNP1207-CAFS735	5.5	3.67	3.2	5.1	17.5
	qFCRM12	12	45.0–46.3	HLJ392-HLJ1093	3.7	4.22	3.9	5.1	22.3
	qFCRM16	16	10.7–12.6	CAFS882-SNP0919	3.1	3.95	3.7	5.1	20.9
	qFCRM21-1	21	24.2–25.3	SNP1392-SNP1055	4.1	3.62	3.6	5.1	17.9
	qFCRM21-2	21[a]	27.3–32.8	SNP1055-SNP0818	5.5	5.47	3.6	5.1	35.6
	qFCRM30	30	38.0–39.1	SNP0375-SNP0044	5.0	4.23	4.0	5.1	26.3
	qFCRM33	33	33.4–45.9	HLJ311-HLJ326	43.2	4.55	3.9	5.1	28.4
	qFCRM38	38	21.4–25.4	HLJ377- HLJE253	10.4	3.62	3.6	5.1	19.4
Hybrid carp	qFCRH5-1	5[a]	11.5–14.5	HLJ318-SNP1119	28.3	5.65	3.9	5.3	32.1
	qFCRH5-2	5	63.2–65.4	SNP1282-SNP0995	3.1	4.23	3.9	5.3	18.6
	qFCRH8	8	30.4–46.0	SNP0044-HLJ866	18.0	4.23	4.0	5.3	17.4
	qFCRH19	19	10.1–13.6	SNP0148-HLJ547	24.8	4.25	3.8	5.3	22.3
	qFCRH21	21[a]	32.0–40.8	HLJE141-SNP1194	27.5	5.54	3.9	5.3	31.6
	qFCRH24	24[a]	13.1–17.2	SNP0156-HLJ1419	21.7	5.75	4.2	5.3	33.4
	qFCRH30	30	0.0–8.3	SNP1391-SNP0050	13.4	4.46	3.7	5.3	17.0
	qFCRH33	33[a]	2.4–6.8	SNP0110-HLJ437	21.0	5.47	4.1	5.3	29.3
	qFCRH41	41	19.2–21.5	SNP1156-HLJE12	16.0	4.79	3.6	5.3	23.7

[a] QTL intervals that are genome-wide significant QTL. PVE, phenotypic variance explained.

4.3. A high efficiency strategy for identify candidate genes of FCR

Identifying candidate genes from a mapped QTL is a difficult and complex research for almost all quantitative traits and most aquaculture breeding animals (Shmookler Reis, 2003; Drinkwater and Gould, 2012). Aquaculture breeding animals tend to have relative large quantities of DNA in their genome and more non-coding DNA than coding DNA, making it difficult to identify particular genes of interest. Further, Aquaculture breeding animals have relatively long generation times, in general three years. Based on phenotypes, one is seldom able to study millions of individuals in order to isolate it from the many thousands of other genes in the organism (Xu et al., 2012). Thus it is difficult to discern the effect of a single gene (QTL). Here, we recommend a simple and efficient approach to identify candidate genes for FCR from QTL in a low-density map. The key points in this strategy are that we are employing a high density genetic map and a reference genome. In addition, most makers are similar between the low- and the high-density maps. In this study, microsatellite markers were employed among experimental panels and the high-density map (Xu et al., 2014b). In the future, researchers can use these microsatellite as well as SNP markers even though we have not used them in this study.

4.4. Comparison with previous studies

FCR is one of the most important economic traits in fish, as fish with a better FCR increase profits. QTLs for FCR have been reported in cattle (Nkrumah et al., 2007), pigs (Houston et al., 2005) and chickens (Van Kaam et al., 1999). Zimmerman et al. (2005) revealed three QTLs for the number of pyloric caeca in three LGs of rainbow trout, an important index associated with FCR in that species. QTL analyses for FCR in aquaculture fishes are rarely reported. A genetic map constructed by AFLP markers was also used to find a QTL associated with FCR in channel catfish (Liu, 2001).

