A genetic linkage map of common carp (Cyprinus carpio L.) And mapping of a locus associated with cold tolerance

Xiaowen Sun*, Liqun Liang

Heilongjiang River Fisheries Research Institute, Chinese Academy Fisheries Science, Harbin 150070, People’s Republic of China

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Abstract

A genetic linkage map has been constructed for the common carp (Cyprinus carpio L.). The segregation of 272 markers, including 105 gene markers, 110 microsatellites, and 57 RAPD markers was studied in a panel with 46 haploid embryos derived from a single hybrid female. The hybrid was produced by crossing a common carp (C. carpio L.) and a Boshi carp (Cyprinus pellegrini pellegrini Tchang). A genetic linkage map was constructed using the panel and 268 markers. The map spans 4111 cm over 50 linkage groups covering the 50 chromosomes of this species. A locus associated with the cold tolerance trait of common carp was mapped on linkage group 5. This map is a starting point for further mapping quantitative traits loci (QTL) linked to important economic traits in common carp.

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1. Introduction

The common carp (Cyprinus carpio L.) is the most extensively cultured fish in the world, as well as in China. Common carp has a very long history of domestication. There are a lot of locally adapted wild and cultured common carp stocks. Many genetic improvement projects including directional selection and cross breeding have been done...
for the common carp in the world (Moav and Wohlfarth, 1976). However, most of the selection methods used in those projects were traits-based selection methods. Traits-based selection has worked best on traits with additive genetic variation, but not well enough on traits with low heritability. Unfortunately, many economically important traits have low heritability. New selection methods based on molecular markers and combined with genetic linkage map are needed to enhance the development of crossbreeding technology on traits with low heritability using marker-assisted selection (MAS).

Genetic linkage maps have been made for aquaculture fishes such as salmon and tilapia (William et al., 1998; Kocher et al., 1998). In carp, microsatellite markers have been reported (Crooijmans et al., 1997; Wei et al., 2001), but a framework genetic linkage map has not been available. Here we are reporting the first genetic linkage map for the common carp.

2. Methods and material

2.1. Haploid gynogenesis

The haploid mapping panel was generated from haploid progeny of a hybrid female, which was produced by crossing a female common carp (C. carpio haematopterus Temminck et Schlegel) with a male Boshi carp (Cyprinus pellegrini pellegrini Tchang). The mature hybrid female was softly stripped by hand and the 20,000–30,000 eggs were divided into batches of 150–300 in Petri dishes. The unfertilized eggs were washed with filtered aquarium water and placed in incubators for development with water temperature at 22 °C. About 80 embryos were collected before those unfertilized eggs died. Normally, those haploid embryos will die when they develop 14–20 h at 22 °C.

2.2. Genomic DNA extraction

Embryos were killed and the yolk sac was removed using watchmaker’s forceps with the aid of a binocular microscope. The embryos were placed into individual sterile 1.5 ml microcentrifuge tubes containing 150 μl TEN buffer (100 mM Tris–HCl, pH 8.0; 10 mM EDTA; 250 mM NaCl), 15 μl 5% SDS, and 5 μl proteinase K (10 mg/ml stock). Tubes were incubated in a water bath at 55 °C for 2 h. The samples were extracted twice using phenol and once using chloroform/isoamyl alcohol (1:24). DNA was precipitated using pure ethanol. Pellets were washed in 70% ethanol, dried, and resuspended in 50 μl sterile dH2O. Approximately 0.5 μg of purified DNA was obtained from each embryo.

2.3. Microsatellite and gene markers

Genotypes were obtained by automated sizing of electrophoresis agarose gel amplified via PCR. We used 25 μl KCl, 10 mM Tris–HCl, pH 8.0, 0.1% Triton X-100, 2.4 mM MgCl₂, 0.16 mM each dNTP, and 0.16 μM each primer, to which we added about 1.5 ng of haploid genomic DNA. The PCR conditions were as follows: initial incubation at 95 °C for 5 min followed by 95 °C for 30 s, 50–55 °C for 30 s, 72 °C for 30 s for 35–38 cycles,
depending on the efficiency of amplification. A final incubation at 72 °C for 5 min was always used after thermal cycles. Volumes of 16 μl of the PCR products were used for analysis. Loading buffer (3 μl of the 6× stocks) was added to the sample and loaded on a 1.5% agarose gel for electrophoresis. Microsatellite markers included 26 common carp microsatellites (Crooijmans et al., 1997), 19 crucian carp microsatellites (Ohara et al., 1999), and 65 zebrafish microsatellites (Knapik et al., 1998). In addition to the microsatellite markers, 57 RAPD and 105 gene markers were used. The zebrafish microsatellite primers were bought from ResGen Invitrogen (Groningen, The Netherlands) and other microsatellite primers were synthesized at ResGen Invitrogen according to the published sequences. Gene markers (herein, gene markers refer to genes of known function, or type I markers) were synthesized according to the primer sequence designed by us (all whole gene sequences were obtained from NCBI (USA), and we designed the primer sequence using the PRIMER3 software on the web site of http://www.genome.wi.mit.edu).

