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Microsatellite Analysis of Genetic Diversity Between Loach with Different Levels of Ploidy

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Abstract: We used microsatellite markers to investigate the genetic parameters of three different polyploidy populations of *Misgurnus anguillicaudatus* from Honghu City, Hubei Province. Polyacrylamide gel electrophoresis banding patterns of diploid (2n), triploid (3n) and tetraploid loaches (4n) were analyzed with PopGen software. A total of 68 alleles were obtained from seven microsatellite loci and the polymorphism information content (*PIC*) indices were all above 0.5. The average expected mean heterozygosity values (*He*) were 0.8420, 0.7186 and 0.8521; the average observed mean heterozygosity values (*Ho*) were 0.9674, 0.9785 and 0.8928; and the Hardy-Weinberg *P* values were 0.3078, 0.3151 and 0.3762, for diploid, triploid and tetraploid individuals, respectively. The results indicated that the three populations were highly polymorphic, with no deviations from Hardy-Weinberg equilibrium observed at all the seven microsatellite loci. This indicated a high level of genetic diversity within the populations. A cluster analysis diagram showed that the shortest genetic distance was between diploid and tetraploid loaches and they shared a close phylogenetic relationship. The triploid and tetraploid individuals had the most distant phylogenetic relationship.

Key words: *Misgurnus anguillicaudatus*, diploid, triploid, tetraploid, microsatellite, genetic diversity

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Introduction

Natural polyploidy is commonly found in aquatic animals, and in the reported karyotype of freshwater fishes, 30 are found to be polyploidy (Ma, 1996). Most of the polyploid fishes are important commercial fishes or cultured species, therefore, researches on polyploidy fishes are very important for ascertaining and making use of the resources. Polyploidy exists in loach, *Misgurnus anguillicaudatus*. A large quantity of triploid are found in Japan, but no tetraploid (Morishima *et al*., 2002; Arai *et al*., 1991; Zhang and Arai, 1999). As it was reported before, the diploid (2*n*=50) and tetraploid (4*n*=100) population existed in China (Li *et al*., 1983; Li *et al*., 1987; Yin *et al*., 2005); however, after ploidy investigation from 29 sites around China, a few natural triploid (3*n*=75) were found (Li *et al*., 2008). Former studies on loach mainly focused on karyptype of tetraploid (Li *et al*., 1983; Li *et al*., 1987; Yin *et al*., 2005), ploidy identification (Gao *et al*., 2007; Guo *et al*., 2009), cyt b sequence (Yang *et al*., 2009), population genetic structure (Zhou *et al*., 2011) and gene polymorphism of mitochondria (Zheng *et al*., 2011). In the last 10 years, we had systematically studied the distribution of natural polyploidy in China (Li *et al*., 2008), chromosome karyotype of somatic cells (Li *et al*., 2009), chromosome band and FISH (Li *et al*., 2010), chromosome behavior of meiosis (Li *et al*.,

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2010), artificially induced gynogenesis (Li *et al*., 2013) and reproductive characteristics (Li *et al*., 2012). However, genetic diversity researches on loaches of different ploidy have not been reported yet. Microsatellite DNA is a newly developed marker with lots of kinds, high polymorphism, high heterozygosity and low recombination rate. It also has lager genetic variation grope and makes up rich polymorphism, highly individual specific and follows the rules of Mendel's Laws. It has many highly provided heritable information of polymorphic locus. Therefore, the microsatellite molecular marker is the most widely used markers for constructing genetic map, and population genetic structure analysis (Norris *et al*., 1999; Du *et al*., 2000; Zhou *et al*., 2001; Sun *et al*., 2001; Liang *et al*., 2002; Dong *et al*., 2007; Zhang *et al*., 2010; Liu *et al*., 2012). Seven microsatellite markers were used to analyze its genctic diversity, in order to provide genetic theory basis for protecting and making use of the loach genetic resources, reveal the origin and evolution mechanism of the polyploid loaches, and to discuss the genetic relationship between loaches of different ploidy for clarifying the formation mechanism of polyploid loaches.

Materials and Methods

Experimental materials

A total of 54 loaches were collected from Honghu City, Hubei Province. Ploidy was detected by measuring the volume of erythrocyte nuclei using a flow cytometer. Of the 54 individuals, 23 were diploid, three were triploid and 28 were tetraploid. Genomic DNA was extracted by using the phenol-chloroform method and diluted to 100 ng·μL^{-1} in 100 μL of TE buffer (pH 8.0) and stored at −20℃.

Microsatellite markers

Microsatellite loci were identified from the simple sequence repeats (SSRs) registered with GenBank. PCR primers were designed on either side of the core sequence. In this study, seven loach microsatellite loci were selected for the analysis (Table 1). The primers were synthesized by Sangon Biotech.

Table 1 Primer sequence of microsatellite DNA

Microsatellite locus	Accession number	Core sequence	Primer sequence (5'-3')	Annealing tempeature (℃)	Size of alleles (bp)
*Mac*3	AB060173	$(CA)_{17}$	F: CAGCCTGTTAACCTTCCACT R: CCCCAAATTCAGAGAGCTAG	56	102-110
*Mac*36	AB060180	$(CA)_{27}$-$(CA)_{9}$	F: TTTAATGTGGGACTGCTGAT R: TCCACACATATGAATTTCCT	56	130-148
*Mac*37	AB060181	$(CA)_{15}$	F: GCAAGTACATGCTCATCCTT R: CACCTGCATTCCTTACATCT	56	107-115
*Mac*45	AB060185	$(CA)_{16}$	F: GTTCAGTACGGCTTTAGCAG R: TGGGTGTTCATTTTTATCCC	56	106-116
*Mac*49	AB060186	$(CA)_{21}$	F: CGCAACAGTGTGCAAACATT R: CTGCAGGACACTTCAAACAG	54	106-124
*Mac*60	AB081631	$(CA)_{21}$	F: CCAGGCTGCAGTTTCAGTTT R: TCAGGTGTCATACATCCTTT	56	125-151
*Mac*63	AB081633	$(CA)_{16}$	F: GGGTCAGAACAAACAGCACA R: GATTGGGGTTGCTGCCCTCTTC	58.8	118-188

PCR

PCR reagent conditions were as follows: 2.5 μL of 10×buffer (Mg^{2+}), 2.5 μL of $MgCl_2$ (2.5 mmol·L^{-1}), 0.5 μL of each dNTP (2.5 mmol·L^{-1}), 1.0 μL of each of the forward and reverse primers (10 μmol·L^{-1}), 0.3 μL