As a few QTLs associated with FCR traits in common carp (Li et al., 2009; Zhang et al., 2010; Wang et al., 2012) have been identified, little genetic variation has been reported by the detected QTLs due to small sample sizes or to a low-density genetic map. In this study, two populations with a significant differ in FCR trait which FCR of mirror carp panel (average FCR: 65.6% ± 10.9%) is higher than in hybride panel (average FCR: 37.0% ± 10.6%), were used to find repeated QTL interval in both populations to certify QTL. Finally, three repeat QTL intervals were found between this families. Thus we not only took advantage of two populations but also employed a high-density map by comparative genome strategy. Hence, eighteen candidate genes close linked to FCR were obtained, which previous studies had not identified (Zhang et al., 2010; Wang et al., 2012).

Similar candidate genes associated with feed conversation rate have been found in other animals. These same function of genes suggested that the techniques and strategies employed were reliable and feasible. For example, IGF-1 as a candidate gene in this study was in a QTL interval on LG17, and this gene was associated with a significant or extremely significant difference in feed conversion ratio in yellow chicken (Zhang et al., 2013a, 2013b). The TGF-β receptor is also a candidate gene in a QTL on LG17 in our study, which is associated with an extremely significant difference in the feed conversion ratio in chicken (Rasal et al., 2015). In addition, two genes, gdf7 in qFCRM12 on LG22 and tgfb2 in qFCRM33 on LG18, involved in TGF-β signaling pathway were also identified as candidate gene affecting FCR.

To today, in various investigations, we have not distinguished between markers that are linked only to growth, and markers that contribute to both growth and FCR. The loci associated with growth and FCR are interrelated, and there are a few associated candidate genes. In future, if these linkages are proven in breeding and phenotypic evaluation studies, they could prove useful for marker-assisted fish breeding (Poompuang and Hallerman, 1997). The results of our study will contribute to realization of this goal.

