A second-generation genetic linkage map for bighead carp (*Aristichthys nobilis*) based on microsatellite markers

C. Zhu*†, J. Tong*, X. Yu*, W. Guo*†, X. Wang*†, H. Liu*†, X. Feng*†, Y. Sun*†, L. Liu*† and B. Fu†‡

*State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China. †University of Chinese Academy of Sciences, Beijing 100039, China. ‡Key Laboratory of Aquatic Biodiversity and Conservation of the CAS, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.

Summary

Bighead carp (*Aristichthys nobilis*) is an important aquaculture fish worldwide. Genetic linkage maps for the species were previously reported, but map resolution remained to be improved. In this study, a second-generation genetic linkage map was constructed for bighead carp through a pseudo-testcross strategy using interspecific hybrids between bighead carp and silver carp. Of the 754 microsatellites genotyped in two interspecific mapping families (with 77 progenies for each family), 659 markers were assigned to 24 linkage groups, which were equal to the chromosome numbers of the haploid genome. The consensus map spanned 1917.3 cM covering 92.8% of the estimated bighead carp genome with an average marker interval of 2.9 cM. The length of linkage groups ranged from 52.2 to 133.5 cM with an average of 79.9 cM. The number of markers per linkage group varied from 11 to 55 with an average of 27.5 per linkage group. Normality tests on interval distances of the map showed a non-normal marker distribution; however, significant correlation was found between the length of linkage group and the number of markers below the 0.01 significance level (two-tailed). The length of the female map was 1.12 times that of the male map, and the average recombination ratio of female to male was 1.10:1. Visual inspection showed that distorted markers gathered in some linkage groups and in certain regions of the male and female maps. This well-defined genetic linkage map will provide a basic framework for further genome mapping of quantitative traits, comparative mapping and marker-assisted breeding in bighead carp.

Keywords *Aristichthys nobilis*, genetic map, interspecific hybrids, pseudo-testcross, simple sequence repeats

Introduction

Genetic linkage maps have become essential tools in many fields of genetic studies including quantitative trait loci (QTL) location (O’Malley *et al.* 2003), marker-assisted selection (MAS) (Fuji *et al.* 2006), comparative genome mapping (Woods *et al.* 2000), genome assembly and position-based cloning (Dietrich *et al.* 1996). Genetic linkage maps have been reported for many economically important aquaculture fishes; however, the majority of them were constructed based mainly on dominant markers, such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), with limited numbers of codominant markers and low resolution. In comparison, second-generation genetic linkage maps, based on codominant markers (microsatellites and SNPs) with high resolution, are more accurate and desirable for their further applications in genetics and genomics studies. Nevertheless, due to the lack of codominant markers, second-generation genetic maps have been reported for a limited number of food fish species, for example, Asian seabass (Wang *et al.* 2011), tilapia (Lee *et al.* 2005) and rainbow trout (Rexroad *et al.* 2008). Compared with SNPs, the microsatellite, also known as a simple sequence repeat (SSR), has become the preferred codominant marker for animal genome and QTL mapping (Wang *et al.* 2011; Liu *et al.* 2012) because of its...
advantages of universality in closely related species, high polymorphism and ease of genotyping via PCR (Chistiakov et al. 2006).

Bighead carp (Aristichthys nobilis) is one of the most important aquaculture fish species in China. The global annual production of bighead carp reached 2.6 million tons with a total value of about 3.3 million US dollars in 2010, and 98.6 percent of the contributions were made by Chinese aquaculture (FAO 2010). Bighead carp is a filter-feeding fish, grows fast and has been introduced into many countries for water quality control and human consumption (Kolar et al. 2005). However, natural populations of bighead carp, as well as other Chinese major carps, have seriously declined during the recent decades due to ongoing human activities such as pollution, dam construction and overfishing. Worse still, wild populations may be the mixture of natural and cultured individuals because of frequent flooding and unscientific artificial releasing (Clifford et al. 1998; Zhu et al. 2007), which has resulted in depression and reduction of growth performance (Liu et al. 1997). This highlights the urgent need for genetic improvement for bighead carp and also for other Chinese major carps in China.

