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ORIGINAL PAPER

Comparative mapping for bighead carp (*Aristichthys nobilis*) against model and non-model fishes provides insights into the genomic evolution of cyprinids

Chuankun Zhu · Jingou Tong · Xiaomu Yu · Wenjie Guo

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Abstract Comparative mapping provides an efficient method to connect genomes of non-model and model fishes. In this study, we used flanking sequences of the 659 microsatellites on a genetic map of bighead carp (*Aristichthys nobilis*) to comprehensively study syntenic relationships between bighead carp and nine model and non-model fishes. Of the five model and two food fishes with whole genome data, *Cyprinus carpio* showed the highest rate of positive BLAST hits (95.3 %) with bighead carp map, followed by *Danio rerio* (70.9 %), *Oreochromis niloticus* (21.7 %), *Tetraodon nigroviridis* (6.4 %), *Gasterosteus aculeatus* (5.2 %), *Oryzias latipes* (4.7 %) and *Fugu rubripes* (3.5 %). Chromosomal syntenic analyses showed that inversion was the basic chromosomal rearrangement during genomic evolution of cyprinids, and the extent of inversions and translocations was found to be positively correlated with evolutionary relationships among fishes studied. Among the five investigated cyprinids, linkage groups (LGs) of bighead carp, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* exhibited a one-to-one relationship. Besides, LG 9 of bighead carp and homologous LGs of silver carp and grass carp all corresponded to the chromosomes 10 and 22 of zebrafish, suggesting that chromosomal fission may have occurred in the ancestor of zebrafish. On the other hand, LGs of bighead carp and common carp showed an approximate one-to-two relationship with extensive translocations, confirming the occurrence of a 4th whole genome duplication in common carp. This study provides insights into the understanding of genome evolution among cyprinids and would aid in transferring positional and functional information of genes from model fish like zebrafish to non-model fish like bighead carp.

Keywords Comparative genome mapping · Bighead carp (*Aristichthys nobilis*) · Cyprinids · Syntenic region · Genomic evolution

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Introduction

With the improvement of sequencing technology and reduction of experimental costs, whole genome sequences and high-resolution genetic linkage maps are available in a fast-growing number of organisms. In teleosts, full genome sequences are presently available for five model fishes, including zebrafish (*Danio rerio*), fugu (*Fugu rubripes*), tetraodon (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*), and three-spined stickleback (*Gasterosteus aculeatus*), as well as some non-model fishes: cave fish (*Astyanax mexicanus*), cod (*Gadus morhua*), tilapia (*Oreochromis*

niloticus), etc. (http://www.ensembl.org/index.html). Well-defined or high-density genetic linkage maps have been constructed for several food fishes, e.g., rainbow trout *Oncorhynchus mykiss* (Guyomard et al. 2012; Rexroad et al. 2008), Atlantic salmon *Salmo salar* (Lien et al. 2011; Brenna-Hansen et al. 2012), gilthead seabream *Sparus aurata* (Sarropoulou et al. 2007), half-smooth tongue sole *Cynoglossus semilaevis* (Song et al. 2012a) and Japanese flounder *Paralichthys olivaceus* (Song et al. 2012b). Physical maps have also been reported in a few commercial fishes, such as channel catfish *Ictalurus punctatus* (Quiniou et al. 2007), Nile tilapia *Oreochromis niloticus* (Katagiri et al. 2005) and European seabass *Dicentrarchus labrax* (Kuhl et al. 2010). In addition, a draft genome assembly for common carp *Cyprinus carpio* has been recently completed (http://carpbase.org/). The increase of genetic and sequence/physical maps has greatly promoted comparative genomics in fishes. Comparative mapping is an efficient way to locate candidate genes on maps of non-model fishes (Guyomard et al. 2012) and perform functional studies of homologous genes (Woods et al. 2000). Orthologous genes in development, growth and disease resistance could be deduced for food fishes, because gene functions are often evolutionarily conserved among vertebrates (Gates et al. 1999; Woods et al. 2000).

Comparative mapping can also provide valuable insights into gene duplications and chromosome rearrangements during evolution in vertebrates. It is expected that whole genome duplication (WGD) plays an important role in the evolution of lineages in which WGD has occurred (Brunet et al. 2006). In teleost, three rounds of WGD were believed to have happened, which was initially deduced through comparative mapping between zebrafish and human (Postlethwait et al. 1998, 2000). Chromosome fission and fusion, interchromosomal translocation and intrachromosomal inversion are all essentials for chromosome reorganization (Nakatani et al. 2007). With the assistance of comparative mapping, these chromosomal rearrangements could be easily observed. Inversion and translocation are quite common among different species, and they have been observed in all previously investigated fishes, including zebrafish (Postlethwait et al. 2000), medaka (Kasahara et al. 2007), rainbow trout (Guyomard et al. 2012) and common carp (Zhang et al. 2013).

Bighead carp (*Aristichthys nobilis*) belongs to the family Cyprinidae and is an important aquaculture species; however, this species had very limited genomic resources for quite a long time. A second-generation genetic linkage map with 659 microsatellites has recently been constructed for bighead carp (Zhu et al. 2014), and it provides an essential tool to perform comparative genome mapping for bighead carp against model and non-model fishes, particularly those in the Cyprinidae of the Cypriniform. Cyprinidae is the largest family of the freshwater fishes in the world, and molecular systematics and phylogenetic relationships among members of this family have been studied through mitochondrial DNA and gene sequences (Saitoh et al. 2006; Wang et al. 2007, 2012b). However, similarity and evolutionary relationships among cyprinid fishes have not been studied based on genetic information throughout the entire genomes. In this study, we selected four species with whole genome sequences or microsatellite-based genetic linkage maps from different cyprinid subfamilies, i.e., silver carp *Hypophthalmichthys molitrix* (Hypophthalmichthyinae), grass carp *Ctenopharyngodon idella* (Leuciscinae), common carp *Cyprinus carpio* (Cyprininae) and zebrafish (Danioninae), to compare their genomes against bighead carp (Hypophthalmichthyinae). We also intended to localize candidate genes on the bighead carp genetic linkage map and identify syntenic regions across genomes of bighead carp and other model and non-model fishes to investigate their genomic similarity and chromosomal rearrangements during evolution. These results would give insights to evolutionary genomics studies and be valuable for positional cloning of genes of interest, gene function analyses, and genome assembly for bighead carp and other cyprinid fishes.

Methods

A second-generation linkage map of bighead carp has been constructed recently (Zhu et al. 2014), using 659 polymorphic microsatellites which were mainly developed from bighead carp and silver carp genomes. The flanking sequences (with an average length of about 2,000 bp) of the 659 microsatellites were used for sequence homology searches against the genomes of five model fishes (zebrafish, medaka, fugu, tetraodon and three-spined stickleback) and four food fishes (Nile tilapia, common carp, grass carp and silver carp) using BLASTn under default settings in NCBI. Hits with E value $<10^{-15}$ and matched sequence length >100 bp were considered to be significant. If a sequence hit two or more homologous loci with an E value difference less than 1,000 folds, it was considered to be a duplicated locus within a genome.

Locations of homologous loci in genomes were identified in Ensembl Genome Browser (http://ensembl.org/index.html) for the five model fishes, CarpBase (http://www.fishbrowser.org/commoncarp/index.php?do=blast) for common carp, genetic linkage maps for grass carp (Xia et al. 2010) and silver carp (Guo et al. 2013) through their accession IDs. Putative conserved synteny regions were identified when at least two loci were located in the same LG and/or chromosome. The comparative maps between bighead carp and zebrafish as well as between bighead

carp and silver carp were drawn using MapDisto software (ver. 1.7.5) (http://mapdisto.free.fr/MapDisto/). Distances between loci were represented with megabase pairs (Mb) in zebrafish, while those in bighead carp and silver carp were represented by centimorgan (cM).