5. Conclusions

The results suggest that QTL associated with FCR exhibited overlapping intervals across two mapping populations. Our results also suggest that a low-density map can be employed to identify candidate genes by matching putative QTL markers to a high-density map and using a reference genome. Eighteen candidate genes for FCR traits were identified, and these genes could be utilized for marker-assisted selection of common carp.
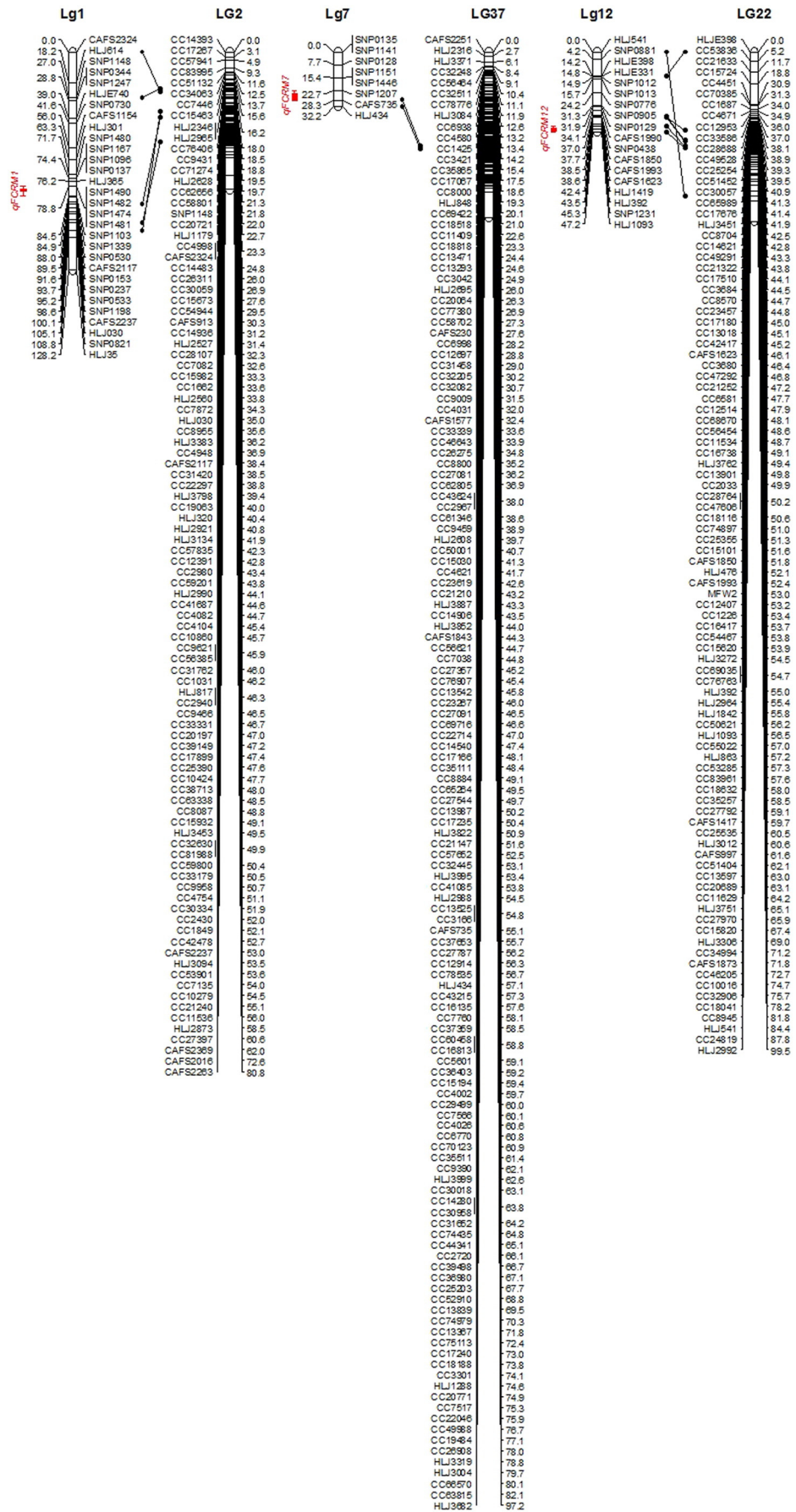