A genetic linkage map is the basis for further genomics, genetics and breeding studies in fish. A first-generation linkage map for bighead carp was constructed using 153 DNA markers (27 SSRs and 126 AFLPs) (Liao et al. 2007a). An extended genetic map for bighead carp, containing 39 linkage groups (LGs) with 168 AFLPs and 120 SSRs (Zhang et al. 2011), still has many small LGs and the resolution is not high enough for QTL analysis and other genomics studies. In this study, we aimed to construct a SSR-based second-generation genetic linkage map for bighead carp using the pseudo-testcross strategy (Liu et al. 2003; Liao et al. 2007a) to provide a framework for further QTL analysis, comparative genomics and MAS study in the species.

**Materials and methods**

**Mapping families and DNA extraction**

A bighead carp × silver carp cross and a reciprocal cross were performed to produce interspecific mapping panels in this study. Four parental fish were collected from the middle reach of the Yangtze River, and artificial reproduction and fertilization were carried out at the Wuhan Donghu Fish Farm under controlled conditions. The F1 progenies were raised in plastic tanks in the laboratory and fed hatched *Artemia* cysts until sampling. At the age of two months, 77 progenies were randomly sampled from each cross and preserved in anhydrous ethanol at 4 °C. Fin clips of four parents were stored under the same conditions. Genomic DNA was extracted following the standard phenol-chloroform protocol (Sambrook & Russell 2001).

**Sources of SSR markers**

The majority of SSR markers in this study were developed from scaffold sequences of genome and transcriptome sequencing projects for bighead carp and silver carp (coordinated by Prof. S.P. He, Institute of Hydrobiology, Chinese Academy of Sciences), and they were named with the prefixes ‘Arsd’, ‘Hysd’ and ‘HySd’ respectively. Other sources included those from three microsatellite-enriched genomic libraries (two for bighead carp with GA and GT repeats and one for silver carp with a GT repeat), which were constructed following the FIASCO (fast isolation by AFLP of sequences containing repeats) method (Cheng et al. 2007), and these microsatellites were named with the prefixes ‘ArGA’, ‘ArGT’ and ‘HyGT’ respectively. In total, 1922 SSR and 260 EST-SSR markers were newly developed from microsatellite-containing sequences of the bighead carp and silver carp in this study. The software PRIMER PREMIER 5.0 (http://www.premierbiosoft.com/primerdesign/index.html) was used to design primers for these microsatellites.

A set of 584 microsatellites published by different authors in bighead carp and its closely related cyprinid fish (silver carp and grass carp), with the prefixes ‘Hym’ (Zhang et al. 2010; Liao et al. 2007a), ‘Ar’ (Cheng et al. 2008), ‘BL’ (Liao et al. 2007b), ‘Hmo’ (Gheyas et al. 2006) and ‘Cid’ (Guo et al. 2009), were also applied in this study.

**Microsatellite genotyping**

The amplifications for SSR markers were carried out through PCR in a total volume of 12.5 μl containing 1.25 μl of 10× reaction buffer, 0.4 μl of dNTP (2.5 mM), 1 Unit of Taq polymerase (TaKaRa), 0.4 μl of forward and reverse primer mixture (2.5 μM), 20–50 ng of template DNA and 9.4 μl of sterile water. PCRs were carried out on a 96-well thermal cycler (Veriti, Applied Biosystems) using the following program: 94 °C for 5 min, followed by 35 cycles of 94 °C for 35 s, optimal annealing temperature (see Table S1) for 35 s and 72 °C for 40 s, and a final extension of 72 °C for 8 min. PCR products were separated by 10% polyacrylamide gel electrophoresis and visualized by the JS-A380 gel imaging system (Peijing) after staining with ethidium bromide. Only those microsatellites that produced visually recognizable alleles were genotyped, and scoring of alleles was based on parental banding patterns with the aid of pBR322 DNA/MSPI molecular standards (Tiangen).

