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A second generation genetic linkage map for silver carp (*Hypophthalmichehys molitrix*) using microsatellite markers

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ABSTRACT

In the study, we constructed a second generation genetic linkage map for silver carp (*Hypophthalmichthys molitrix*) using anonymous and EST-derived microsatellite markers in a mapping panel containing 156 "pseudo BC" progenies from two interspecific crosses between silver carp and bighead carp (*Aristichthys nobilis*). A total of 703 markers were ordered on 24 linkage groups (LGs) which are equal to chromosome numbers of the haploid genome of the species. The consensus map spanned 1561.1 cM covering 93.1% of the silver carp genome with an average resolution of 2.2 cM/locus. Length of LGs ranged from 42.1 cM to 97.8 cM (mean 65.0 cM). Total number of markers on individual LG varied from 13 to 56 (mean 29.3). Estimated total length of the female map (1809.0 cM) was 1.52 times longer than that of the male map (1188.5 cM), and the recombination ratio between sexes (female vs. male) was 2.2, showing markedly higher recombination in the females. Percentage of distorted loci in the male map was obviously higher than that in the female map, and 5 segregation distortion regions were identified in the male linkage groups. This second generation genetic linkage map evidently extends previous genetic maps for silver carp, and provides a basis for such studies as quantitative trait locus mapping, comparative genomics and marker-assisted selection.

1. Introduction

Genetic linkage map is an essential tool to understand genome organization, evolutionary relationships, and to verify DNA sequence contig order and orientation for genome assembly (Botstein et al., 1980; Stapley et al., 2008; Wang et al., 2011; Xia et al., 2010). The advance of molecular genetic techniques and sophisticated statistical tools for linkage analyses made genetic linkage map construction possible for many aquaculture organisms. To date, dominant DNA markers such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) have been generally replaced by co-dominant markers such as microsatellites (SSRs) and single nucleotide polymorphisms (SNPs) in genome mapping (Xia et al., 2010). Compared with other molecular markers, microsatellites have a number of advantages: they are PCR-based, hyperallelic, and widely distributed throughout eukaryotic genomes (Jewell et al., 2006). Furthermore, the high information content and transferability of microsatellite markers provide an important tool for integration of different maps and for comparative genomics studies (Xia et al., 2010; Yu et al., 2004). So far, first generation genetic maps with low or medium resolution have been reported for many aquaculture animals (e.g., Coimbraa et al., 2003; Li et al., 2003; Li et al., 2005; Li et al., 2006), and now microsatellites are extensively applied in the preparation of second generation genetic linkage maps in some economically important aquaculture fishes, such as tilapia (Lee et al., 2005), rainbow trout (Palti et al., 2012; Rexroad et al., 2008), Japanese flounder (Castaño-Sánchez et al., 2010; Song et al., 2012), channel catfish (Kucuktas et al., 2009; Ninwichian et al., 2012), grass carp (Xia et al., 2010) and common carp (Zhang et al., 2013; Zheng et al., 2011).

The silver carp (*Hypophthalmichthys molitrix*) is a filter-feeding cyprinid fish and also one of the most important aquaculture fishes (Fu and He, 2012). It has great values not only for food fish but also for biological control of bloom-forming cyanobacteria in lakes, ponds and reservoirs (Ke et al., 2009). Due to fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value, commercial harvest of silver carp has steadily increased in recent years, especially in China. According to recent statistics (FAO, 2010), global annual production of silver carp has exceeded 4.0 million metric tons. However, in China, natural populations of silver carp have declined dramatically due to habitat fragmentation and over-fishing, and production traits are also declining due to long-term artificial reproduction and inappropriate management of broodstock resources. The initiation of breeding programs for silver carp and other Chinese major carps is thus required for genetic improvement of economically important traits such as growth, and disease and stress resistance.

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Genetics and genomics studies on silver carp are in their infancy. Approximately 600 SSR sequences for silver carp are available in GenBank or other public databases. A few first-generation genetic maps have been previously reported for silver carp, primarily based on AFLP markers and limited number of SSRs, resulting in relatively low resolution and more linkage groups than expected (Liao et al., 2007b; Zhang et al., 2010, 2011). A well-defined genetic map with co-dominant DNA markers is not available for this species. The objective of this study is to produce a second generation genetic linkage map for silver carp using anonymous and EST (expressed sequence tag)-derived microsatellite markers to supply a framework for subsequent genome-wide searches for quantitative trait loci (QTL) and for comparative genomics studies among Chinese carps.