Fig. 3. Locations of QTL intervals related to the feed conversion ratio trait on high-density linkage map and whole genomic scaffold of *Cyprinus carpio*. Eight linkage groups containing QTL regions were aligned to the high linkage group including Lg1 to LG2, Lg7 to LG37, Lg12 to LG22, Lg16 to LG17, Lg21 to LG26, Lg30 to LG21, Lg33 to LG18, and Lg38 to LG4.
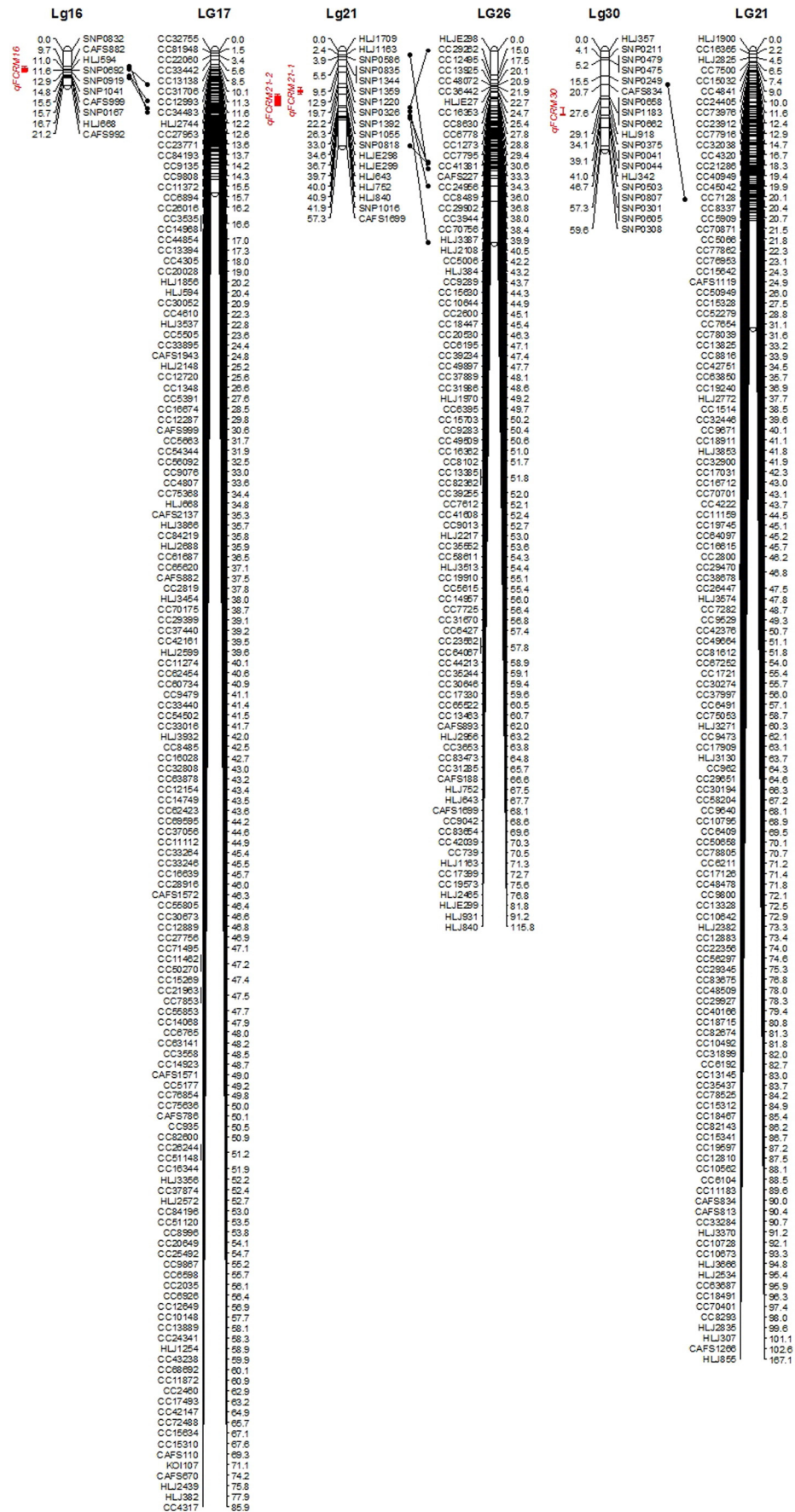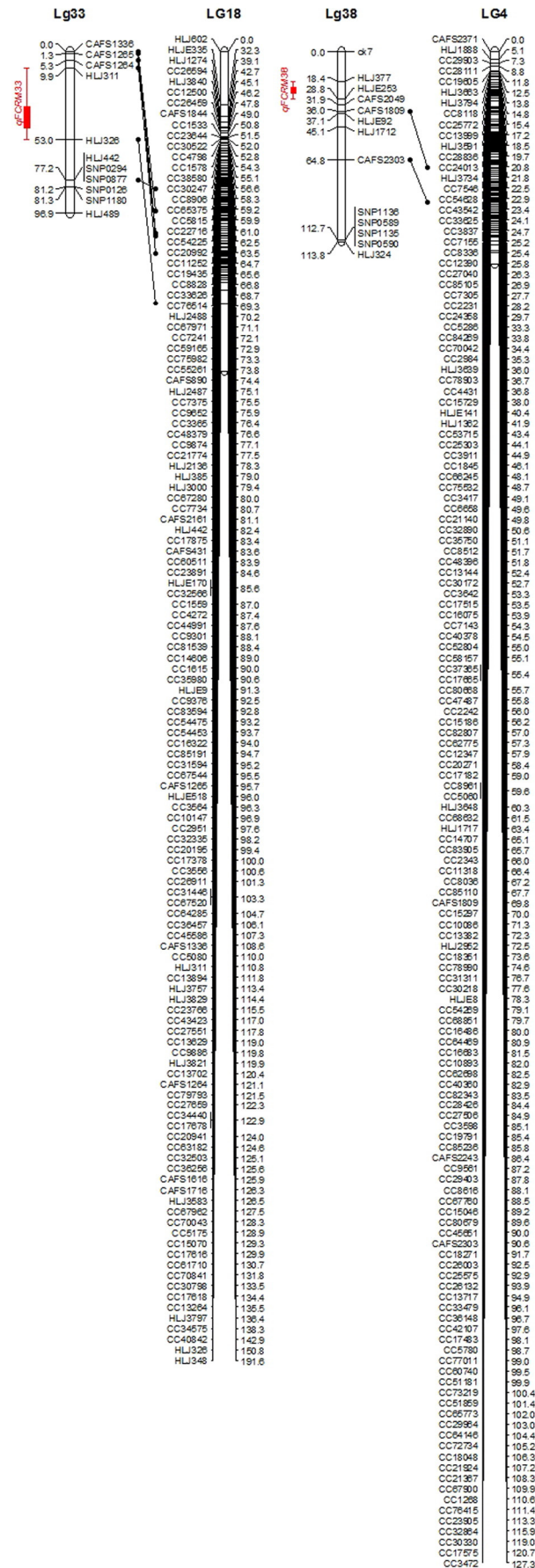
Fig. 3 (continued).

