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Genetic analysis of QTL for eye cross and eye diameter in common carp (*Cyprinus carpio* L.) using microsatellites and SNPs

S.B. Jin[1,2], X.F. Zhang[1], J.G. Lu[1], H.T. Fu[2], Z.Y. Jia[1] and X.W. Sun[1]

[1]Heilongjiang River Fisheries Research Institute,
Chinese Academy of Fishery Sciences, Heilongjiang, Harbin, China
[2]Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization,
Ministry of Agriculture, Freshwater Fisheries Research Center,
Chinese Academy of Fishery Sciences, Wuxi, China

Corresponding author: X.W. Sun
E-mail: sunxw2002@163.com

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ABSTRACT. A group of 107 F_1 hybrid common carp was used to construct a linkage map using JoinMap 4.0. A total of 4877 microsatellite and single nucleotide polymorphism (SNP) markers isolated from a genomic library (978 microsatellite and 3899 SNP markers) were assigned to construct the genetic map, which comprised 50 linkage groups. The total length of the linkage map for the common carp was 4775.90 cM with an average distance between markers of 0.98 cM. Ten quantitative trait loci (QTL) were associated with eye diameter, corresponding to 10.5-57.2% of the total phenotypic variation. Twenty QTL were related to eye cross, contributing to 10.8-36.9% of the total phenotypic variation. Two QTL for eye diameter and four QTL for eye cross each accounted for more than 20% of the total phenotypic variation and were considered to be major QTL. One growth factor related to eye diameter was observed on LG10 of the common carp genome, and three growth factors related to eye cross were observed on LG10,

LG35, and LG44 of the common carp genome. The significant positive relationship of eye cross and eye diameter with other commercial traits suggests that eye diameter and eye cross can be used to assist in indirect selection for many commercial traits, particularly body weight. Thus, the growth factor for eye cross may also contribute to the growth of body weight, implying that aggregate breeding could have multiple effects. These findings provide information for future genetic studies and breeding of common carp.

Key words: Mirror carp *Cyprinus carpio* L.; Quantitative trait loci; Eye cross and Eye diameter; Functional growth factor; Breeding; Correlation analysis

INTRODUCTION

The common carp (*Cyprinus carpio* L.) is the most extensively farmed fish species in China, and worldwide (Sun and Liang, 2004). Although productivity of this species has dramatically increased in recent decades, limited genetic research has been conducted on common carp breeding because of the low density of single nucleotide polymorphism (SNP) markers in linkage maps.

Genomic information can be used to advance genetic improvement programs in aquaculture, particularly the use of quantitative trait loci (QTL) mapping, which can lead to marker-assisted selection of desirable traits, as has already occurred in terrestrial livestock programs (Dekkers, 2004). However, genetic linkage maps are required to enable more detailed genetic studies. Such in-depth genetic studies may include the mapping of traits of commercial interest to a particular region of the genome, comparative genomics, physical cloning of mutants, and whole genome sequencing (Cnaani et al., 2004; Naruse et al., 2004). Fortunately, significant advances have been made in the construction of genetic linkage maps and the identification of QTL locations in the common carp in recent decades, including the construction of three genetic linkage maps using molecular markers (Sun and Liang, 2004; Zhang et al., 2010; Wang et al., 2011a) and the construction of comparative maps (Zheng et al., 2011). In addition, studies have also identified important commercial traits of the common carp on these maps in recent years. Of these studies, one conducted genetic analysis of the German mirror carp (Hou et al., 2007), others identified genes involved in the control of quantitative traits in common carp using QTL, such as cold tolerance (Sun and Liang, 2004) and muscle fiber density (Zhang et al., 2010), and others have conducted QTL analyses in other species (Zimmerman et al., 2005). Despite this progress, problems remain with previous studies. For example, some linkage maps were constructed using dominant markers (Zhang et al., 2010) or with a limited population, which makes it difficult to construct maps with greater levels of resolution (Sun and Liang, 2004).