The sequences of 659 markers on the bighead carp genetic map were also subjected to search against zebrafish genome through BLASTx under default settings in NCBI to deduce potential genes linking to these microsatellite markers under the same searching criteria as BLASTn. Positions of the putative orthologues of these putative genes were then identified in genomes of medaka, tetraodon, three-spined stickleback and human to find conserved syntenic regions and perform genomic comparisons between bighead carp and these model species. Some syntenic regions were selected to analyze translocation, inversion and gene duplication among species in detail.

Genome comparison for cyprinid fishes were performed among bighead carp, silver carp, grass carp, common carp and zebrafish through homologous sequences, and LGs and/or chromosomes (chrs) for these species were abbreviated as Ar_LG, Hy_LG, Ci_LG, Cc_LG and Dr_chr, respectively.

To compare the results of genome evolution obtained in this study and the historical evolutional relationship among the 11 species compared in this study, we presented an overview diagram of these species based on COII sequences (Fig. 1).

Results

Sequence homology

Compared with the seven fishes with whole genome information (zebrafish, medaka, fugu, tetraodon, three-spined stickleback, Nile tilapia and common carp), the flanking sequences of 659 microsatellites in the bighead carp genome had most homologies in common carp with a proportion of 95.3 % (628/659, $E < 10^{-15}$), followed by zebrafish of 70.9 % (467/659, $E < 10^{-15}$) and Nile tilapia of 21.7 % (143/659, $E < 10^{-15}$) (Fig. 1). Nevertheless, the similarities between bighead carp and the rest four model fishes, which were percomorph fish and distant related with bighead carp phylogenetically, sharply reduced to very low levels with 6.4 % (42/659, $E < 10^{-15}$) for tetraodon, 5.2 % (34/659, $E < 10^{-15}$) for three-spined stickleback, 4.7 % (31/659, $E < 10^{-15}$) for medaka and 3.5 % (23/659, $E < 10^{-15}$) for fugu (Fig. 1). Although whole genome information of silver carp and grass carp were limited presently (3200 and 24000 nucleotides in GenBank, respectively), orthologs with bighead carp sequences could still be detected from available sequences of these two species with 167 in silver carp and 20 in grass carp (Fig. 1).

Of the total 486 loci (data not shown) with significant hits against at least one of the five model fish genomes, 4 (0.8 %) matched all five species, 6 (1.2 %) matched four species, 20 (4.1 %) matched three species, and 50 (10.3 %)

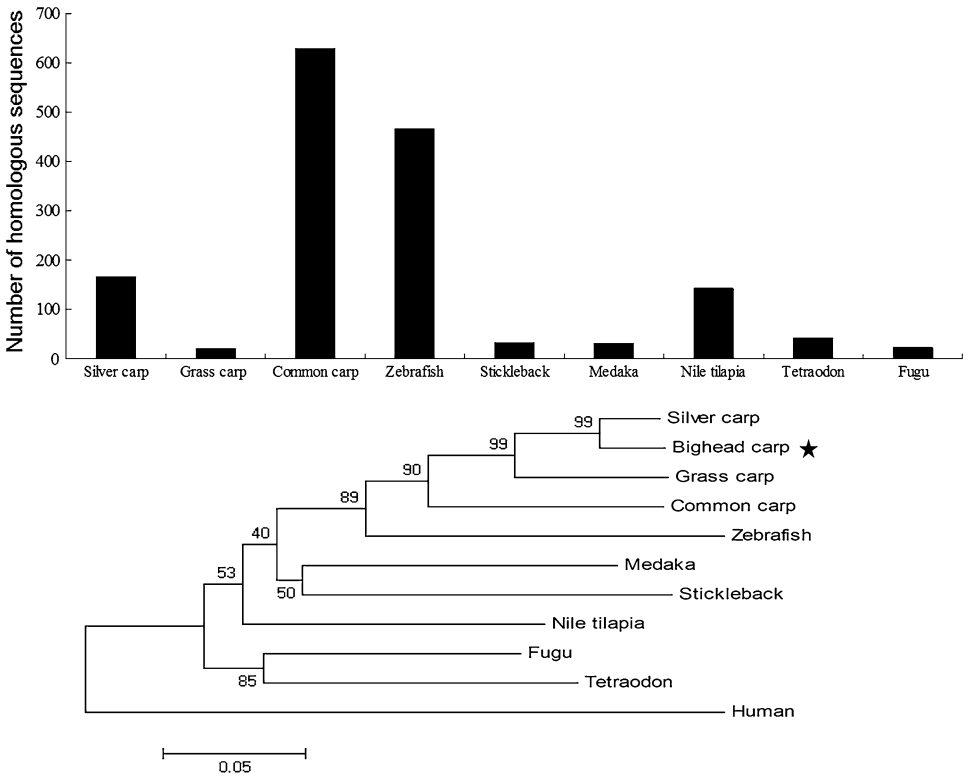

Fig. 1 Homologs of 659 microsatellites on bighead carp genetic linkage map (Zhu et al. 2014) in genomes of zebrafish, tetraodon, medaka, three-spined stickleback, fugu, common carp, Nile tilapia, silver carp and grass carp and their phylogenetic relationship based on COII sequences. The neighbor-joining tree of the species analyzed in this study was constructed through the software Mega 4.0 with a bootstrap of ×10,000, and COII sequences of silver carp (FJ827138.1), bighead carp (FJ827140.1), grass carp (NC_018134.1), common carp (FJ655382.1), zebrafish (NC_002333.2), medaka (NC_012975.1), three-spined stickleback (AP002944.1), Nile tilapia (NC_013663.1), fugu (AJ421455.1), tetraodon (AP006046.1) and human (NC_012920.1) were downloaded from NCBI

matched two species; in other words, 80 of the 486 loci (16.5 %) matched at least two model fishes. Homologous analyses between bighead carp and four cyprinid fishes (zebrafish, common carp, silver carp and grass carp) revealed that, of the 628 loci with significant hits, 148 (23.6 %) were common between bighead carp and at least three other cyprinid genomes (data not shown).

Syntenic regions

Through BLASTx against the zebrafish genome, homologous genes were detected for 152 of the 659 loci on bighead carp genetic map (Online Resource 1). Majority of these genes have orthologs in genomes of medaka, tetraodon, three-spined stickleback and human, forming different syntenic regions (Online Resource 1). Syntenic blocks with more than two anchor loci were found to be the most in zebrafish and the fewest in human (Table 1). In spite of this, some highly conserved blocks were identified, for instance, a syntenic block defined by markers *Arsd703* and *HysdE115-2* on Ar_LG 2 was conserved among all six species (Online Resource 1). Information for more conserved syntenic blocks was shown in Online Resource 1.

Syntenic blocks between bighead carp and human distributed on 15 Ar_LGs, and seven of them contained two or more blocks homologous to various human chromosomes, which is a reflection of interchromosomal translocations. Thus, the rates of interchromosomal translocation between bighead carp and human could be roughly estimated as 46.7 % (7/15) (Fig. 2). In the same way, the rates between bighead carp and zebrafish, medaka, tetraodon, three-spined stickleback were 8.3 % (2/24), 33.3 % (6/18), 40.0 % (6/15) and 50.0 % (9/18), respectively (Fig. 2).

