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A genetic map of large yellow croaker *Pseudosciaena crocea*

Yue Ning [a], Xiande Liu [a], Zhi Yong Wang [a,*], Wei Guo [a,b], Yiyun Li [a], Fangjing Xie [a]

[a] *The Key Laboratory of Science and Technology for Aquaculture and Food Safety, Fisheries College, Jimei University, Xiamen 361021, China*
[b] *Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

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Abstract

Genetic linkage maps were constructed for large yellow croaker *Pseudosciaena crocea* (Richardson, 1846) using AFLP and microsatellite markers in an F_1 family. Five hundred and twenty-three AFLP markers and 36 microsatellites were genotyped in the parents and 94 F_1 progeny. Among these, 362 AFLP markers and 13 SSR markers followed the 1:1 Mendelian segregation ratio ($P>0.05$). The female genetic map contained 181 AFLP and 7 microsatellite markers forming 24 linkage groups spanning 2959.1 cM, while the male map consisted of 153 AFLP and 8 microsatellite markers in 23 linkage groups covering 2205.7 cM. One sex linked marker was mapped to the male map and co-segregated with the AFLP marker agacta355, suggesting an XY-male determination mechanism and this may be useful in the breeding of monosex populations.

Keywords: *Pseudosciaena crocea*; AFLP; Microsatellite; Genetic linkage map; Sex determination

1. Introduction

Genetic maps have become essential tools in many fields of genetic studies and have been constructed in various organisms (Postlethwait et al., 1994; Dib et al., 1996; Dietrich et al., 1996; Groenen et al., 2000), including several aquaculture species (Kocher et al., 1998; Young et al., 1998; Sakamoto et al., 2000; Robison et al., 2001; Waldbieser et al., 2001). These maps have been efficiently used for various biological analyses, such as quantitative trait loci (QTL) (Jackson et al., 1998; Sakamoto et al., 1999; Ozaki et al., 2001; O'Malley et al., 2003), marker-assisted selection (Lande and Thompson, 1990; Fuji et al., 2006), comparative genome mapping (Naruse et al., 2000; Woods et al., 2000) and position-based cloning (Dietrich et al., 1996).

Large yellow croaker (*Pseudosciaena crocea* Richardson, 1846), one of the most economically important marine fish in China, is mainly distributed in coastal regions of East Asia (Feng and Cao, 1979). Its wild population has severely declined since 1970's, and the commercial characteristics (growth rate, flesh quality and disease resistance) of the cultured stocks have also declined (Wang et al., 2002). The genetic improvement of farmed large yellow croaker has been relatively slow compared with other aquaculture species (Fjalestad et al., 2003; Garber and Sullivan, 2006). To date only a few breeding programs have been initiated through selective breeding and no genetic map has been constructed for large yellow croaker.

A moderately dense linkage map can be made rapidly using amplified fragment length polymorphism (AFLP) markers. As a PCR-based technique, AFLP

* Corresponding author. Tel.: +86 592 6183816; fax: +86 592 6181476.
E-mail address: zywang@jmu.edu.cn (Z.Y. Wang).

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could generate a large number of polymorphic markers without any prior knowledge of DNA sequences for the organism (Vos et al., 1995). Many initial genetic maps based on molecular markers have been successfully constructed primarily relying on AFLP markers in aquaculture species, such as tilapia, *Oreochromis niloticus* (Kocher et al., 1998) and rainbow trout, *Oncorhynchus mykiss* (Young et al., 1998). Nevertheless, transferring AFLP markers between labs, species, and even crosses are questionable; this deficiency could be overcome by using co-dominant markers such as microsatellites. But for large yellow croaker, only limited microsatellites are available. So in this study, AFLP markers with a small set of microsatellites were used to construct the maps of large yellow croaker.

2. Materials and methods

2.1. Mapping population

The mapping population used in this study is an inter-population hybrid family of large yellow croaker. Its female parent was sampled from a commercial pond. This pond stock was derived from around 36 mature large yellow croakers that had been trawled from a wild population on the East China Sea in 1986. The male parent was wild and caught from the East China Sea in the spawning season. The mapping population was obtained by artificial propagation. Ninety four two-year-old F_1 progeny from this family were sampled, and the sex of the fish was determined by dissection to examine the gonad.

2.2. Genomic DNA extraction

Genomic DNA was extracted from fin of the F_1 progeny and their parents using standard phenol–chloroform technique with slight modifications (Wang et al., 2000). Fin samples (20–30 mg) were placed into individual sterile of 1.5 ml microcentrifuge tubes containing 550 μl TE buffer (100 mM NaCl, 10 mM Tris, pH 8, 25 mM EDTA, 0.5% SDS, and freshly added proteinase K, 0.1 mg/ml). The samples were incubated at 55 °C overnight, and subsequently extracted twice using phenol and then phenol/chloroform (1:1). DNA was precipitated by adding two and a half volumes of ethanol, collected by brief centrifugation, washed twice with 70% ethanol, air dried, re-dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5), and quantified with a spectrophotometer.

2.3. AFLP analysis

AFLP analysis was performed according to Vos et al. (1995) as modified by Wang et al. (2004). Briefly, genomic DNA was digested with two enzymes, *Eco*RI and *Mse*I, and ligated to adapters to provide the complementary sequence for AFLP primers. Pre-amplification reactions were performed using *Eco*RI and *Mse*I primers each with a single selective base. For the selective amplification two additional selective bases were used in both primers. The amplification products were resolved in the 6% denaturing polyacrylamide gels and run at 80 W using a Sequi-Gen GT (38 × 50) cm gel apparatus (BioRad, USA). The amplification products were visualized by silver staining (Wang et al., 2004).

2.4. Microsatellite analysis

Total of 36 microsatellite loci containing (CA) repeats that were isolated from an enriched large yellow croaker genomic DNA library were screened for mapping (Guo et al., 2004, 2005). All primers were synthesized by Sangon Biological Engineering Technology CO., Ltd (Shanghai, China). PCR amplification was performed in a 20 μl reaction volumes containing 40–100 ng template DNA, 1× PCR buffer (10 mM Tris, 50 mM KCl, pH 9.0, 200 μM of each dNTP (Promega, USA), the concentrations of $MgCl_2$ varied depending on the locus, 0.5 U *Taq* polymerase, and 4 pmol of each primer. PCR cycling was carried out on an Autorisierter Thermocycler (Eppendorf, German) with the initial denaturing at 95 °C for 2 min, followed by 30 cycles of 30 s denaturing at 95 °C, 30 s annealing at locus-specific temperatures, 30 s extension at 72 °C, and a final extension for 10 min at 72 °C. The PCR products were denatured and visualized using denaturing polyacrylamide gels (6%) followed by silver staining.

2.5. Markers scored and nomenclature

AFLPs were scored as dominant markers. Then the genotypes of the parents for a given marker were inferred from the marker phenotypes of the offspring. Only polymorphic bands that were presented in one (cross type Aa × aa) or both (Aa × Aa) parents and segregated in the progeny were scored. The AFLP markers were named according to the combination of the selective amplification primers used and the approximate product size, which were determined by using a 10 bp DNA Ladder (Invitrogen, USA). For example, for AFLP marker agacag255, the first three letters (aga) represent the selective nucleotides in the *Eco*RI primer, the next three