SNPs are the most common type of variation in the genome, and they provide the best genome coverage for analyzing performance and production of traits. A genome with high-density SNP coverage serves as a powerful tool for whole genome association studies because it can be used to detect linkage disequilibrium (Liu et al., 2011). Research has shown that SNP markers are very powerful markers for linkage mapping, construction of large-number populations, and identification of complex traits in humans (Wang et al., 1998), along with other

model species (Zimdahl et al., 2004; Guryev et al., 2006). SNP markers have also been widely used to construct aquaculture fish linkage maps (Zheng et al., 2011; Lien et al., 2011; Wang et al., 2011b). The marker densities on these maps are believed to be relatively low because of insufficient markers. Consequently, genetic maps with low-density co-dominant marker coverage only provide small QTL regions of commercial traits, limiting functional gene analysis of the relatively large QTL regions. Recently, a number of microsatellites (Yue et al., 2004; Sun et al., 2005; Wang et al., 2007) have been identified as a practical tool for accurate construction of genetic linkage maps for large populations of common carp. Sufficient markers would enable QTL regions to be narrowed down and would increase the accuracy of common carp linkage maps. Such markers can also provide accurate QTL regions for commercial traits, including the detection of functional genes controlling the growth of different traits.

Eye diameter and eye cross are both important fish morphological characteristics and play essential roles in fish taxonomy (Yan et al., 2007; Liu et al., 2009). Furthermore, these traits are related to fish behavior, such as predation and predator avoidance (Kröger and Fernald, 1994; Ben-Simon et al., 2009). In a recent study, 445 polymorphic markers were used to identify the QTL location of head length, eye diameter, eye cross, body length, and body weight of carp on a carp linkage map, including 265 amplified fragment length polymorphism markers, 127 microsatellite markers, 37 EST-SSR markers, and 16 RAPD markers (Liu et al., 2009). In another study, 186 SSR and 321 SNP markers were used to carry out QTL location studies of eye diameter and eye cross in the common carp (Jin et al., 2012). Further in-depth investigations of the functional genes that control the characteristics of eye diameter and eye cross are required.

In this study, a high-density linkage map was constructed using common carp, and the genetic regions of eye diameter and eye cross were identified. In addition, the major functional regions of eye diameter and eye cross were individually identified by constructing a genetic linkage map using both microsatellite and SNP markers. The candidate functional genes responsible for the development of eye cross and eye diameter were determined from these major functional regions, significantly enhancing further breeding work.

MATERIAL AND METHODS

Experimental fish

German mirror common carp were obtained from the Songpu Aquaculture Experimental Station in Harbin, China. To obtain the largest genetic variation, males and females with relatively distant genetic descent were selected for breeding. Male and female individuals with the largest genetic variance were used to obtain the F_1 generation. The mating of broodstock and production of the full-sib family were carried out at the Songpu Research Station (Harbin, China). Mature (3 years old), healthy broodfish in good condition were selected and stored at a hatchery in two tanks, with females and males kept separately. Eggs from a single female were fertilized with sperm from a single male and were spawned and hatched in a bowl. Water temperature during the incubation period was 22°C. Following incubation, 107 embryos hatched and the fry were transferred to 107 rectangular tanks (120 L) with individual filtration circulation. Fish were fed every day and cultured under experimental conditions for six months. Eye cross and eye diameter were measured at six months post-hatching using Vernier calipers to an accuracy of 0.02 mm, following the methods of Sun and Liang (2004),

then the genotypes and phenotypes of the fish were determined. Eye diameter was measured as the inside diameter of the orbital, which is parallel to the head-to-tail axis and eye cross was measured as the straight-line distance between the edges of the fish orbitals.

DNA extraction

Fresh blood was extracted from each of the 107 individuals, and DNA was extracted from the fresh blood using a standard phenol-chloroform protocol (Sambrook and Russell, 2001). Genomic DNA was dissolved in double-distilled water, and the DNA quality was assessed on a 1% agarose gel. The DNA concentration was adjusted to 2.5 μg/μL based on the primary DNA concentration measured by spectrophotometer.