Gene duplication

Some of the 152 potential gene loci were found to have two or three locations in genomes of zebrafish, medaka, tetraodon and three-spined stickleback, but not in human genome (Online Resource 1). In tetraodon, 40 of the 161 detected homologous sites were duplicated, with a duplication rate of 24.8 %; in three-spined stickleback and medaka, the duplication rates were 19.7 % (28/142) and 16.9 % (22/130), respectively; while in zebrafish, the rate was only 6.1 % (10 duplications from 164 sites) (Table 1). In bighead carp, 12 loci were treated as gene duplications with a rate of 7.9 % (12/152) (Online Resource 1). In addition, gene duplication patterns were different among teleosts. For instance, locus F on Ar_LG 1 was duplicated in all five teleost species investigated, loci A and E were duplicated in medaka, tetraodon and three-spined stickleback, however, loci B, C and H did not duplicate in any of those teleost species (Fig. 3).

Comparative genome mapping among selected cyprinids

Of the 659 microsatellites on bighead carp map, 467 were present on 25 zebrafish chromosomes. Through these loci, all 24 Ar_LGs were clearly associated with Dr_chrs, with the numbers of shared markers varying from 2 to 38 (18.7 on average) per LG/chr pair on the comparative map (Figs. 4, 5). On the orthologous map between bighead carp and zebrafish (Fig. 5), each Ar_LG apparently corresponded to a particular Dr_chr in most cases. However, four cases of exceptions (Ar_LG 9 vs Dr_chr 10 and Dr_chr 22, Ar_LG 1 vs Dr_chr 5 and Dr_chr 24, Ar_LG15 vs Dr_chr 8 and Dr_chr 24, Ar_LG 24 vs Dr_chr 14 and Dr_chr 15) were also observed (Fig. 4). In addition, 46 of the 467 homologous loci were scatterly located on different Dr_chrs without formation of syntenic blocks (Fig. 4).

Compared with the second-generation linkage map of silver carp (Guo et al. 2013), 167 of 659 microsatellites on the bighead carp map were shared by the two maps. The LGs on bighead carp and silver carp maps exhibited a strict one-to-one relationship using 167 common markers as anchor loci, and common markers on each homologous LG pair ranged from 2 to 17 with an average of 7.0 (Figs. 4, 6). No Ar_LGs correspond to two or more Hy_LGs; however, the orders of loci may slightly differ between bighead carp and silver carp in some cases (Fig. 6).

The bighead carp linkage map has 628 common markers with common carp genome, and 108 (17.2 %) of them have duplicated positions. In addition, markers on each Ar_LG distributed widely in common carp genome (Fig. 4). A total of 122 syntenic blocks were identified by these homologous loci with the number of common markers ranged from

Table 1 Syntenic comparison between bighead carp and five vertebrates based on deduced gene sites

	Zebrafish	Tetraodon	Medaka	Stickleback	Human
Number of homologous gene sites	164	161	130	142	141
Number of duplicated sites	10	40	22	28	0
Rates of duplication (%)	6.1	24.8	16.9	19.7	0.0
Number of syntenic regions with at least 2 anchors	25	24	27	30	25
Number of syntenic regions with more than 2 anchors	21	14	13	15	9

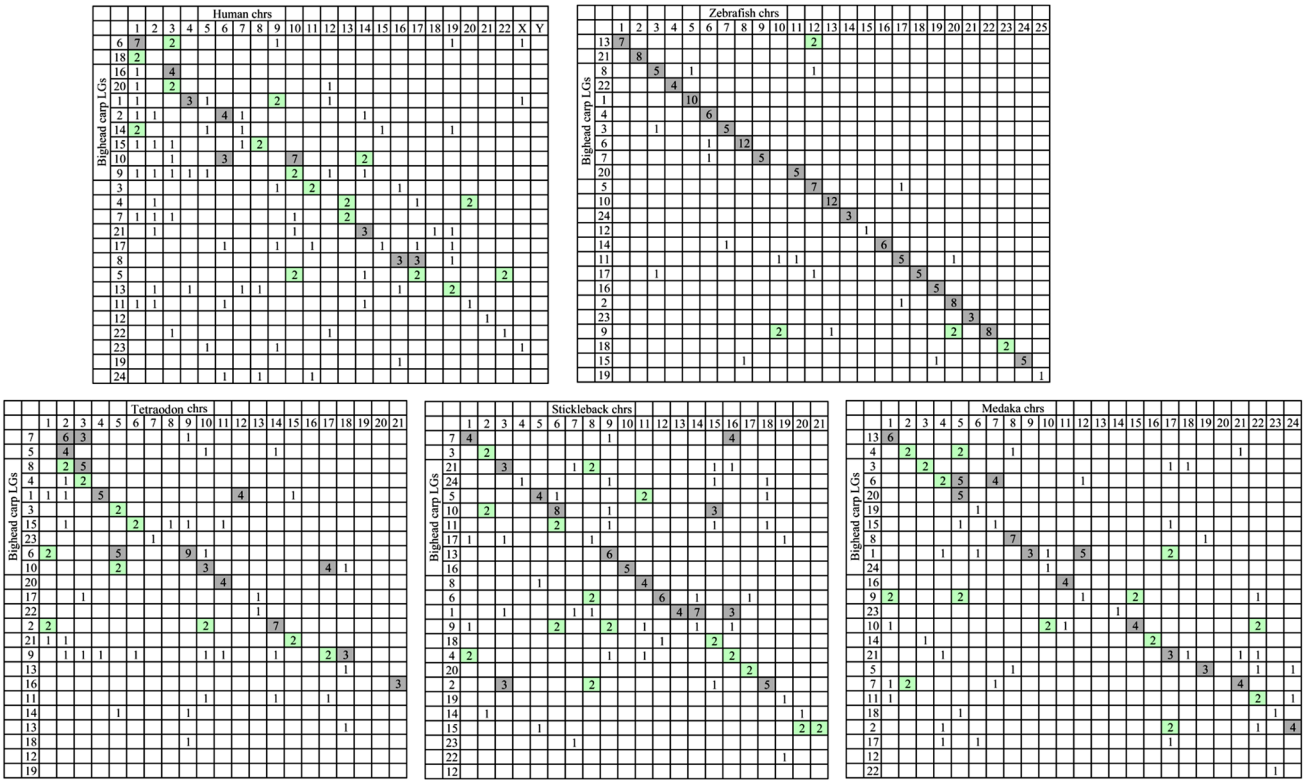

Fig. 2 Syntenic relationships among bighead carp, human and model species based on candidate gene sites. Digital in each *square* is the number of matched genes between two species; *gray shades* refer to matches of more than two genes; *light gray shades* refer to syntenic blocks with two anchor genes. *LG* and *chr* are abbreviations of linkage group and chromosome, respectively

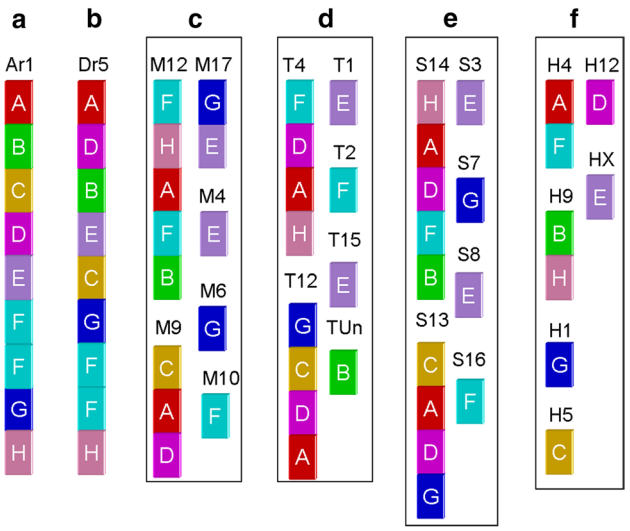

Fig. 3 Locations of nine gene loci from bighead carp (Ar, **a**) linkage group 1 on chromosomes of zebrafish (Dr, **b**), medaka (M, **c**), tetraodon (T, **d**), stickleback (S, **e**) and human (H, **f**). The genes A to H are *pdlim5a*, *scai*, *fst*, *ssh1b*, *efnb1*, *lysosome membrane protein 2-like*, *lactbl* and *pappa1*, respectively

2 to 13 (6.0 markers/block on average), and 52 (42.6 %) of these blocks contained more than two anchor loci. Although an Ar_ LG may correspond to several Cc_LGs, in most cases one particular Ar_ LG mainly matched two Cc_LGs (Fig. 4), exhibiting a 1:2 relationship.