**Map construction and genome coverage**

Chi-square tests were performed to determine the fitness of marker segregation data to the expected 1:1 ratio. Distorted markers were also used for linkage analysis, and they were indicated with asterisks on male and female LGs. For those distorted markers on the map, the more
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Markers with more than 10% missing data were eliminated from further analysis. The construction of both female and male maps was implemented by the JOINMAP 3.0 program (Van Ooijen & Voorrips 2001) using the Kosambi’s function under the BC1 algorithm with the logarithm of odds (LOD) value of 4.0 at a stringency of LOD > 1.00 and REC (recombination fraction) < 0.400. The consensus map was constructed under the same conditions by joining the female and male linkage maps through common markers, which played the role of bridging the two maps. Graphical linkage maps were drawn using the software MAPCHART 2.1 (Voorrips 2002).

The estimated genome lengths for female, male and consensus bighead carp maps were calculated through two different approaches. One method is adding 2s, where s is the average space of different maps, to the length of each LG, which accounts for chromosome ends (Fishman et al. 2001); the other is multiplying the length of each LG using the formula (m + 1)/m, where m is the number of markers in each LG, and summing them up for all LGs (Chakravarti et al. 1991). The estimated genome lengths are the averages of the lengths calculated by these two methods.

Map characteristics

SPSS 13.0 software was used to obtain the characteristics of the genetic linkage maps. The Shapiro–Wilk test was implemented to determine whether distributions of standardized map distances between adjacent markers fit the normal distribution, and the Pearson’s correlation coefficient between the number of markers and the total length of each LG was calculated to evaluate whether markers distributed randomly throughout the map. In addition, a G-test was performed to estimate whether there were significant recombination differences between the female and male maps of bighead carp.

Results

Informative markers and segregation distortion

A total of 2766 SSRs were screened in the two mapping families. Ultimately, 461 loci were informative for the female bighead carp of the bighead carp × silver carp cross, and 85 (18.4%) of them were distorted. Meanwhile, 418 loci segregated for the male bighead carp of the reciprocal cross with a distortion ratio of 15.3% (64 markers).

Linkage mapping and genome coverage

For the female map, 431 of 461 informative SSRs were ordered in 24 LGs with 68 distorted loci. For the male map, 356 SSRs were assigned into 24 LGs with 57 distorted markers. There were 125 common markers between the female and male maps, and a consensus map consisting of 659 SSRs in 24 LGs was produced based on these markers shared between the maps of the two sexes (Fig. 1). Detailed information, along with GenBank accession numbers for these 659 SSRs, is provided in Table S1. The total length of the consensus map was 1917.3 cM, covering 92.8% of the estimated genome size (2067.1 cM) of bighead carp, with the maximum LG 133.5 cM, the minimum LG 52.2 cM and an average space of 2.9 cM. The number of markers per LG ranged from 11 to 55, with an average of 27.5 (Table 1).

Comparison between sex-specific maps

The female map covered 1814.6 cM with a genome coverage rate of 89.3% (estimated genome length was 2032.9 cM), whereas the male map spanned 1620.0 cM covering 86.7% of the estimated genome length (1869.5 cM; Table 1). Therefore, the female map is 1.12 times the length of the male map, with 16 homologous LGs in the female map being longer than in the male map. A total of 125 common markers distributed in 24 LGs bounded a set of common intervals between the two maps, adding up to 982.0 cM in the female map and 954.4 cM in the male map. The average recombination ratio between the female and male maps was 1.10, which was calculated by averaging the ratios of mean distances of each LG in the female and male maps (Table 2). The result of the G-test indicated that the recombination difference between the sexes in bighead carp was not statistically significant (P = 0.191).