Microsatellite and SNP genotyping

One thousand microsatellite loci from BAC end sequences and 3000 microsatellite loci from genomic shotgun sequences, generated from the Roche 454 platform, were selected for marker development. A total of 1024 microsatellite loci were genotyped for the mapping population. Identification of SNP markers was conducted using the SLAF-seq method (Sun et al., 2013) and 4026 SNPs were identified for the population. JoinMap 4.0 was used to perform the linkage analysis. A total of 978 microsatellite markers and 3899 SNPs were amplified in the DNA samples. The total volume of the polymerase chain reaction was 15 μL, containing 1 U Taq DNA polymerase, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, and 0.01% gelatin, pH 8.3), 200 mM dNTPs, 0.1 mM of both the forward primer and reverse primer, and 100 ng DNA templates. Thermal cycling was performed in a PCR System (Eppendorf Mastercycle pro S) in three steps. Cycling conditions consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles of denaturing at 94°C for 30 s, annealing for 30 s (at a temperature specific for the primers being used), extension at 72°C for 30 s, and a final extension step at 72°C for 5 min. The amplified PCR products were visualized on an 8% polyacrylamide gel, using 1X TBE buffer, and the bands were analyzed using silver staining. The lengths of the PCR products were estimated using the Vision WorkLS software.

Construction of linkage map and QTL location

JoinMap 4.0 was used to construct the linkage map (Jacobs et al., 1995; Viruel et al., 1995) and MapQTL 4.0 was used to locate QTL using interval mapping (IM) methods (Van Ooijen, 2004). A limit of detection (LOD) score was set as the significant threshold for declaration of candidate QTL for both eye cross and eye diameter performed using IM. This threshold was set following a permutation test applied to each data set (1000 repetitions) ($P = 0.05$). QTL affecting these two traits were identified on a genome-wide scale, and the genome-wide LOD significance threshold was 2.5.

Detection of functional genes

To identify genes contributing to the expression of eye diameter and eye cross traits, a blast search of QTL markers was performed against the draft genome of carp as a query and specific chromosome regions were extracted for gene prediction. Functional annotation of

genes was performed using a BLASTX search (e-value < $1e^{-5}$) against the National Center for Biotechnology Information (NCBI) non-redundant protein database.

Correlation analysis

We measured the following traits six months after hatching: body weight, full length, body length, head length, eye diameter, eye cross, height, body thickness, caudal peduncle length, caudal peduncle height, and snout length. Microsoft Excel 2010 was used to perform the correlation analysis (Xie, 2010). When a population comprises more than 60 individuals, correlation values of >0.193 and >0.254 are considered to indicate correlation ($P < 0.05$) and significant correlation ($P < 0.01$) of variables, respectively (Zhou and Zheng, 1997). As such, these values were applied in this study.

RESULTS

Measurements of eye diameter and eye cross

The average difference is an index that indicates measurement accuracy, where the smaller the average difference, the more accurate the measurement is considered to be (Jin et al, 2012). Measurements of eye diameter ranged from 0.75-1.14 cm with an average (± SD) of 0.9311 ± 0.0077 cm. The measurements of eye cross ranged from 1.69-2.70 cm with an average (± SD) of 2.10 ± 0.0205 cm. These values indicate that the measurements could be used for further genetic analysis and QTL location research.

Construction of the genetic map

The linkage map containing 50 linkage groups was constructed using a total of 4877 markers (3899 of the 4026 SNP markers and 978 of the 1042 microsatellite markers). The total length of the inherited linkage map was 4775.90 cM. The map distance ranged from 47.498 cM on LG38 to 191.62 cM on LG18, with an average distance between markers of 0.98 cM. The number of markers on each linkage group ranged from 32-166 with an average of 97.54 markers per group.

QTL location

Ten QTL were found to be responsible for the characteristics of eye diameter: qED1-5 (CA1602-SNP83693), qED2-5 (HLJ346-HLJ2013), qED3-5 (HLJ377-HLJ1909), qED1-21 (SNP49664-SNP67252), qED1-27 (HLJ3294-SNP27486), qED1-30 (SNP1629-SNP52392), qED1-44 (SNP32671-SNP4483), qED2-44 (SNP28085-SNP35232), qED1-45 (HLJ3407-HLJ3396), and qED2-45 (SNP7445-SNP81574) (Table 1). Among these QTL linkage groups, the largest LOD value was held by the qED1-5 (CA1602-SNP83693) group. This group was responsible for 12.1% of the total phenotypic variation. The qED3-5 (HLJ377-HLJ1909) group had the smallest LOD value (2.55), explaining 10.5% of the total phenotypic variation. Of the total phenotypic variation, these ten linkage groups explained between 10.5% [group qED3-5 (HLJ377-HLJ1909)] and 57.2% [group qED1-45 (HLJ3407-HLJ3396)] (Figure 1). The marker interval for eye diameter ranged from 1.786 cM on LG 7 to 44.257 cM on LG 42.