The common markers between maps of bighead carp and grass carp were too limited to perform comparative mapping. However, genetic linkage maps of these two fishes could be linked using zebrafish genome as a stepping stone because a comparative map between grass carp and zebrafish has been reported (Xia et al. 2010). Therefore, we obtained genomic relationship for bighead carp and grass carp through this indirect comparison. Each Ar_LG was clearly associated with one of the Ci_LGs (Fig. 4).

At the LG/chr level, the overall evolutionary relationships among five cyprinids (bighead carp, silver carp, zebrafish, common carp and grass carp) could be clearly demonstrated based on the results of comparative mapping. Compared with three species with 24/25 chromosomes in their haploid genomes, bighead carp ($n = 24$) has the highest genetic similarity with silver carp, followed by grass

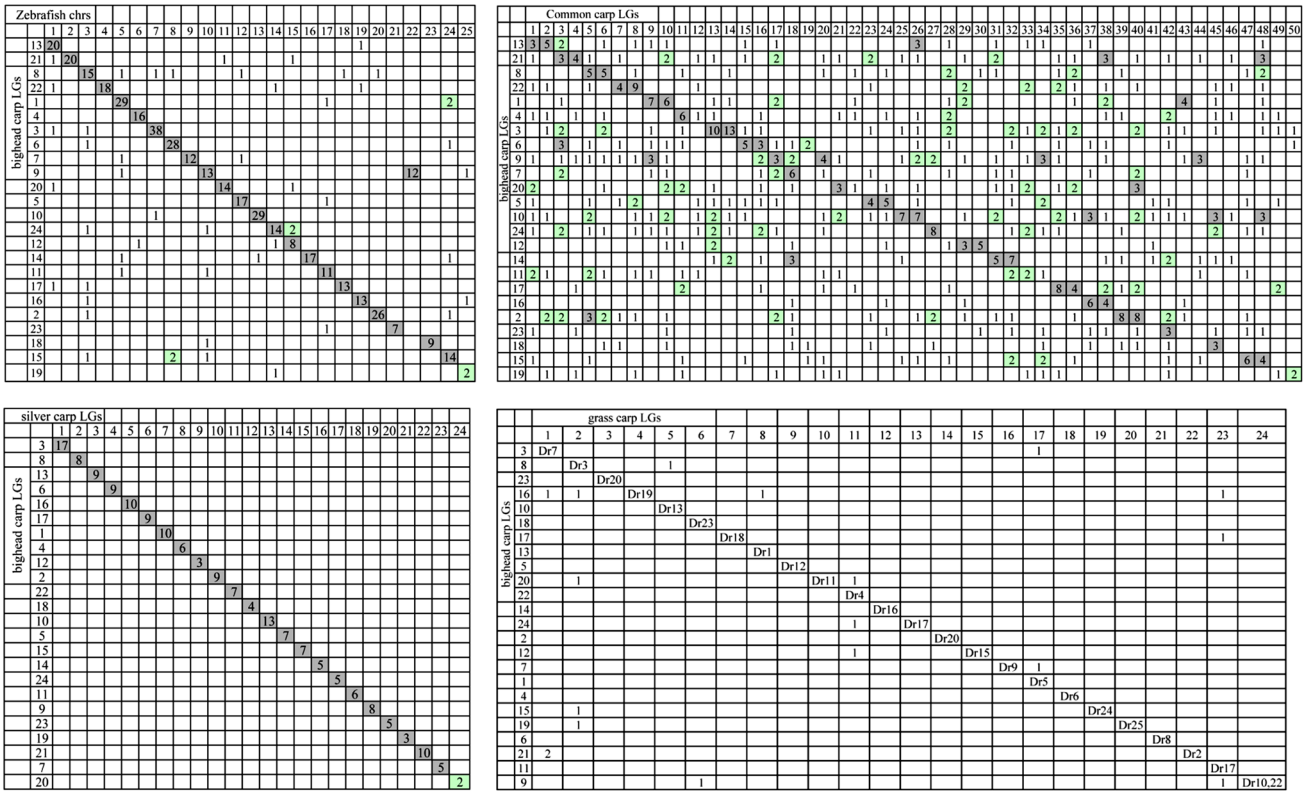

Fig. 4 Syntenic relationships between bighead carp and four cyprinid fishes based on 659 microsatellite sequences on bighead carp genetic linkage map. Digital in each *square* is the number of matched loci between two species; *gray shades* refer to matches of more than two loci; *light gray shades* refer to syntenic blocks with two anchor loci. *LG* and *chr* are abbreviations of linkage group and chromosome, respectively. The oxford grid between bighead carp and grass carp are constructed according to their correspondence of LGs with zebrafish chromosomes. *DrN* stands for the Nth chromosome of zebrafish

carp and zebrafish (Table 2). Bighead carp LGs showed complicated patterns of correspondence to common carp genome ($n = 50$) (Table 2), indicating large differences in chromosomal structure between these two cyprinid species.

Translocation and inversion

Both translocations and inversions were detected when bighead carp genome was compared with those of model and non-model fishes and human, although interchromosomal translocations are rare between some cyprinid fishes. For example, nine potential gene loci on Ar_LG 1 were all positioned on Dr_chr 5 but the gene orders were different (Fig. 3). However, in genomes of medaka, tetraodon, threespined stickleback and human, these loci all distributed on six different chromosomes (Fig. 3), indicating that interchromosomal translocations were quite common during evolution of genomes and speciation in vertebrates.

In conserved syntenic regions between bighead carp and other vertebrates, gene orders were not conserved. We analyzed three syntenic blocks among bighead carp, zebrafish and human, making human as the outgroup and assuming gene orders in human to be ancestral type. Under this assumption, we found that there were fewer chromosomal inversions between human and zebrafish than that between human and bighead carp (Fig. 7).