Distribution of distorted markers

We found that distorted markers did not randomly distribute throughout the bighead carp genome; instead, they gathered in some regions of certain female or male LGs. For instance, 33 of the 85 distorted markers were clustered in LG4, LG17 and LG20 of the female map. And interestingly, LG3 of the male map contained 22 of the 64 distorted markers (Fig. 1). Globally, 13 female LGs and 17 male LGs contained distorted markers.

Map characters

All three distance distribution histograms showed a cluster of map intervals on the left side of the charts (Fig. 2). The results of Shapiro–Wilk tests showed that the interval distances in three maps were all non-normally distributed (data not shown). However, the correlations between the number of markers and length of each LG were significant at the 0.01 level and 0.05 significance level (two-tailed) in the consensus (P = 0.007) and female (P = 0.018) maps with a Pearson’s correlation coefficient of 0.53 and 0.48 respectively. However, this correlation was not significant in the male map (P = 0.088) with a Pearson’s correlation coefficient of 0.36.

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Discussion

Informativeness rate of microsatellites

Informativeness rates of microsatellites in the two interspecific mapping families were relatively low compared with previous studies (Lee et al. 2005; Zhang et al. 2010). There may be several reasons responsible for this phenomenon in this study. First, the genetic polymorphism of bighead carp was low. Our previous population genetics studies indicated that the average observed heterozygosity of bighead carp populations in the middle reach of the Yangtze River was

Figure 1 Genetic linkage map for bighead carp based on microsatellite markers. On the left side of each linkage group (LG) is the consensus map, with male and female maps in the middle and on the right respectively. The genetic distances in Kosambi centimorgans are listed on the left of each LG, and markers are listed on the right. Distorted markers are marked with ‘*’.
relatively low \((H_o = 0.5)\) compared with that of other cyprinid fish species such as silver carp \((H_o = 0.8)\) (Zhu C., Yu X. & Tong J.; Guo C., Yu X. & Tong J., unpublished data). Second, the efficiency of cross-species markers was relatively low in the two mapping families. Of the 2766 SSRs, only 850 were developed from bighead carp, and 272 of them were assigned to the linkage map with an efficiency rate of 32%; the remaining 1916 were from silver carp and grass carp, and only 20\% (387 SSRs) of them were useful. Finally, a small number of the cross-species amplified markers were not included in the final data set because of too much missing data (probably due to null alleles).

Genetic linkage map

In this study, a pseudo-testcross strategy and interspecific hybrids between bighead carp and silver carp, together with both genomic and genic microsatellites, were applied to construct a second-generation genetic linkage map for bighead carp, an important food fish in China. This mapping strategy allowed us to construct genetic linkage maps for both species. A total of 754 SSRs were informative in the two mapping families, and 431 and 356 of them were localized in the female and male maps respectively of bighead carp. Based on 125 common markers between the two sex-specific maps, a consensus map with 659 markers was constructed for bighead carp. Expectedly, these markers were assigned into 24 LGs that coincided with the chromosome numbers of the haploid genome of bighead carp \((2n = 48)\) (Bozhko et al. 1976; Zan & Song 1980). The number of codominant markers in this map is more than five times that of previous maps (up to 120 SSRs) (Liao et al. 2007a; Zhang et al. 2011) of bighead carp, a little higher than that of grass carp \((2n = 48)\) (Xia et al. 2010) but similar to that of Asian seabass (Wang et al. 2011). The map length \((1917.3 \text{ cM})\) was about twice that of the previous map \((965.8 \text{ cM})\) (Zhang et al. 2011), and was longer than that of silver carp \((1555.2 \text{ cM}; 2n = 48)\) (Guo et al. 2013) and grass carp \((1176.1 \text{ cM})\) (Xia et al. 2010) but significantly shorter than that of zebrafish \((3011 \text{ cM}; 2n = 50)\) (Kelly et al. 2000) and common carp \((5183.9 \text{ cM}; 2n = 100)\) (Xu et al. 2012). These simple comparisons show that information derived from genetic linkage maps in the Cyprinidae (bighead carp, silver carp, grass carp, zebrafish and common carp) may reflect a correlation between estimated map length and real genome size in this fish family. The coverage rate (92.8\%) of the bighead carp map in this study is higher than that of many food fish species reported previously. As shown in a QTL analysis for seabass with a similar map resolution (Wang et al. 2011), the density of our genetic linkage map would be sufficient for future QTL fine mapping and MAS studies in bighead carp.