Twenty QTL were found to control the expression of eye cross, including eleven QTL on linkage group 6. These QTL were located on the linkage groups qEC1-5 (SNP50851-SNP79571), qEC2-5 (SNP8987-SNP3496), qEC1-6 (SNP6373-SNP9469), qEC2-6 (SNP59840-SNP14880), qEC3-6 (SNP26196-SNP25935), qEC4-6 (HLJ2556-SNP16225), qEC5-6 (SNP29602-SNP4802), qEC6-6 (SNP54288-SNP3418), qEC7-6 (SNP5961-SNP1566), qEC8-6 (SNP3047-SNP3263), qEC9-6 (SNP20906-SNP29343), qEC10-6 (SNP53263-CA1598), qEC11-6 (SNP15767-HLJ853), qEC1-14 (SNP6081-HLJ2920), qEC1-33 (HLJE310-SNP14543), qEC2-33 (CA2183-HLJ1972), qEC3-33 (HLJ3143- SNP8212), qEC1-35 (SNP26965-CA1931), qEC1-39 (SNP29265-SNP16825), and qEC1-44 (SNP82796-SNP71135) (Table 1). Among these QTL linkage groups, qEC1-6 (SNP6373-SNP9469) had the largest LOD value (4.09), explaining 24.8% of the total phenotypic variation. In contrast, qEC11-6 (SNP15767-HLJ853) had the smallest LOD value (2.53), explaining 12.8% of the phenotypic variation. The total phenotypic variation explained by these 20 linkage groups ranged from 10.8% by group qEC2-5 (SNP8987-SNP3496) to 36.9% by group qEC1-39 (SNP29265-SNP16825) (Figure 2). The marker interval for eye cross ranged from 0.581 cM on LG 35 to 20.186 cM on LG 32.

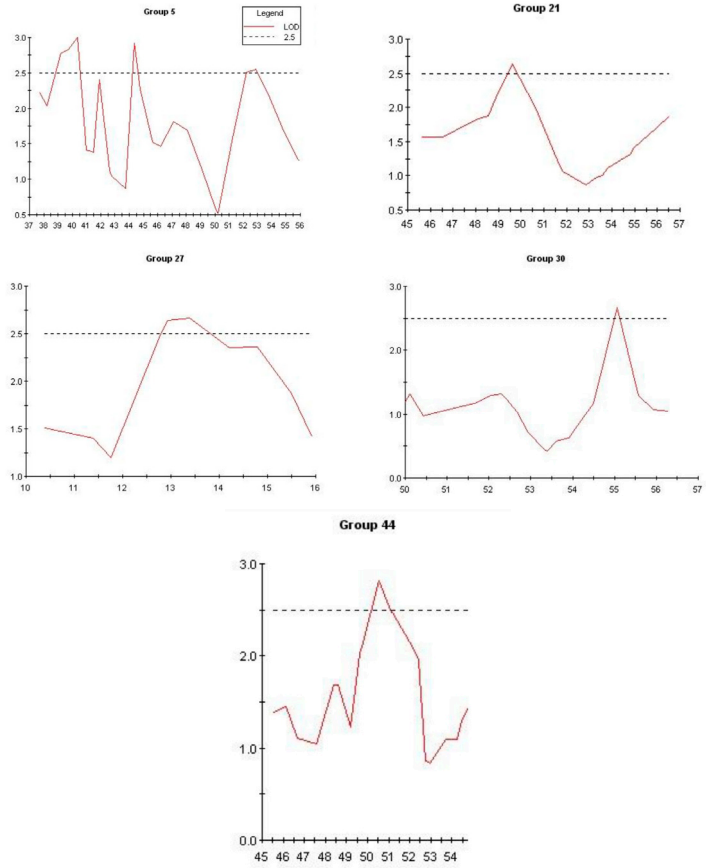

Figure 1. LOD curves for QTLs contributing to the eye diameter. The X-axis indicates marker distance and the Y-axis represents LOD with the dashed line indicating the threshold value of 2.5.

Table 1. Eye diameter (ED) and eye cross (EC) QTLs and estimates of genetic effects.