Discussion

Studies on comparative mapping have revealed that the rates of nucleotide sequence similarity were positively correlated with phylogenetic relationships among fish species (Xia et al. 2010; Zheng et al. 2011; Guyomard et al. 2012). In this study, we found that bighead carp (Hypophthalmichthyinae) genome has a higher similarity with common carp (Cyprininae) (95.3 %) than that with zebrafish (Danioninae) (70.9 %). It has been reported that cyprinids may have originated about 46–49 million years ago (MYA) in the mid-Eocene, followed by the emergence of Danioninae and Cyprininae at about 30–31 MYA (early Oligocene) and 26 MYA (mid-Oligocene), respectively, while the ancestors of bighead carp and common carp separated about 14–15 MYA (Wang et al. 2007). Therefore,

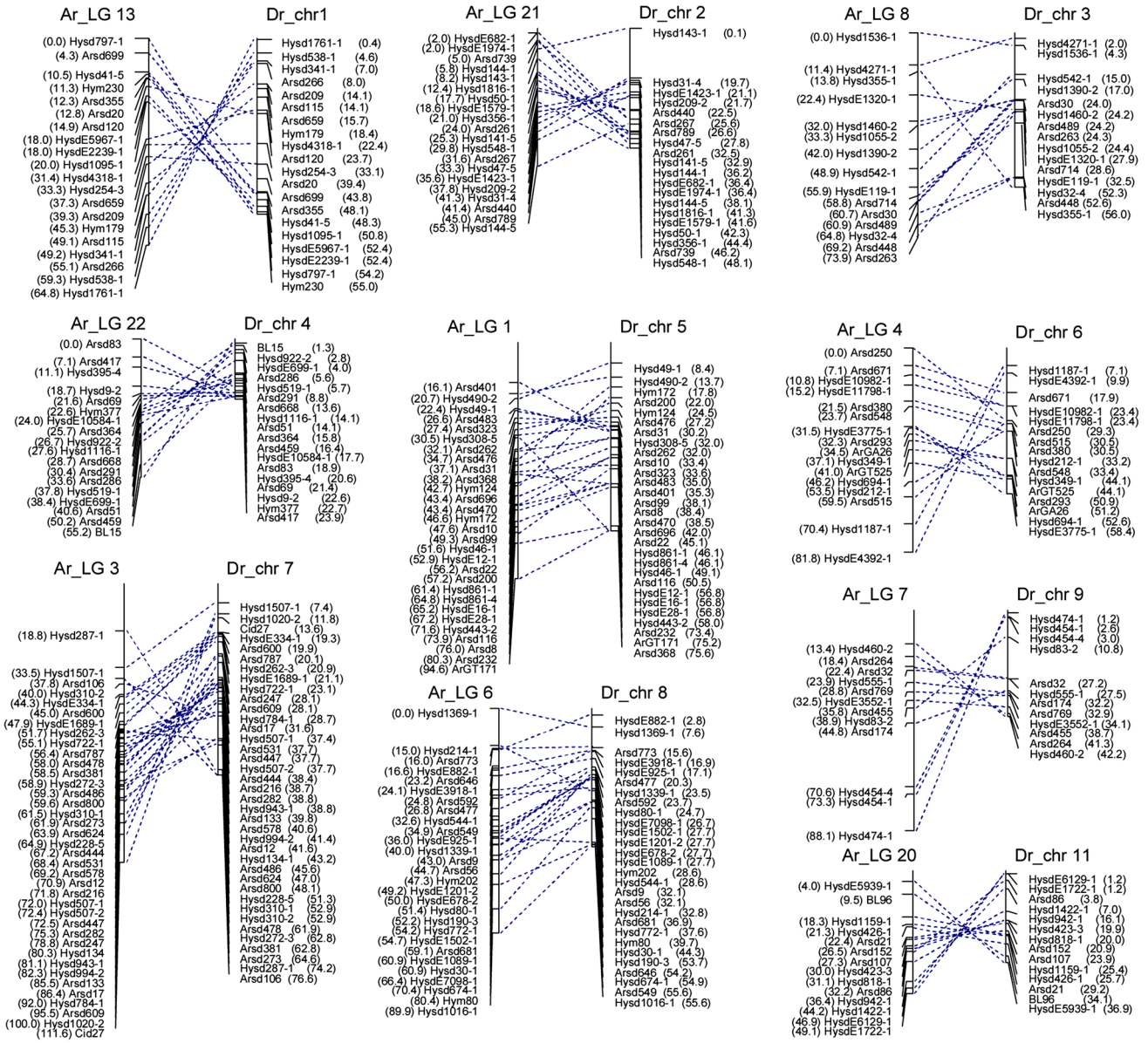

Fig. 5 Syntenic relationship between bighead carp linkage groups (Ar_LG) and zebrafish chromosomes (Dr_chr). Each *vertical line* represents individual bighead carp linkage group (*left*) or zebrafish chromosome (*right*). Marker names and genetic distances (cM) of each LG are shown on *left side*, and the marker names and their physical distances (Mb) for zebrafish chromosomes are given on *right side*. Markers distributed randomly in zebrafish genomes are not included

our findings that the relationship between bighead carp and common carp is closer than that between bighead carp and zebrafish coincided with the results of previous evolutionary studies for Cyprinids (Wang et al. 2007). The common ancestor of medaka, fugu, tetraodon and three-spined stickleback segregated with cyprinid about 290 MYA (Steinke et al. 2006), resulting in low genome similarities between bighead carp and these four model fish species. Based on the similarity results of this present study, we could deduce that genomes of cyprinid fishes are quite conserved.

Comparative mapping is an efficient way to locate genes on linkage maps of non-model species (Guyomard et al. 2012). Like most food fishes, genome information for bighead carp is also quite limited, and gene orders are unclear so far in this species. In this study, 23.1 % (152/659) of the markers on bighead carp genetic linkage map were deduced to be linked with candidate genes, which was similar to the ratio (22.9 %) (510/2226) in rainbow trout (Guyomard et al. 2012). Most of these genes could be found in genomes of model fishes and even human; therefore, our study confirmed that genes are highly conserved among