2.4. RAPD markers and nomenclature

RAPD PCR condition were as follows: initial incubation at 95 °C for 5 min, 95 °C for 30 s, 50–55 °C for 30 s, 72 °C for 30 s for 35–38 cycles, and a final incubation at 72 °C for 5 min. Genotypes were obtained as MS marker. Formal locus names for RAPD markers in common carp consist of the name of the 10 nucleotide long primer, followed by the approximate size of the amplification product. Thus, the locus 2H.451c, is amplified by primer H02 (Operon Technologies, USA), and results in a 451-bp amplification product from common carp.

2.5. Map construction and linkage analysis

The Macintosh porting of MAP MANAGER (Manley and Cudmore, http://mcbio.med.buffalo.edu/mapmgr.html) was used to construct the linkage map. Linkage groups and marker order were produced by using commands ‘Chrom’ and ‘Locus’ to identify linkage groups and to determine marker order. Genotype data were entered in two phases, M and P, to satisfy the requirements of the software, where M means the genotype of a haploid identical to its maternal grandmother, and P means the genotype of a haploid identical to its maternal grandfather.

2.6. Identification of the markers associated with cold tolerance

Boshi carp (Cyprinus (Cyprinus) pellegrini pellegrini Tchang) is a local stock in Yunnan province of China. It is highly sensitive to low temperatures and unable to overwinter in ponds in the Heilongjiang province. A local stock of common carp (Cyprinus (Cyprinus) carpio haematopterus Temminck et Schlegel) in Heilongjiang province of China is highly resistant to low temperatures and is overwintering well in ponds in the Helongjiang Province. Boshi carp and common carp have a similar karyotype, and same numbers of chromosomes (Shi, L., personal communication).

The F2 generation was obtained from F1 hybrids of a cross between a common carp and a Boshi carp. After hatching, the F2 generation was raised as normal common carp. Prior
to the winter, 100 F2 offspring weighing about 30–50 g were tagged and stocked into a winter pond. DNA was extracted from the pectoral fin tissue of those F2 individuals. During the spring, the F2 individuals were identified by their tags. The DNA samples were divided into two groups, one group containing the survivors and the other containing the missing (dead) fish. The survivors were assumed to have cold tolerance characteristics.

The DNA panels of the two groups were screened using RAPD PCR. Several bands were amplified from the DNA samples in the survivors group, but not from the DNA samples of the dead group. To double check, the DNA samples of the grandparents of the F2 fish were also amplified with the same RAPD primers.

The mapping panel was genotyped with the markers associated with cold tolerance traits, and the data were treated as in Map construction and linkage analysis.

3. Results

3.1. Genotypes

The grandparents and 46 haploid progeny of a single female were screened for a total of 272 DNA markers. A total data set containing 12,512 individual genotypes were obtained and transformed into the two phases, M and P, to satisfy the requirements of the software.

Most markers tested showed detectable linkage to another marker with a threshold of LOD 2.8 and a 95% limit, except for the four markers that were not linked to any other marker. The linkage map (Fig. 1) consists of 50 linkage groups (LG) spanning 4111 cm. The biggest group is 227 cm long and the smallest group is only about 11 cm.

A total of 107 gene markers were mapped on the genetic linkage map, with some genes known to be important for economic traits. For example, the growth hormone gene was mapped on LG36, gonadotropin1 on LG1, gonadotrophin2 on LG29 and cysteine proteinase on LG36.

3.2. Identification of the markers associated with cold tolerance

To identify markers associated with cold tolerance, RAPD analysis was conducted to identify bands that have been amplified only from DNA samples of the cold tolerance grandparent and the F2 survivors after over wintering in pond, but not from DNA samples of the cold sensitive grandparent or F2 hybrid individuals that did not survive over winter, using the same primers. Four such bands were identified as putative markers associated with cold tolerance. The markers were named 5N1451c, 10C900c, 10C1300c, and 19C1200c, respectively (Fig. 2).