2. Materials and methods

2.1. Mapping family and DNA extraction

Two couples of bighead carp and silver carp were sampled from the middle reach of the Yangtze River, and a mapping panel including two parents and 156 progenies from two F_1 pseudo-testcross families (Fig. 1) were produced by reciprocal crossing between silver carp and bighead carp at the Wuhan Donghu Fish Farm. 78 "pseudo BC" progenies at the age of two months were randomly sampled from each family and stored in 100% ethanol. Fin clips of parental fish were also sampled. Genomic DNA was extracted from ethanol-preserved fin tissues using a phenol–chloroform method (Taggart et al., 1992). DNA quality was checked using 1% agarose gel electrophoresis, and the concentrations were estimated using NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.2. Sources of microsatellites and marker nomenclature

Major SSRs in this study were developed from scaffold sequences of the genome and transcriptome sequencing projects for silver carp and bighead carp (coordinated by Prof. S.P. He, Institute of Hydrobiology, the Chinese Academy of Sciences). These sources of SSR markers were named with "Hysd", "HysdE", and "Arsd" prefixes, respectively. Other sources of SSRs included those from three microsatellite-enriched genomic libraries prepared by following the AFLP of sequences containing repeats (FIASCO) method (Zane et al., 2002): one from silver carp with GT repeat (named with "HyGT" prefix), and two from bighead carp with GA and GT repeats (named with "ArGT" and "ArGA" prefixes, respectively). In total, 1922 anonymous SSR and 260 EST-SSR markers were newly developed in this study using the software Primer premier 5.0 (http://www.premierbiosoft.com/primerdesign/index. html) for design of primers. Detailed information for those SSR markers mapped on the silver carp consensus map is available in Appendix A.

In addition, a total of 584 SSRs previously published for silver carp and bighead carp were also used for initial segregation screening in this study. These SSRs were named using their original nomenclatures with the prefixes of "Hym" (Zhang et al., 2010; Zhang et al., 2011), "Ar" (Cheng et al., 2008), "BL" (Liao et al., 2007a), "Hmo" (Gheyas et al., 2006), and "Cid" (Guo et al., 2009).

2.3. Microsatellite genotyping

All candidate SSR markers were initially screened in the two parents of silver carp, and polymorphic loci segregated in either female or male parents were subsequently genotyped in the two mapping families. Amplification of SSRs was carried out through PCR reaction in a thermocycler (Veriti, ABI) with a total volume of 12.5 μl, containing 50 ng of template DNA, 1.25 μl of 10× reaction buffer, 1 U of *Taq* polymerase (TaKaRa, Japan), 0.5 μl of dNTP (2.5 mmol/l), 0.5 μl of forward and reverse primer mixture (2.5 μmol/l each) and water to the final volume. The cycling profiles were as follows: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 35 s, optimal annealing temperature (see Appendix A and references) for 35 s, 72 °C for 40 s, and a final 72 °C extension for 10 min. The PCR products were separated on 10% polyacrylamide gels and visualized by staining with ethidium bromide.

2.4. Linkage analyses and genome coverage

The data from Family 1 was used for female map construction, and those from Family 2 for male map construction. Linkage analyses for

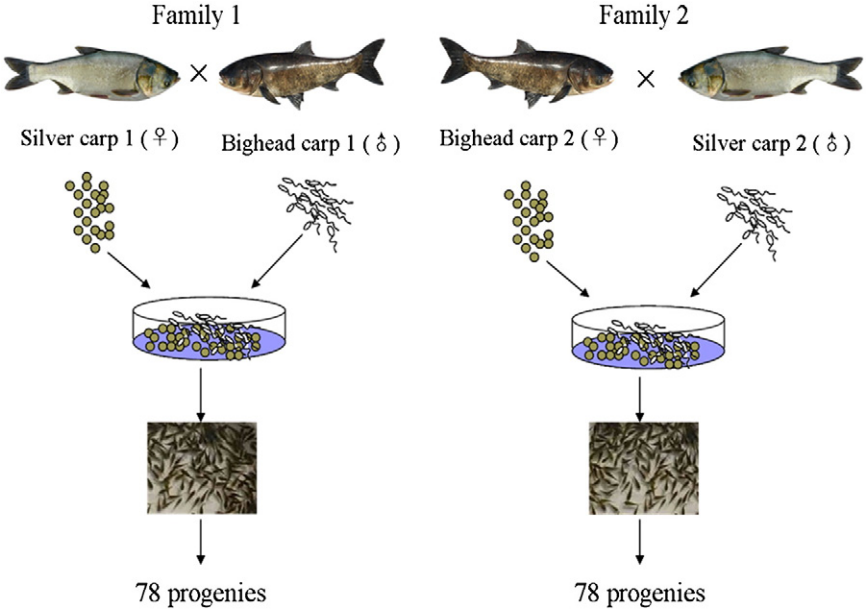

Fig. 1. A flowchart for the production of two interspecific mapping families between silver carp and bighead carp.