Fig. 3 (*continued*).

C. Lu et al. / Aquaculture 468 (2017) 585–596* 595

Table 2
Candidate genes of FCR identified by comparative genomics.

QTL	LG[a]	Gene Symbol	Gene name	Function of gene	Pathway involved
qFCRM1	2	rasgrf2a	RAS protein-specific guanine nucleotide-releasing factor 2a	Molecular switches for pathways regulating cell proliferation, survival, growth, migration, and differentiation	Ras signaling pathway
		insra	Insulin receptor a	Metabolism of carbohydrates, fats, and protein	Insulin signaling pathway
qFCRM7	37	fgfbp2	Fibroblast growth factor binding protein 2	Angiogenesis, wound healing, embryonic development	Signaling regulating pluripotency of s-cells
		egf	Epidermal growth factor	Stimulates cell growth, proliferation, and differentiation	Pancreatic cancer
qFCRM12	22	gdf7	Growth differentiation factor 7	Growth factor activity	TGF-beta signaling pathway
		mdh1ab	Malate dehydrogenase 1Ab, NAD (soluble)	Malate dehydrogenase activity, oxidoreductase activity, carbohydrate and carboxylic acid metabolic process, malate metabolic process, tricarboxylic acid cycle	Carbon metabolism; glucose metabolism
qFCRM16	17	tgfbr2(*)	Transforming growth factor, beta receptor II	Growth, development, tissue homeostasis, and regulation of the immune system	Cytokine-cytokine receptor interaction
		Smad7	SMAD family member 7	Controls organ size from flies to humans	Hippo signaling pathway
		igf1ra(*)	Insulin-like growth factor 1a receptor	Childhood growth and anabolic effects in adults	IGF-1 pathway
qFCRM21-1	26	arhgap24	Rho GTPase-activating protein 24	Molecular switches for signaling pathways regular cell proliferation, survival, growth, migration	Ras signaling pathway
		rap1gap2b	RAP1 GTPase-activating protein 2b	Molecular switches for signaling pathways regular cell proliferation, survival, growth, migration	Ras signaling pathway
qFCRM30	21	lipeb	Lipase, hormone-sensitive b	Releases fatty acids (FAs) and glycerol for use by other organs as energy substrates	Regulation of lipolysis in adipocytes
		lsl-2a	Insulin gene enhancer protein	Metabolism of carbohydrates, fats, protein	Insulin signaling pathway
qFCRM33	18	mecr	Mitochondrial trans-2-enoyl-CoA reductase	Oxidoreductase activity, trans-2-enoyl-CoA reductase (NADPH) activity	Carbohydrate and lipid metabolism; fatty acid metabolism
		tgfb2	Transforming growth factor, beta 2	Cytokine activity, growth factor activity, TGF-beta receptor binding	TGF-beta signaling pathway
		gapdh	Glyceraldehyde-3-phosphate dehydrogenase	NADP binding	Metabolism of carbohydrates
qFCRM38	4	eif2b1	Eukaryotic translation initiation factor 2B, subunit 1 alpha	NOT L-methionine biosynthetic process from methylthioadenosine	Metabolism of proteins
		pip5kl1	Phosphatidylinositol-4-phosphate 5-kinase-like 1	Phosphatidylinositol phosphate kinase activity	Inositol phosphate metabolism

