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Construction of genetic linkage maps of guppy (*Poecilia reticulata*) based on AFLP and microsatellite DNA markers

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Abstract

Genetic linkage maps of guppy (*Poecilia reticulata*) were constructed using microsatellite DNA and AFLP markers and a pseudo-testcross mapping strategy. $(AC)_n$ microsatellite DNA markers were developed and used to genotype sixty-one full-sib progenies (F_1) and their two parents. Of 293 microsatellite DNA markers, 101 segregated at 1:1 or 1:1:1:1 ratios. In addition, 336 AFLP markers segregated also in F_1 progenies at 1:1 ratio, which were produced using 91 primer combinations. All these markers were mapped with two linkage maps produced, one each parent. Female map included 135 markers in 22 linkage groups, covering a total of 1267.7 cM in length. Male map included 172 markers in 20 linkage groups, covering a total of 1771.2 cM in length.
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Keywords: *Poecilia reticulata*; Linkage map; AFLP; Microsatellite DNA

1. Introduction

Guppy fish (*Poecilia reticulata*) belongs to class Actinopterygii (ray-finned fish), order Cyprinodontiformes, family Poeciliidae and genus *Poecilia*. It mainly inhabits the coastal streams and rivers of Venezuela, Guyana, Surinam and several of the Lesser Antilles including Trinidad and Tobago (Haskins and Haskins, 1951; Yamamoto, 1975). Since 1950s, breeders have domesticated, artificially selected and genetically improved wild guppy for traits such as brilliant color, longer fin and larger body size. At present, different guppy strains have been created by intensive selection of spontaneous mutant genes that affect the coloration as well as the shape and size of the body and fins (Kirpichnikov, 1981; Fernando and Phang, 1985). Guppy has become popular among aquarists and hobbyists and played an important role in the freshwater ornamental fish industry worldwide. Guppy is also a model fish for studying the genetic bases of heterosis, aging and inbreed depletion crucial for aquaculture because of its relatively short life cycle, easiness of breeding and reproduction, availability of commercial strains homologous for the traits such as body color and

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tail shape, and very importantly the availability of evaluation methods of heterosis, aging and inbreed depletion (Nakajima and Fujio, 1993; Fujio et al., 1995; Shikano et al., 1997).

Genetic linkage maps have become powerful tools of research in many organisms (Dib et al., 1996). A complete linkage map is necessary for efficiently carrying out molecular based analyses, e.g. location of quantitative trait locus and map-based cloning (Lander and Botstein, 1989). Genetic linkage maps have been developed for almost all major and important cultured fish, e.g., tilapia, rainbow trout, catfish, Atlantic salmon, medaka and Arctic char (Young et al., 1998; Sakamoto et al., 2000; Gilbey et al., 2004; Woram et al., 2004; Lee et al., 2005). For guppy, a preliminary linkage map has been constructed (Winge, 1927), which included 18 genes on sex chromosomes. In addition, Khoo et al. (2003) constructed RAPD marker linkage map and Watanabe et al. (2005, 2004) constructed microsatellite DNA and AFLP marker linkage map of guppy, respectively. However, these previous maps were restricted by the number of markers appropriate for their applications.

In the present study, two parent-specific genetic linkage maps of guppy were constructed by analyzing the segregation of AFLP and microsatellite DNA markers in 61 F_1 progenies. Our objectives were to: (1) estimate the genome lengths of guppy; and (2) construct moderately dense linkage maps.

2. Materials and methods

2.1. Mapping population

In Chinese ornamental fish market, the dealers used to self-cross relatively pure commercial strains, which will cause the inbreeding depression in a few generations. In order to recover the vigor, the dealers used to cross the females of a strain with the males of the other, producing morphologically similar but genetically different fish for sale. Fortunately, this facilitates our mapping trial; the number of loci segregating in the offspring of a cross between the relatively pure strains is less than that in the offspring of a cross between the lines purchased from the market.

Two parental lines used in this study were purchased from the local ornamental fish market, Qingdao, China. Both female and male individuals of one line (red line) had pink body and red tail while both female and male individuals of the other line (black line) had black (rare part) and grey (front part) body and red tail. These parental lines are homozygous for desired morphological traits as were observed in their self-crossing offspring, but heterozygous in genetic background to some extent as was determined in microsatellite DNA marker screening (of 221 markers amplified robust bands, 128 were polymorphic in at least one parent). The first filial generation was obtained by making a cross between a female individual of red line and a male individual of black line, with F_1 progenies reared for more than 50 days to sexual maturation. All F_1 progenies were similar to black line in body and tail color. A total of 61 progenies (31 female and 30 male) were used for constructing parent-specific linkage maps.

2.2. DNA extraction

DNA was extracted from muscle tissue following the method described by Dinesh et al. (1993) with minor modifications. Approximately, 100 mg of tissue was cut into small pieces which were then placed in 1 ml STE buffer (10 mM Tris–HCl, pH 8.0; 50 mM EDTA, pH 8.0; 200 mM NaCl and 0.5% SDS) and digested with 0.5 mg/ml proteinase K at 55 °C overnight (>8 h). The resulting solution was extracted with phenol and chloroform. DNA was precipitated with ethanol and dissolved in TE buffer. The quality and concentration of DNA were assessed by agarose gel electrophoresis.

2.3. AFLP analysis

AFLP analysis was carried out essentially as described by Vos et al. (1995). DNA was digested with *Eco*R I and *Mse* I before ligation to restriction site-specific adaptors. Pre-amplification was carried out using adaptor-specific primers with one selective base overhang each primer (*Eco*R I adaptor primer with A and *Mse* I adaptor primer with C). The pre-amplification product was diluted (20-fold) and used for selective amplification. Selective amplification was carried out with diluted pre-amplification product and primers with three selective bases overhang each primer. In total, 91 primer combinations were selected for AFLP analysis. The products of selective amplification were separated on polyacrylamide gel with the bands visualized by silver staining.

Mse I and *Eco*R I selective primers were named with capital letters and numbers, respectively (Table 1). AFLP marker was named with the name of *Mse* I selective primer and that of *Eco*R I selective primer followed by letter f (fragment) and the size of the fragment in base pairs (Zhang et al., 2006), e.g., A8f169 referred to the 169 bp fragment generated by *Mse* I primer A (CTA) and *Eco*R I primer 8 (AGC).

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