Linkage Group	QTL name	Marker interval	Marker interval (cM)	Linkage group size (cM)	LOD	Variation (%)
LG5	qED1-5	CA1602-SNP83693	39.8-41	55.933	3	12.1
LG5	qED2-5	HLJ346-HLJ2013	43.7-44.8	55.933	2.92	13.5
LG5	qED3-5	HLJ377-CA1909	50.2-55.9	55.933	2.55	10.5
LG21	qED-21	SNP49664-SNP67252	48.9-51.8	167.116	2.64	11.1
LG27	qED1-27	HLJ3294-SNP27486	12.9-14.2	108.167	2.64	12.5
LG30	qED1-30	SNP1629-SNP52393	54.5-55.6	124.087	2.67	11.4
LG44	qED1-44	SNP32671-SNP4483	49.8-51.1	109.257	2.82	11.9
LG44	qED2-44	SNP28085-SNP35232	61.1-62.1	109.257	2.67	11.9
LG45	qED1-45	HLJ3407-HLJ3396	68.4-112.1	126.423	2.85	57.2
LG45	qED2-45	SNP7445-SNP81574	61.2-63.1	126.423	2.73	56.8
LG5	qEC1-5	SNP50851-SNP79571	10.3-11.8	55.933	3.01	12.1
LG5	qEC2-5	SNP8987-SNP3496	14.4-15.1	55.933	2.65	10.8
LG6	qEC1-6	SNP6373-SNP9469	11.7-14.4	79.982	4.09	24.8
LG6	qEC2-6	SNP59840-SNP14880	31.1-31.8	79.982	3.29	15.4
LG6	qEC3-6	SNP26196-SNP25935	29.5-30.4	79.982	3.20	13.3
LG6	qEC4-6	HLJ2556-SNP16225	39.6-40.2	79.982	3.18	15.2
LG6	qEC5-6	SNP29602-SNP4802	34.7-35.3	79.982	3.08	15.2
LG6	qEC6-6	SNP54288-SNP3418	30.7-31.2	79.982	2.94	12.1
LG6	qEC7-6	SNP5961-SNP1566	37.2-37.4	79.982	2.82	21.8
LG6	qEC8-6	SNP3047-SNP3263	36.5-36.8	79.982	2.77	11.3
LG6	qEC9-6	SNP20906-SNP29343	32.2-32.4	79.982	2.73	11.4
LG6	qEC10-6	SNP53263-CA1598	14.9-15.8	79.982	2.60	13.4
LG6	qEC11-6	SNP15767-HLJ853	18.3-19.9	79.982	2.53	12.8
LG14	qEC1-14	SNP6081-HLJ2920	35.4-37.7	84.565	2.84	11.7
LG33	qEC1-33	HLJE310-SNP14543	64.6-65.6	88.901	3.63	19.3
LG33	qEC2-33	CA2183-HLJ1972	51.0-51.5	88.901	3.07	23.2
LG33	qEC3-33	HLJ3143-SNP8212	41.2-42.3	88.901	2.64	13.1
LG35	qEC1-35	SNP26965-CA1931	52.9-53.6	95.554	2.94	12.3
LG39	qEC1-39	SNP29265-SNP16825	0-5.2	88.062	2.72	36.9
LG44	qEC1-44	SNP82796-SNP71135	32.7-37.0	109.257	2.81	11.6

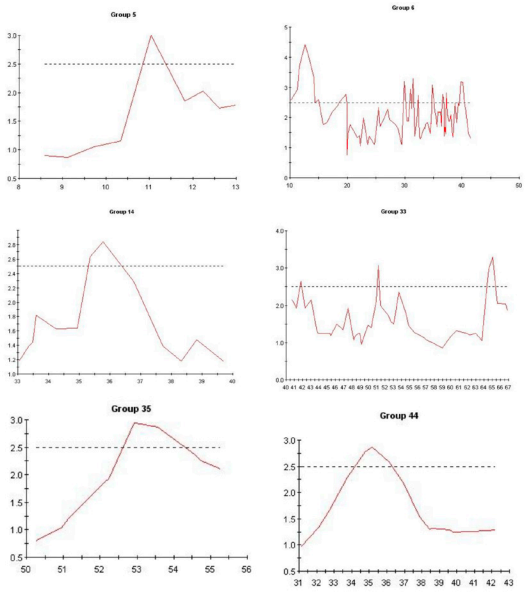

Figure 2. LOD curves for QTLs contributing to eye cross. The X-axis indicates marker distance and the Y-axis represents the LOD with the dashed line indicating the threshold value of 2.5.