* genes mapped to the FCR traits in chicken.
[a] The number of highly linkage group Xu et al. (2014b).

Acknowledgements

References

Andersen, B.B., 1977. Genetic Studies Concerning Growth Rate in Cattle, Body Development and Feed Efficiency. Report no 488. National Institute of Animal Science, Copenhagen.

Basarab, J.A., Price, M.A., Aalhus, J.L., Okine, E.K., Snelling, W.M., Lyle, K.L., 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83, 189–204.

Churchill, G.A., Doerge, R.W., 1994. Empirical threshold values for quantitative trait mapping. Genetics 138, 963–971.

Crawford, R.D., 1990. Poultry Breeding and Genetics. Elsevier, Amsterdam.

de Oliveira, P.S., Cesar, A.S., do Nascimento, M.L., Chaves, A.S., Tizioto, P.C., Tullio, R.R., Lanna, D.P., Rosa, A.N., Sonstegard, T.S., Mourao, G.B., Reecy, J.M., Garrick, D.J., Mudadu, M.A., Coutinho, L.L., Regitano, L.C., 2014. Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC Genet. 15, 100.

Doerge, R.W., Churchill, G.A., 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics 142, 285–294.

Doupé, R.G., Lymbery, A.J., 2003. Toward the genetic improvement of feed conversion efficiency in fish. J. World Aquacult. Soc. 34, 245–254.

Drinkwater, N.R., Gould, M.N., 2012. The long path from QTL to gene. PLoS Genet. 8, e1002975. http://dx.doi.org/10.1371/journal.pgen.1002975.

Gjedrem, T., Baranski, M., 2009. Selective Breeding in Aquaculture: An Introduction. Springer, New York.

Goddard, S., 1996. Feed management in intensive aquaculture. Am. J. Roentgenol. Radium Ther. Nucl. Med. 149, 159–179.

Harvest, M., 2012. The Marine Harvest Salmon Industry Handbook. Marine Harvest, Bergen, p. 41.

Houston, R.D., Haley, C.S., Archibald, A.L., Rance, K.A., 2005. A QTL affecting daily feed intake maps to chromosome 2 in pigs. Mamm. Genome 16, 464–470.

Jin, S., Zhang, X., Jia, Z., Fu, H., Zheng, X., Sun, X., 2012. Genetic linkage mapping and genetic analysis of QTL related to eye cross and eye diameter in common carp (Cyprinus carpio L.) using microsatellites and SNPs. Aquaculture 358, 176–182.

Laghari, M.Y., Lashari, P., Zhang, Y., Sun, X., 2014. Identification of quantitative trait loci (QTLs) in aquaculture species. Rev. Fish. Sci. Aquacult. 22, 221–238.

Li, O., Cao, D., Zhang, Y., Gu, Y., Zhang, X., Lu, C., Sun, X., 2009. Studies on feed conversion ratio trait of common carp (Cyprinus carpio L.) using EST-SSR marker. J. Fish. China 33, 624–631.