The interval distances of our present genetic map seem to be non-normally distributed with a cluster of distance between 0 and 4 \text{ cM} (Fig. 2) on the left side of the distance distribution chart (Fig. 2). It is believed that the frequency of genetic distances in a saturated genetic map should be a normal distribution (Krutovskii et al. 1998). These characteristics of distance frequencies suggest that more markers are needed in the future to make the map saturated. Nevertheless, previous studies indicated that the number of markers and the length of the LGs must be linear when markers are distributed throughout the genome randomly (Cervera et al. 2001). As expected, the results of the correlation analyses in our study showed a significant correlation between these two indexes; therefore, we conclude that the SSRs in this consensus map distribute randomly among different LGs of bighead carp, similar to

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the previous genetic linkage analysis in common carp (Cheng et al. 2010).

In comparison with the previous genetic map of the same species (Zhang et al. 2011), our study improved the genetic linkage map of bighead carp in the number of codominant markers (from 120 to 659), the number of LGs (from 39 to 24), the genome coverage rate (from 77.3% to 92.8%) and the map resolution (from 3.9 to 2.9 cM). Further, in this study, we constructed the male map and consensus map for bighead carp for the first time, as previously reported genetic maps for bighead carp were actually female maps only (Liao et al. 2007a; Zhang et al. 2011). Syntenic comparison between genetic linkage maps of the two generations showed that, for 17 LGs in our present map, we could find homologous LGs in the previous map (Zhang et al. 2011) through 24 common markers (Fig. S1). However, macro-syntenic relationships could not be obtained due to the lack of more SSRs shared between these two maps for bighead carp.

### Common markers between female and male maps

Of the 659 microsatellites located on the consensus map of bighead carp, 125 were common between the female and male maps. Compared with previous studies (Xia et al. 2010; Zhang et al. 2010), the rate of common markers was at a low level in this study, which may be a reflection of genetic differences of the male and female bighead carp parents of the two families used for mapping. Usually, to link as many markers as possible on linkage maps using F1 mapping populations, a reasonably high level of genetic difference between the parents is required (Liu et al. 2003; Liao et al. 2007a; Xia et al. 2010). In this present study, interspecific hybrid F1 families between bighead carp and silver carp were applied as mapping populations; in addition, the dams and sires of the two families were originally collected from different sites of the Yangtze River when they were juveniles. This strategy helped us to use female and male parents with remarkable genetic differences to construct mapping panels. However, these genetic differences may be expressed as different heterozygous patterns: For a given marker, one parent may be heterozygous but the other may be homozygous; therefore, this marker could not be mapped for the homozygous parent. If more mapping families were used, heterozygous parents would be present for both sexes, and the level of common markers would be higher. In fact, the rate of common markers between sexes of bighead carp (19.0%, 125/659) in this study was higher

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**Table 1** Statistics of the female, male and consensus maps of bighead carp.