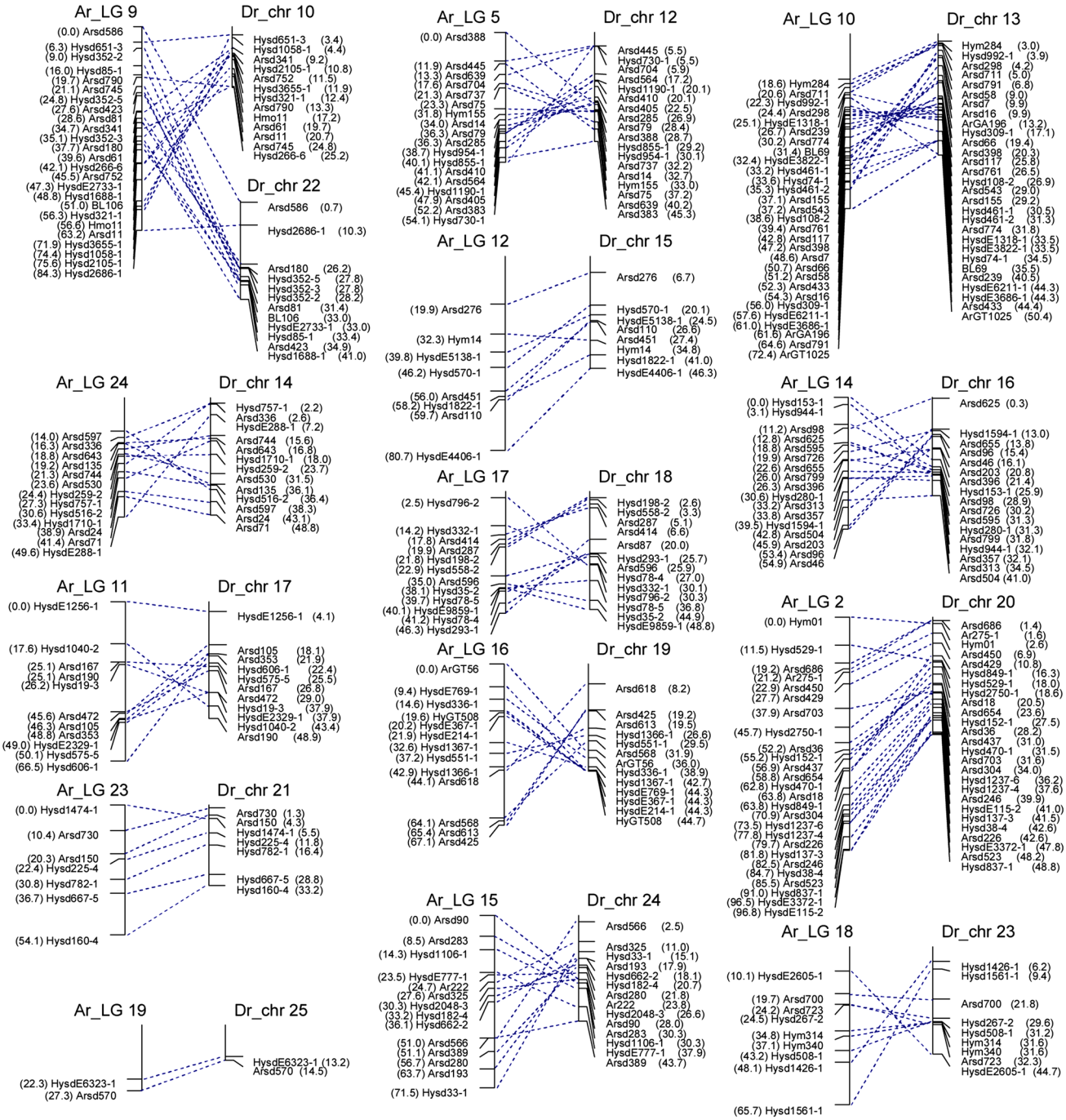

Fig. 5 continued

different lineages of vertebrates. These potential gene sites would be useful in further studies once they were found to be linked with phenotypes of interest. For instance, a candidate gene, named HIV-EP2/MBP-2, was found to be associated with infectious salmon anemia virus resistance QTL in Atlantic salmon via comparison with tetraodon and medaka genomes (Li et al. 2011). Comparative mapping between closely related species is proposed to be a valid strategy for identifying QTLs, and has been successfully used to identify four QTLs affecting spawning time in coho salmon *Oncorhynchus kisutch* through comparative mapping against rainbow trout (Araneda et al. 2012). QTL mapping has not been reported in bighead carp yet, but a series of QTLs have been identified in common carp (Zhang et al. 2011; Liu et al. 2009; Zhang et al. 2007) and zebrafish (Wright et al. 2006; Waits and Nebert 2011). If

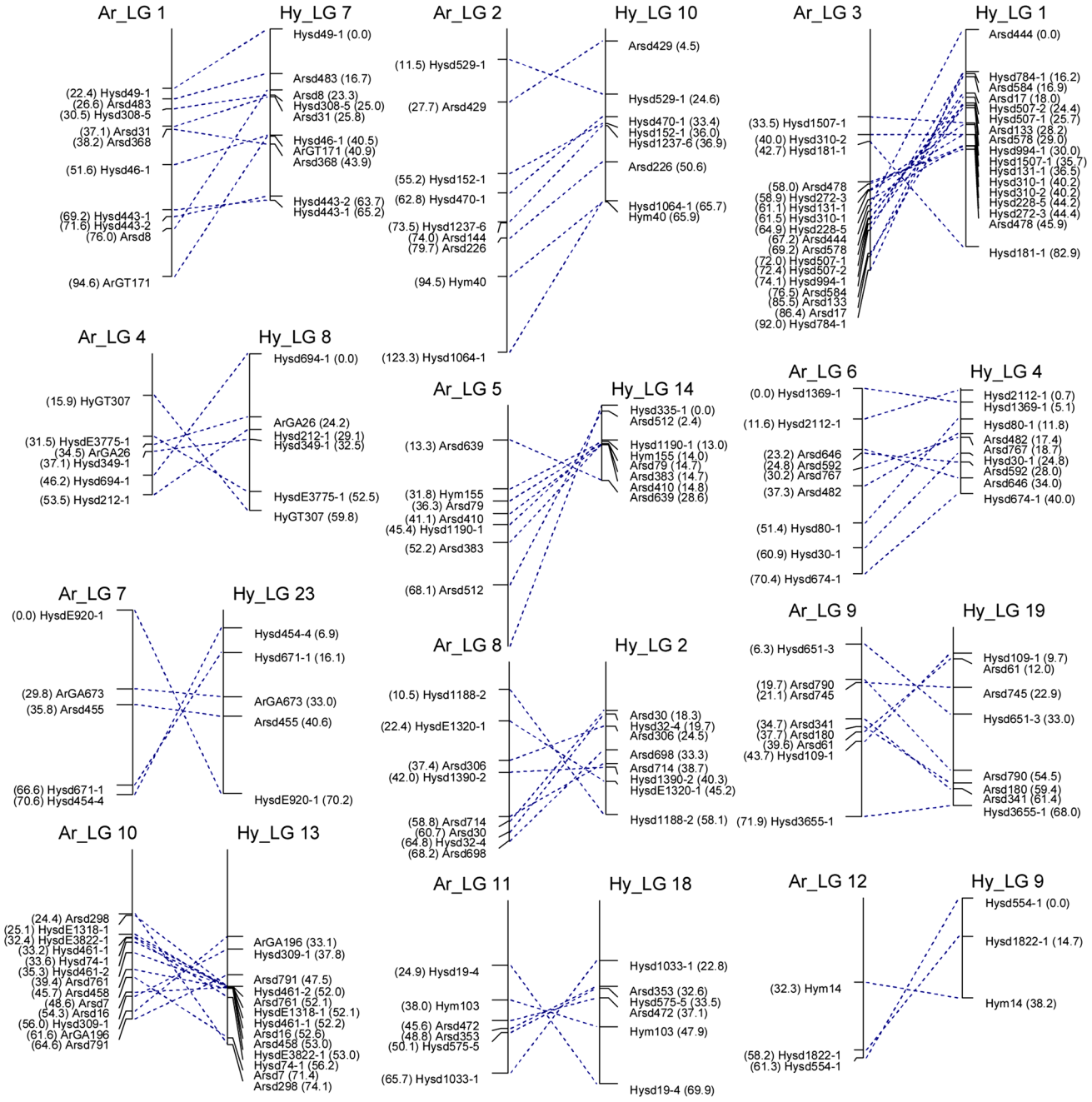

Fig. 6 Syntenic relationship between linkage groups of bighead carp (Ar_LG) and silver carp (Hy_LG). Each *vertical line* represents individual bighead carp linkage group (*left*) or silver carp linkage group (*right*). Marker names are shown with their genetic distances (cM) of each linkage group

syntenic regions contain one or more QTLs in common carp or zebrafish, similar QTL(s) may also exist in bighead carp genome.