Li, Y., Xu, P., Zhao, Z., Wang, J., Zhang, Y., Sun, X.W., 2011. Construction and characterization of the BAC library for common carp Cyprinus carpio L. and establishment of microsynteny with zebrafish Danio rerio. Mar. Biotechnol. 13, 706–712.

Liu, Z.J., 2001. Gene mapping, marker-assisted selection, gene cloning, genetic engineering and integrated genetic improvement programs at Auburn University. In: Gupta, M.V., Acosta, B.O. (Eds.), Fish Genetics Research in Member Countries and Institutions of the International Network on Genetics in Aquaculture. ICLARM Conference Proceedings 64, p. 179.

Nkrumah, J.D., Sherman, E.L., Li, C., Marques, E., Crews, D.H., Bartusiak, R., Murdoch, B., Wang, Z., Basarab, J.A., Moore, S.S., 2007. Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. J. Anim. Sci. 85, 3170–3181.

Ponzoni, R.W., Nguyen, N.H., Khaw, H.L., Ninh, N.H., 2008. Accounting for genotype by environment interaction in economic appraisal of genetic improvement programs in common carp Cyprinus carpio. Aquaculture 285, 47–55.

Poompuang, S., Hallerman, E.M., 1997. Toward detection of quantitative trait loci and marker-assisted selection in fish. Rev. Fish. Sci. 5, 253–277.

Rasal, K.D., Shah, T.M., Vaidya, M., Jakhesara, S.J., Joshi, C.G., 2015. Analysis of consequences of non-synonymous SNP in feed conversion ratio associated TGF-β receptor type 3 gene in chicken. Meta Gene 4, 107–117.

Saatchi, M., Beever, J.E., Decker, J.E., Faulkner, D.B., Freetly, H.C., Hansen, S.L., Yampara-Iquise, H., Johnson, K.A., Kachman, S.D., Kerley, M.S., Kim, J., Loy, D.D., Marques, E., Neibergs, H.L., Pollak, E.J., Schnabel, R.D., Seabury, C.M., Shike, D.W., Snelling, W.M., Spangler, M.L., Weaber, R.L., Garrick, D.J., Taylor, J.F., 2014. QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC Genomics 15, 1004.

Shen, R., Fan, J.B., Campbell, D., Chang, W., Chen, J., Doucet, D., Yeakley, J., Bibikova, M., Wickham Garcia, E., McBride, C., Steemers, F., Garcia, F., Kermani, B.G., Gunderson, K., Oliphant, A., 2005. High-throughput SNP genotyping on universal bead arrays. Mutat. Res. 73, 70–82.

Sherman, E.L., Nkrumah, J.D., Li, C., Bartusiak, R., Murdoch, B., Moore, S.S., 2014. Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. J. Anim. Sci. 87, 37–45.

Shmookler Reis, R.J., 2003. From QTL mapping to genes: the long and winding road. J. Bone Miner. Res. 18, 186–189.

Sun, X.W., 2010. Molecular Breeding of Fish (in Chinese). China Ocean Press, Beijing.

Sun, X., Liang, L., 2004. A genetic linkage map of common carp (*Cyprinus carpio* L.) and mapping of a locus associated with cold tolerance. Aquaculture 238, 165–172.

Tong, J., Sun, X., 2015. Genetic and genomic analyses for economically important traits and their applications in molecular breeding of cultured fish. Sci. China Life Sci. 8, 168–186.

Van Kaam, J.B., Groenen, M.A., Bovenhuis, H., Veenendaal, A., Vereijken, A.L., Van Arendonk, J.A., 1999. Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. Poult. Sci. 78, 15–23.

Van Ooijen, J., 2006. JoinMap 4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma, BV, Wageningen, Netherlands.

Van Ooijen, J., 2009. MapQTL 6.0: Software for the Mapping of Quantitative Trait Loci in Experimental Populations of Diploid Species. Kyazma BV, The Netherlands: Wageningen.