| Linkage group | Female | | Male | | Consensus | |
|---------------|--------|---|--------|---|----------|---|
|               | No. of loci | Length (cM) | No. of loci | Length (cM) | No. of loci | Length (cM) |
| LG1           | 24     | 99.2 | 22     | 100.3 | 37     | 133.5 |
| LG2           | 22     | 102.2 | 20     | 97.0  | 36     | 123.3 |
| LG3           | 34     | 118.9 | 32     | 46.5  | 55     | 111.6 |
| LG4           | 13     | 46.2  | 15     | 81.8  | 26     | 97.6  |
| LG5           | 12     | 75.8  | 15     | 59.7  | 23     | 91.5  |
| LG6           | 22     | 91.0  | 23     | 65.9  | 37     | 89.9  |
| LG7           | 17     | 84.1  | 9      | 69.0  | 22     | 88.1  |
| LG8           | 16     | 92.2  | 21     | 65.0  | 31     | 85.4  |
| LG9           | 23     | 93.1  | 15     | 71.9  | 34     | 84.3  |
| LG10          | 28     | 74.4  | 29     | 97.2  | 47     | 82.4  |
| LG11          | 13     | 82.5  | 9      | 61.7  | 20     | 82.4  |
| LG12          | 10     | 81.2  | 9      | 75.8  | 16     | 80.7  |
| LG13          | 22     | 87.9  | 11     | 65.7  | 28     | 75.5  |
| LG14          | 19     | 79.3  | 17     | 65.3  | 31     | 74.9  |
| LG15          | 17     | 90.3  | 14     | 99.0  | 26     | 74.6  |
| LG16          | 11     | 67.6  | 11     | 54.9  | 20     | 72.3  |
| LG17          | 20     | 55.9  | 6      | 37.3  | 24     | 58.2  |
| LG18          | 12     | 70.8  | 8      | 47.8  | 16     | 65.7  |
| LG19          | 8      | 64.1  | 5      | 51.3  | 11     | 65.3  |
| LG20          | 16     | 79.7  | 12     | 45.1  | 21     | 63.3  |
| LG21          | 24     | 69.1  | 15     | 70.4  | 31     | 55.3  |
| LG22          | 18     | 45.2  | 13     | 55.9  | 26     | 55.2  |
| LG23          | 10     | 24.4  | 10     | 65.0  | 13     | 54.1  |
| LG24          | 20     | 39.5  | 15     | 70.5  | 28     | 52.2  |
| Total         | 431    | 1814.6| 356    | 1620.0| 659    | 1917.3|
| Average       | 18.0   | 75.6  | 14.8   | 67.5  | 27.5   | 79.9  |
| Expected genome length (cM) | 2032.9 | 1869.5 | 2067.1 |
| Genome coverage (%) | 89.3   | 86.7  | 92.8   |
| Average space (cM) | 4.2    | 4.6   | 2.9    |
Recombination differences between sexes

Different recombination rates between sexes have been reported in many teleosts, which is usually higher in the homogametic sex than in the heterogametic sex. In many fish, the recombination ratio of female to male was about 2:1 (Chistiakov et al. 2008; Rexroad et al. 2008; Xia et al. 2010; Nomura et al. 2011), whereas in other fish species these differences may be extremely significant, for example, 8.26:1 in Atlantic salmon (Moen et al. 2004). On the other hand, in some fish species the recombination rates may be higher for males than for females, and an extreme male to female recombination ratio even reached 7.4:1.0 in Japanese flounder (Coimbra et al. 2003). Recombination is more restricted in the sex that has a heterogametic gamete than in the sex that has a homogametic gamete (Haldane 1922). Because either female or male fish may be heterogametic sex if sex chromosomes occur (Gold 1979), both female and male bias in recombination rates could be observed. In red drum, family-specific recombination rates were also detected, whereas the overall recombination rates between female and male differed slightly, with the female to male ratio of 1.03:1 (Portnoy et al. 2010).

The average female to male recombination ratio of this bighead carp map was 1.10:1, which was similar to that of red drum (Portnoy et al. 2010). The slight difference between female and male recombination rates may reflect the absence of detectable sex chromosomes (Portnoy et al. 2010) and, indeed, no sex chromosomes were observed in bighead carp (Yu et al. 1989). However, sex-specific recombination rates varied in different LGs of the bighead carp map (Table 2), and a similar phenomenon was also detected in other fish species, for example, silver carp (Zhang et al. 2010), Asian seabass (Wang et al. 2011) and rainbow trout (Sakamoto et al. 2000). In addition, regional differences in recombination rates within a specific chromosome have also been observed (Sakamoto et al. 2000; Wang et al. 2011), and the causation came partly from increased homogametic-sex recombination in regions proximal to centromeres and increased heterogametic-sex recombination in regions proximal to telomeres (Portnoy et al. 2010).