Growth factor detection

One growth gene was detected from the QTL qED5-3 with the function of growth arrest-specific 2 like 1. Three growth genes were detected from the QTL qEC5-2, qEC6-5, and qEC33-3 with the functions of putative growth hormone-regulated TBC protein, transforming growth factor-beta (TGF-β) family, and inhibitor of growth protein 5, respectively. Their functions are detailed in Table 2 and Figure 3. These candidate genes were identified as being involved in the regulation of growth cycles, and they have previously been cited as being key candidate genes regulating growth rates (Lee et al., 1999; Moustakas and Heldin, 2008; Xing et al., 2011).

Table 2. Eye diameter (ED) and eye cross (Ed) QTLs refined for the draft carp genome and candidate growth genes

QTL name	Associated markers	Chr.	Marker location		Gene ID	Gene location		Total length	Gene function
			From	To		From	To		
qED-5-3	CAFS1909	10	20321935	20321309	16558.gff.p.0.0	19658977	19655410	22665327	Growth arrest-specific 2 like 1
qEC-5-2	SNP8987	10	5854802	5854492	430.gff.p.0.0	5854802	5854492	22665327	Growth hormone-regulated TBC protein, putative
qEC-6-5	CAFS82	35	28025365	28024893	17348.gff.m.6.0	28013213	28011048	29126390	Transforming growth factor-beta (TGF-beta) family
qEC-33-3	HLJE310	44	2767791	2768471	4563.gff.m.0.4	2126678	2126567	15901873	Inhibitor of growth protein 5

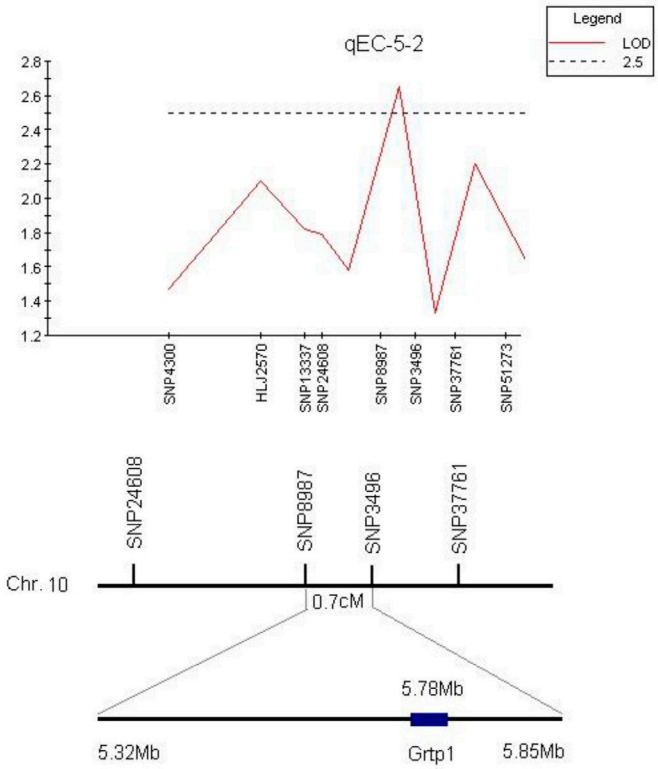

Figure 3. Candidate growth gene in the marker interval region of the carp genome.

Correlation analysis

The results of the correlation analysis between eye diameter and eye cross and the other traits indicate that eye diameter and eye cross are positively correlated with all commercial traits ($P < 0.05$; Table 3). Eye diameter and eye cross showed highly significant positive correlation with the important commercial traits ($P < 0.01$), including body weight, full length, body length, height, and thickness. In particular, correlation was greater than 0.8 between eye cross and the following traits: body weight, full length, body length, height, and thickness.

Table 3. Correlation coefficients between different growth traits in the common carp.