Vangen, O., 1984. Future breeding programme in pigs in a situation with artificial insemination. Aktelt Fra Statens Fagtjeneste for Landbruket 1, 300–306.

Voorrips, R., 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93, 77–78.

Wang, X.P., Zhang, X.F., Li, W.S., Zhang, T.Q., Li, C., Sun, X.W., 2012. Mapping and genetic effect analysis on quantitative trait loci related to feed conversion ratio of common carp (*Cyprinus carpio* L.) (in Chinese). Acta Hydrobiol. Sin. 36, 177–196.

Williams, D.R., Li, W., Hughes, M.A., Gonzalez, S.F., Vernon, C., Vidal, M.C., Jeney, Z., Jeney, G., Dixon, P., Mcandrew, B., 2008. Genomic resources and microarrays for the common carp *Cyprinus carpio* L. J. Fish Biol. 72, 2095–2117.

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., Xu, J., Zheng, X., Ren, L., Wang, G., Zhang, Y., Huo, L., Zhao, Z., Cao, D., Lu, C., Li, C., Zhou, Y., Liu, Z., Fan, Z., Shan, G., Li, X., Wu, S., Song, L., Hou, G., Jiang, Y., Jeney, Z., Yu, D., Wang, L., Shao, C., Song, L., Sun, J., Ji, P., Wang, J., Li, Q., Xu, L., Sun, F., Feng, J., Wang, C., Wang, S., Wang, B., Li, Y., Zhu, Y.,

Xue, W., Zhao, L., Wang, J., Gu, Y., Lv, W., Wu, K., Xiao, J., Wu, J., Zhang, Z., Yu, J., Sun, X., 2014b. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nat. Genet. 46, 1212–1219.

Xu, J., Zhao, Z., Zhang, X., Zheng, X., Li, J., Jiang, Y., Kuang, Y., Zhang, Y., Feng, J., Li, C., 2014a. Development and evaluation of the first high-throughput SNP array for common carp (*Cyprinus carpio*). BMC Genomics 15, 307.

Xu, Y., Lu, Y., Xie, C., Gao, S., Wan, J., Prasanna, B.M., 2012. Whole-genome strategies for marker-assisted plant breeding. Mol. Breed. 29, 833–854.

Xu, P., Wang, J., Wang, J., Cui, R., Li, Y., Zhao, Z., Ji, P., Zhang, Y., Li, J., Sun, X., 2011. Generation of the first BAC-based physical map of the common carp genome. BMC Genomics 12, 537.

Yue, G.H., 2014. Recent advances of genome mapping and marker-assisted selection in aquaculture. Fish Fish. 15, 376–396.

Zhang, Y., Huang, X.L., Ji, C.L., Peng, Z.J., Zhang, D.X., 2013a. The study on the relations of chicken *IGF-1* gene and feed conversion traits. Heilongjiang Animal Science and Veterinary Medicine. 4, pp. 37–39.

Zhang, Y., Wang, S., Li, J., Zhang, X., Jiang, L., Xu, P., Lu, C., Wan, Y., Sun, X., 2013b. Primary genome scan for complex body shape-related traits in the common carp *Cyprinus carpio*. J. Fish Biol. 82, 125–140.

Zhang, L., Zhang, X., Cao, D., Yang, J., Chu, Z., Sun, X., 2010. QTL analysis related to feed conversion efficiency in common carp (*Cyprinus carpio*) using SSR and EST markers (in Chinese). Chin. J. Agric. Biotechnol. 18, 963–967.

Zheng, X., Kuang, Y., Lv, W., Cao, D., Zhang, X., Li, C., Lu, C., Sun, X., 2013. A consensus linkage map of common carp (*Cyprinus carpio* L.) to compare the distribution and variation of QTLs associated with growth traits. Sci. China Life Sci. 56, 351–359.

Zimmerman, A.M., Wheeler, P.A., Ristow, S.S., Thorgaard, G.H., 2005. Composite interval mapping reveals three QTL associated with pyloric caeca number in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 247, 85–95.