Many scientists have been asserting that teleost has undergone one more WGD event than mammals (Postlethwait et al. 1998; Gates et al. 1999; Woods et al. 2000). Our present study provides further evidence to support this 3rd WGD because gene duplications have been detected in all fishes investigated but not in human (Table 1 and Online Resource 1). For the duplication rate, bighead carp was similar to zebrafish, but much lower than medaka, tetraodon and three-spined stickleback, suggesting that different teleosts may have retained different sets of duplicated genes, which was also proved by other studies (Woods et al. 2005; Naruse et al. 2004). It was believed that different sets of gene duplications in teleosts may be mainly caused by gene

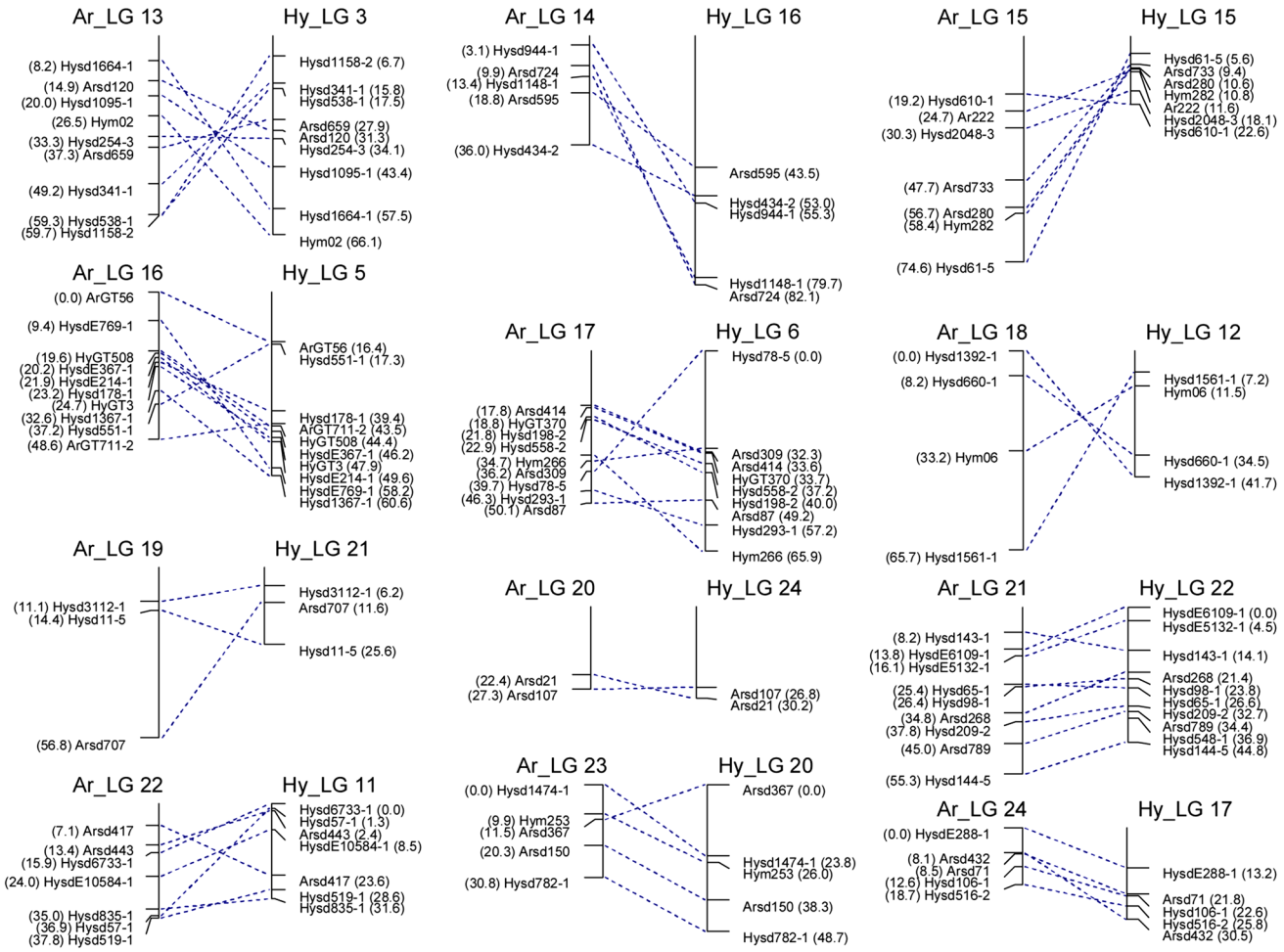

Fig. 6 continued

loss, in other words, after WGD in the ancestor of teleost, different natural selection pressures caused various patterns of gene loss, resulting in lineage divergence and speciation (Brunet et al. 2006; Lynch and Conery 2000).

Compared with ancestral genome of teleost, extensive translocations have been experienced during the evolution of zebrafish genome (Kasahara et al. 2007). Similar phenomenon may have occurred in bighead carp, because some translocations were detected between bighead carp and zebrafish, although majority of Ar_LGs exhibited a one-to-one relationship with Dr_chrs. In fact, translocation is a common chromosomal rearrangement in teleost, which has also been detected between genomes of zebrafish and grass carp (Xia et al. 2010), common carp (Zhang et al. 2013; Zheng et al. 2011) and silver carp (Guo et al. unpublished). However, Ar_LGs and Hy_LGs presented a strict one-to-one relationship, and no translocations were detected, indicating that genomes of these two carps have highly conserved synteny and therefore supporting their close evolutionary relationship as shown by Wang et al. (2007). Comparison of translocation rates in zebrafish, medaka, tetraodon, three-spined stickleback and human against bighead carp indicated that the closer lineage relationships, the lower translocation rates.

Using a third or even fourth species as a bridge is an efficient way to compare genomes of fishes with limited homologous information (Sarropoulou and Fernandes 2011; Sarropoulou et al. 2008). In this study, we successfully connected genomes of bighead carp and grass carp and obtained information for correspondence of LGs between the two species by applying zebrafish as a bridge. Since zebrafish is a model fish with whole genome, all sequence-based genetic linkage maps of cyprinids could be compared with its genome, therefore, using zebrafish as a stepping stone, genetic and evolutionary relationships among these cyprinid fishes could be deduced, and genomic information (such as gene order of orthologs and QTLs) may be transferred from model fishes (such as zebrafish) to commercial species such as bighead carp, silver carp and common carp.

Table 2 Correspondences of linkage groups/chromosomes among five cyprinid fishes

Bighead carp LG	Silver carp LG	Zebrafish chr	Common carp LG	Grass carp LG
1	**7**[a]	**5,** 24	**9, 10,** 17, 29, 38, **43**	**17**
2	**10**	**20**	2, 3, **5,** 6, 17, 27, **39, 40,** 42	**14**
3	**1**	**7**	3, 6, **13, 14,** 28, 32, 34, 36, 40	**1**
4	**8**	**6**	**11,** 28, 42	**18**
5	**14**	**12**	8, **23, 24,** 34	**9**
6	**4**	**8**	**3, 15, 16,** 19	**21**
7	**23**	**9**	3, **17, 18,** 40	**16**
8	**2**	**3**	**5, 6,** 28, 36, 48	**2**
9	**19**	**10, 22**	**9,** 16, **17,** 18, **20,** 26, 27, **34, 44**	**24**
10	**13**	**13**	5, 10, 13, 21, **25, 26,** 31, 35, **37,** 40, **45, 48**	**5**
11	**18**	**17**	1, 5, 32, 33	**23**
12	**9**	**15**	13, **29, 30**	**15**
13	**3**	**1**	**1, 2,** 3, 26	**8**
14	**16**	**16**	14, **18, 31, 32,** 42	**12**
15	**15**	8**, 24**	32, 34, **47, 48**	**19**
16	**5**	**19**	**37, 38**	**4**
17	**6**	**18**	11, **35, 36,** 38, 40, 49	**7**
18	**12**	**23**	**45**	**6**
19	**21**	**25**	**50**	**20**
20	24	**11**	1, 10, 11, **21,** 33, 36, **40**	**10**
21	**22**	**2**	**3, 4,** 10, 17, 31, **38, 48**	1, **22**
22	**11**	**4**	**7, 8,** 29, 33, 35	**11**
23	**20**	**21**	**42**	**3**
24	**17**	**14,** 15	3, 13, 16, **27,** 33, 45	**13**

[a] Homologous LGs/chrs containing more than two anchor loci with corresponding bighead carp LGs are showned in bold characters

In some tetraploidized fish species, such as common carp and rainbow trout, a 4th round WGD was believed to have occurred (Allendorf and Thorgaard 1984; Friedman and Hughes 2001). Comparative mapping between common carp and zebrafish revealed that Cc_LGs and Dr_chrs showed a one-to-two relationship, and this relationship was thought to be an evidence for occurrence of the extra WGD in common carp (Zheng et al. 2011). Comparison between bighead carp and common carp indicated that Ar_LGs and Cc_LGs also exhibited a one-to-two relationship, although there were some exceptions, confirming the occurrence of a 4th WGD in common carp (Friedman and Hughes 2001). Extensive translocations between bighead carp and common carp were detected, which were much more than those between bighead carp and other fishes. The possible reason for such extensive translocations may be the chromosomal rearrangement or translocation during the diploidization progress of the tetraploid common carp ancestor (Larhammar and Risinger 1994).