Fig. 1. The genetic linkage map of common carp. Gene markers identified by two or three letters; microsatellite (MS) markers begin with “Z” and followed by three, four or five digits for zebrafish MS markers, “MFW” and “GF” for common carp MS markers, “GON” for golden carp MS markers. “EST” markers begin with one or two letters and followed with six digits. RAPD markers begin with a number and capital letters that refer to the manufacturer’s primer name followed by the size of the band in base pairs. For example, the locus 2H.451, is amplified by primer H02 (Operon Technologies, USA), and results in a 451-bp amplification product from common carp.
Fig. 2. The result of identifying the markers associated with cold tolerance. M lane is a molecular weight marker. Y lane is PCR products from the genomic DNA of the cold tolerant grandparent, B lane is PCR products from the genomic DNA of the cold sensitive grandparent, and K lane is PCR products from the genomic DNA of the over winter surviving group of fish. OPN or OPC are the manufacturer’s primer name. Top: with markers 10C900c and 10C1300c. Bottom: with marker 5N1451c and 19C1200c.

Fig. 3. Mapping of 5N1451, the marker associated with cold tolerance, on the linkage map.
3.3. Mapping markers associated with cold tolerance on map

In order to map the putative markers associated with cold tolerance, the four putative markers were analyzed for their map locations using RAPD analysis and the mapping panel. However, only 5N1451c could be mapped on LG5 (Fig. 3), whereas the other three cold tolerance markers were not mapped on any linkage group yet.

4. Discussion

Lie et al. (1994) have showed a good strategy of mapping through haploid gynogenesis. Postlethwait et al. (1994), Johnson et al. (1996) and Kocher et al. (1998) have constructed genetic maps for zebrafish and tilapia using this mapping strategy. In spite of its advantage of easy genotyping, particularly for dominant RAPD markers, the major problem for genotyping with haploid material is that few phenotypic traits can be measured (Kocher et al., 1998). Another major problem for genotyping with haploid material is that only very small DNA samples may be obtained from each embryo and only about 300 markers could be mapped with this haploid mapping panel. Since mapping quantitative trait loci is one of the most important goals of genomic research, diploid panels should be more useful for construction of the second generation of linkage maps in carp. Fifty linkage groups were identified in this study, equal to the number of chromosomes in a haploid common carp. These linkage groups cover 4111 cm. This genome size is quite large, although genetic distances are not the same as the physical size. The most important factor may be owing to so many chromosomes of common carp. Other factors affected the size of map are chromosome interferences. The positive interference will decrease the size of map, and the negative interference will increase the size (Rieger et al., 1983). Thorgaard et al. (1983, 1998), Johnson et al. (1995, 1996) and Kocher et al. (1998) all have noticed a high level of interference in teleost fish genomes. Crooijmans et al. (1997) suggested that common carp had been tetraploidized 50 million years ago and the tetraploidy resulted from allo-tetraploidization (species hybridization) rather than from autotetraploidization (genome doubling), which would result in high levels of chromosome interference. In fact, whether the interference of common carp enlarged the map remained a question. Additionally, Crooijmans et al. (1997) have noticed that the microsatellite markers produced a high numbers of alleles. It is assumed that if a marker has more than one locus, the LOD value will be reduced and the estimated genome size becomes larger.

The cold tolerance trait of fish has long been studied, due to its importance for aquaculture. However, most research to date has been focused on physiological factors such as blood cells, cell membranes, and the enzyme esterase. We report here the first marker linked to cold tolerance in carp. One of the difficulties for cold tolerance research in fish is the lack of well-defined lines contrasting with respect to cold tolerance. In this study, we used a hybrid system with two carps, one of which is cold tolerant and the other cold sensitive. This hybrid system provided an ideal system for analysis of cold tolerance. Segregation of the chromosomes and thereby the traits in F2 offered a good system for further mapping and identification of cold tolerance genes and QTLs. This study is the first step toward eventual molecular cloning of cold tolerant genes in carps. One marker
associated with cold tolerance has been mapped on a linkage group. Another three markers could not be mapped on any linkage group due to poor data and the too small panel of DNA samples left which prevented repeating the mapping work. An attempt to map them will soon be made using a new mapping panel.

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