	Body weight	Full length	Body length	Height	Thickness	Head length	Snout length	Eye diameter	Eye cross	Caudal peduncle length	Caudal peduncle height
Body weight	1.0000										
Full length	0.9309	1.0000									
Body length	0.9128	0.9900	1.0000								
Height	0.9716	0.8947	0.8706	1.0000							
Thickness	0.8590	0.7318	0.7021	0.8578	1.0000						
Head length	0.7804	0.8092	0.7944	0.7540	0.6778	1.0000					
Snout length	0.6148	0.5795	0.5796	0.5838	0.5321	0.4736	1.0000				
Eye diameter	0.5917	0.5498	0.5481	0.5527	0.4864	0.4069	0.5100	1.0000			
Eye cross	0.8899	0.8470	0.8351	0.8633	0.7442	0.7853	0.6412	0.5661	1.0000		
Caudal peduncle length	0.4005	0.5745	0.5959	0.3712	0.2818	0.4007	0.0498	0.1935	0.3491	1.0000	
Caudal peduncle height	0.8811	0.8330	0.8216	0.8725	0.7632	0.6747	0.5650	0.5276	0.7431	0.3750	1.0000

DISCUSSION

This study aimed to identify the QTL regions for eye cross and eye diameter and to determine the candidate functional genes responsible for the development of these traits. A correlation analysis was performed to test whether the functional genes identified for eye diameter and eye cross potentially play an essential role for these characteristics and other important traits.

Recombination length was used to estimate the genome size of the common carp in this project and it was much larger than previously published for this species (Sun and Liang, 2004; Zhang et al., 2010; Wang et al., 2011b). The first linkage map for the common carp was constructed using dominant markers with a total recombination length of 4111 cM based on 214 microsatellites and 54 RAPD markers (Sun and Liang, 2004). Further, additional co-dominant markers, including SNPs and microsatellites, were synthesized, making it possible to measure the genetic variation between different species and generations. Zhang et al. (2010) observed a genome length of 1852 cM for a consensus map based on only 161 microsatellite markers. Wang et al. (2011b) obtained a total recombination length of 2805.85 cM with an average linkage group length of 6.31 cM based on 186 SSR markers and 321 SNP markers. In this study, the population size and number of markers were dramatically larger than those used in the previous studies. A total of 4877 polymorphic markers (3899 of 4026 SNP markers and 978 of 1042 microsatellite markers) were used to construct the genetic map with a population of 107. We obtained a total recombination length of 4777.09 cM with an average linkage group length of 0.98 cM. Using plenty of markers can result in smaller average lengths between different markers. The linkage map comprised 50 linkage groups, which is consistent with the karyotype of the common carp (Yu et al., 1989), suggesting that sufficient markers

were used to construct this high-quality linkage map. By using these results, the QTL of the common carp can be accurately pinpointed and can provide accurate information for future breeding programs.

Eye diameter and eye cross reported by Liu et al. (2009) and Jin et al. (2012) have been identified to be quantitative traits. According to previous studies, only two QTL were identified as being related to eye diameter and eye cross, explaining 5.62-9.77% and 8.29-8.88% of the total phenotypic variation, respectively (Liu et al., 2009). Additionally, eight QTL were related to eye diameter and eleven QTL were related to eye cross, explaining 16.60-36.70 and 13.60-31.60% of the total phenotypic variation, respectively. In the current study, ten QTL were related to eye diameter. Of the total variation, these linkage groups explained 10.5% on LG5 qED3-5 (HLJ377-HLJ1909) and 57.2% on qED1-45 (HLJ3407-HLJ3396). The QTL on LG45 explained the highest phenotypic variation (Table 1), thus, it is suggested as the main functional region for eye diameter. Twenty QTL were related to eye cross, and of the total phenotypic variation, these linkage groups explained 10.8% on qEC2-5 (SNP8987-SNP3496) to 36.9% on qEC1-39 (SNP29265-SNP16825). Eleven of these twenty QTL were located on LG6, implying that LG6 may be the main functional group controlling the expression of eye cross (Figure 2). Generally, the value of explained variation was closely related to trait variance within a family. Altogether, 30 QTL regions were found to be correlated to these two quantitative traits, with six regions (two regions for eye diameter and four regions for eye cross) considered to be significant because they each explained more than 20% of the total phenotypic variation. LG5 and LG44 were important for both eye diameter and eye cross because both QTL for eye diameter and eye cross were located on LG5 and LG44 simultaneously. In the present study, further QTL regions were identified for eye diameter and eye cross. Most importantly, these QTL regions are significantly smaller, and the accuracy of the genetic map is much higher, than reported in previous reports because many searches have been conducted in relation to the sequencing of the whole genome of common carp in recent years (Lien et al., 2011; Xu et al., 2011). Thus, sufficient SNP markers were available to construct the linkage map with a high-density of markers. As a consequence, the functional genes responsible for the growth and expression of eye cross and eye diameter were identified. Linkage groups 5 and 44 may contain functional genes that simultaneously control the growth of eye diameter and eye cross.