According to previous studies, the ancestor of teleosts had 24 chromosomes, and chromosomal fission/fusion may have occurred in fishes with various chromosome numbers (Kasahara et al. 2007; Nakatani et al. 2007). Among the five cyprinids investigated, bighead carp, silver carp and grass carp all retained the ancestral chromosome number in their haploid genome ($n = 24$), while zebrafish ($n = 25$) and common carp ($n = 50$) did not. The fact that Ar_LG 9 (this study), Hy_LG 19 (Guo et al. 2013) and Ci_LG 24 (Xia et al. 2010) all corresponded to the same two chromosomes of zebrafish may have revealed that a chromosome homologous to Ar_LG 9, Hy_LG 19 and Ci_LG 24 fissured in the ancestor of zebrafish and formed the Dr_chr 10 and Dr_chr 22. However, further studies were needed to prove this hypothesis. The formation of 50 chromosomes in common carp should be the results of a 4th round WGD and a more recent segmental duplication in its genome (David et al. 2003; Wang et al. 2012a) rather than chromosomal fission/fusion.

Karyotype evolution in teleosts occurred mainly by inversions (Kai et al. 2005; Jaillon et al. 2004). The results of this study also support this viewpoint, because we found that among the most closely related cyprinid species, such as bighead carp and silver carp, inversion is the sole type of chromosomal rearrangements. Moreover, the occurrence

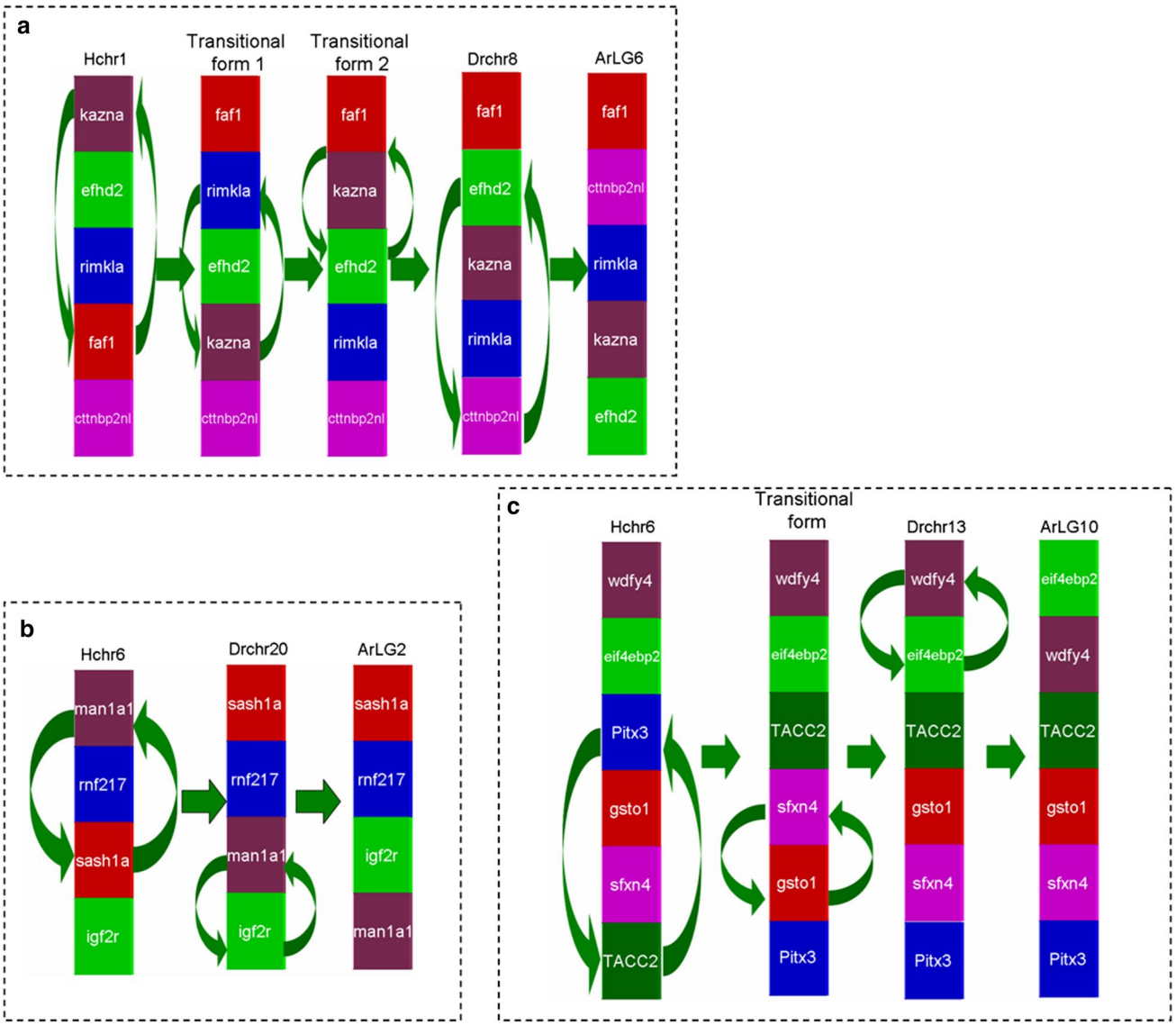

Fig. 7 Diagrams of chromosomal inversions for three syntenic regions among bighead carp, zebrafish and human. *Light gray arrows* indicate the inversion process between zebrafish and bighead carp, and *gray arrows* show that between zebrafish and human. *ArLG* stands for linkage group of bighead carp, *Zchr* and *Hchr* refers to chromosome of zebrafish and human, respectively

of inversions may differ among species with different lineage relationships, that is, the more closely related, the less inversion. Furthermore, in genomes of species with a relatively distant relationship, both inversion and translocation could occur.

Conclusions

Comparative mapping between bighead carp and nine model and non-model fishes in this study revealed syntenic and evolutionary relationships among these species. Homologous analyses showed that although genomes of five cyprinids are quite conserved, genetic similarities are positively correlated with their phylogenetic relationships. Inversion was proved to be the basic chromosomal rearrangement during genome evolution of cyprinid fishes, although other chromosomal rearrangements including translocation, chromosomal fission and WGD were all detected. Furthermore, the extent of inversions and translocations was found to be positively correlated with evolutionary relationships between fish species. Results of these comprehensive genome comparisons give insights into the elucidation of cyprinid evolution and provide basic resources for further studies on functional genomics analyses, QTL identification and genome assembly in bighead carp and other closely related cyprinid fishes.

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