Marker orders between female and male maps

In most cases of this study, the order of common markers were similar between the male and female maps of bighead carp, but in some cases, there were also differences in marker order between the two maps. Changes of marker order between sexes are common phenomena and have also been detected in other fish species, for example, silver carp.
(Zhang et al. 2010; Guo et al. 2013) and grass carp (Xia et al. 2010). Usually, the differences in marker order have been considered a result of chromosomal rearrangements (Sakamoto et al. 2000). The rates of chromosomal rearrangements during evolution may differ between sexes and populations, and these distant or local recombination events may be reflected by different marker order (Portnoy et al. 2010). Slight differences in marker order may suggest that average rates of chromosomal rearrangements are quite similar between sexes in bighead carp.

Segregation distortion

Markers with segregation distortion were also used for map construction in this study, as reported in other aquatic species (Guo et al. 2012; Liu et al. 2012). The average percentage of skewed loci in bighead carp (16.0%) was similar to that found in channel catfish (~16%) (Liu et al. 2003) but lower than that found in Pacific oysters (27%) (Li & Guo 2004), Zhikong scallop (17.8%) (Li et al. 2005) and blue mussel (29.0%) (Lallias et al. 2007b). On the other hand, relatively lower percentages of segregation distortion were previously reported in tilapia (8%) (Kocher et al. 1998), swimming crab (10%) (Liu et al. 2012) and rainbow trout (13.3%) (Young et al. 1998).

Several reasons have been proposed to explain the observed marker segregation. These include the amplification of a single-sized fragments derived from several genomic regions (Faris et al. 1998), limited individuals in mapping families (Liu et al. 2003) and the presence or absence of specific genes or haplotypes that are not compatible with the survival and/or fertilization of eggs (Liu et al. 1994; Cheng et al. 2010). Some authors pointed out that distorted markers tended to cluster in specific LGs and small segments of LGs (Li et al. 2005; Lallias et al. 2007a), and we confirmed this phenomenon in bighead carp. A deleterious gene was found to be located in the area with a cluster of distorted markers in Eastern oyster (Yu & Guo 2003), and at least four major deleterious recessive genes of the Pacific oyster were proved to be linked to distorted marker clusters (Li & Guo 2004). Moreover, large regions of LGs containing blocks of distorted markers were estimated to be linked to sublethal genes in rainbow trout (Young et al. 1998). Similar to previous studies, distorted markers also gathered in some LGs and certain areas of LGs in our study, which would be useful for localizing potential deleterious or lethal genes and further eliminating these detrimental genes in MAS breeding of bighead carp.

Conclusion

Based on a large set of genomic and EST-derived microsatellite markers, a second-generation genetic linkage map was constructed for bighead carp in this study, with a genome coverage of 92.8%, a resolution of 2.9 cM and 24 LGs, which were equal to the chromosome numbers of the haploid genome of the species. This well-defined genetic linkage map will facilitate further studies in such areas as QTL mapping, comparative genomics between food fish (e.g., bighead carp) and model fish (e.g., zebrafish), MAS for genetic breeding programs and genome assembly in bighead carp.

Acknowledgements

The authors would like to thank Prof. S. He and Mr. F. Zeng for laboratory technical assistance, and S. Dan, D. Tan, X. Tan, D. Wang and L. Cheng for sample preparation. This work was supported by NSFC (30771643, 31272647), MOST973 (2010CB126305), MOA Specials (200903045, 2011-G12) and FEBL (2011FBZ20) of China.
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**Supporting information**

Additional supporting information may be found in the online version of this article.

**Figure S1** Homologous LGs between present genetic map and previous map (Zhang et al. 2011) of bighead carp.

**Table S1** Information of markers located in the linkage map of bighead carp.