When the genome of a species is unknown, QTL may be useful for determining the putative functions of genes by observing their similarity to genes with known functions in other genomes through sequencing the identified region. It is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene (Jannink et al., 2001). This study is the first to identify functional genes responsible for the growth of commercial traits from QTL regions in common carp. In addition, a number of growth factors were observed that may regulate the growth of eye diameter and eye cross. Our results represent the first observations of major genetic growth factors and contribute toward understanding the regulation of eye diameter and eye cross growth in common carp.

In the present study, one growth factor (growth arrest-specific 2 like 1) was observed in the QTL region of eye diameter, and this growth factor is located on LG10 of the common carp genome. Three growth factors (growth hormone-regulated TBC protein, putative; TGF-beta family; inhibitor of growth protein 5) were found in the QTL regions of eye cross, and these growth factors are located on LG10, LG33 and LG45 of the common carp genome (Figure 3). Growth arrest-specific 2 like 1 is a member of the GAS2 family (Goriounov et

al., 2003). GAS2 may play an important role in regulating chondrocyte proliferation and differentiation. This gene may also be involved in executing the apoptotic program in hindlimb interdigital tissues, acting as a death substrate for caspase enzymes (Lee et al., 1999). Growth hormone-regulated TBC protein (Grtp1) is a novel gene, and contains the TBC signature motif of GTPase activator proteins of Rab-like small GTPases. TGF-β regulates cellular behavior in embryonic and adult tissues by binding to serine/threonine kinase receptors on the plasma membrane, which activates Smad molecules and additional signaling proteins that together regulate gene expression or cytoplasmic processes, such as cytoskeletal dynamics (Moustakas and Heldin, 2008). Inhibitor of growth protein 5 (ING5) is a new member of the ING family, identified by a computational homology search. Thus far, little research has been conducted on the function of ING5. ING5 can physically interact with p300 and p53 *in vivo*, and the over-expression of ING5 induces apoptosis in colorectal cancer cells. A study on mutation and downregulation of ING5 mRNA in oral squamous cell carcinoma suggests that this is a tumor suppressor gene (Xing et al., 2011). Correlation analysis can be used to examine the relationship between different commercial traits, and several studies have already proven that one commercial trait may assist the indirect selection for other commercial traits based upon their correlation relationship (Rasaei et al., 2011). In previous studies, eye cross and eye diameter were identified as being significantly correlated with body weight, and these two commercial traits share some QTL regions with body weight in the same generation of common carp (Jin et al., 2012). Based on correlation analysis, eye diameter and eye cross were found to be significantly positively correlated with many commercial traits, especially those with a higher positive correlation with body weight, which is consistent with the previous study (Jin et al., 2012). This implies that these two commercial traits may share QTL regions with body weight, and, thus, these two commercial traits may assist the indirect selection for body weight (Table 3; Jin et al., 2012).

Cluster phenomenon exists in many species and its ecological significance has been discussed because some QTL regions responsible for different traits have been observed clustered on the same QTL groups (Cai and Morishima, 2002). “Multifactorial linkages” may be the key factor for this cluster phenomenon followed by natural selection favoring co-adapted traits. Furthermore, pleiotropy based on a number of unknown key factor(s) controlling various traits through diverse metabolic pathways may be another possible reason for the clustering phenomenon (Cai and Morishima, 2002). Therefore, multiple traits could be improved simultaneously when closely linked markers are used to assist the breeding work based on close genetic characteristics, and aggregate breeding could achieve multiple effects. This indicates that the growth factors responsible for the regulation of eye diameter and eye cross in this study may also simultaneously control the growth of body weight. Therefore, studies on the growth factors observed here may also advance improvements in body weight, enhancing the commercial value of the carp population. These findings provide useful information for genetic studies and will benefit future breeding programs for common carp.

In conclusion, this study has accurately located eye diameter and eye cross on the linkage map of common carp. A number of growth factors related to the growth of eye diameter and eye cross have been identified, which has valuable implications for aquaculture breeding programs. This research also showed that eye diameter and eye cross may be used for indirect selection for important commercial traits and aggregate breeding could achieve multiple effects.

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