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CORRELATION OF MICROSATELLITE DNA MARKERS WITH GROWTH TRAITS IN STRIPED CATFISH (*Pangasianodon hypophthalmus*)

Huria Marnis#, Evi Tahapari, and Jadmiko Darmawan

Research Institute for Fish Breeding
Jl. Raya Pantura No. 2, Patokbeusi, Subang, Sukamandi 41263, West Java

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ABSTRACT

Marker-assisted selection in genetic improvement of striped catfish is useful in the breeding program of the fish. Five microsatellite markers were characterized in the largest (4.03 kg ± 1.67 kg) and smallest (1.41 kg ± 0.22 kg) individuals. Five polymorphic loci were then used to genotype 160 individuals and the associations between their genotypes and growth traits were examined. The result showed that twenty-seven alleles were detected in striped catfish. The number of alleles per locus (N_A) ranged from 4 to 7, with an average of 5.4 alleles per locus. The effective number of alleles per locus (N_E) ranged from 3.940 to 6.939, with an average of 5.32 alleles per locus. H_O and H_E ranged from 0.125 to 0.944 (mean value of 0.472) and from 0.564 to 0.775 (mean value of 0.697), respectively. PIC ranged from 0.573 to 0.799 (mean value of 0.706), showing that they were highly polymorphic loci. Only one microsatellites loci (Pg13) that showed significant differences ($P<0.01$) in the associations between their genotypes and growth traits, while Pg3 and Pg14 were significantly associated with the standard length ($P<0.01$) and body weight ($P<0.05$). However, the Pg1 and Pg2 were not significantly associated with the body weight and standard length. Four genotypes of three loci were positively correlated with the growth traits (body weight and standard length) i.e. genotypes 194/194 for Pg3, 227/227 and 229/229 for Pg13, 279/279 for Pg14. These four genotypes can be used to identify growth traits in the molecular marker-based selection of a breeding program.

KEYWORDS: **microsatellite; growth traits; allele; fast-growing; genetic diversity; *Pangasianodon hypophthalmus***

INTRODUCTION

Nowadays, the development of molecular genetic markers has stimulated a renewed interest in the study of growth trait improvement of fish. In aquatic animals, molecular markers are not only used in genetic monitoring of selective breeding lines (Hao *et al*., 2010; Yu *et al*., 2011) but also in the association analysis of target traits (Kang *et al*., 2002). Many researchers have reported that certain genotypes of microsatellite loci are positively correlated to growth traits (Fan *et al*., 2009; Liu *et al*., 2012).

Microsatellite markers have been used as functional markers to identify genes that play important roles in productive traits. The characterization of gene-associated microsatellites in some species including *Oreochromis* sp. (Yue & Orban, 2002), *Cyprinus carpio* (Yue *et al*., 2003), *Salmo salar* (Vasemägi *et al*., 2005), *Gadus morhua* (Stenvik *et al*., 2006), *Epinephelus bruneus* (Kessuwan *et al*., 2016), *Larimichthys crocea* (Han *et al*., 2017), and *Colossoma macropomum* (Ariede *et al*., 2018) had already demonstrated the usefulness of these markers in the detection of economically important quantitative traits and its application in breeding studies. Nonetheless, this characterization has not been observed in striped catfish.

This study was conducted to study the growth performance of striped catfish and identify molecular markers that are associated with the growth traits in striped catfish using microsatellite. The markers could provide a valuable theoretical basis for marker-assisted selection (MAS) to breed faster growing strain and to preserve fish germplasm.

Correspondence: Research Institute for Fish Breeding.
Jl. Raya Pantura No. 2, Patokbeusi, Subang, Sukamandi 41263, West Java, Indonesia
Phone: + 62 260 520500
E-mail: *marnis.huria@gmail.com*

MATERIALS AND METHODS

Fish and DNA Samples

The research spanned in the period of six years from January 2012 until December 2017. The identification of broodstock bearing fast growth markers was performed at the Genetic and Physiology Laboratory, Research Institute for Fish Breeding Sukamandi, Ministry of Marine Affairs and Fisheries of the Republic of Indonesia.

Three generations of striped catfish have been produced from the fish breeding selection program i.e. G-0, G-1, and G-2 populations. These fish were tagged using microchips. The average weight of the largest individuals was 4.03 kg ± 1.67 kg and the smallest was 1.41 kg ± 0.22 kg from a total of 160 fish of an isolated population at the Research Institute for Fish Breeding. Approximately 1 cm^2 of the caudal fin of each fish was cut using a sterile section scissor. Each sample was placed in a microtube containing 1 mL of 70% ethanol and stored at room temperature until the DNA extraction process was performed.

DNA Extraction

The genomic DNA of each sample was extracted using DNA extraction kit (GeneJet Genomic DNA Purification Thermo Scientific, Lithuania) following the protocols recommended by the manufacturer (Thermo Scientific, Lithuania). The genome sample was analyzed in a mini horizontal gel electrophoresis. The sample was loaded into 1.5% (w/v) agarose gel, powered with 65-volt electricity and run for 60 min. The gel was then stained with peqGREEN (Vwr, UK) 1 μg/mL and viewed using ultraviolet transilluminator gel documentation system.

Microsatellite Primers

Five microsatellite loci (Pg1, Pg2, Pg3, Pg12, and Pg14) were used in this study (Na-Nakorn *et al*., 2006; Na-Nakorn & Moeikum, 2009). It showed polymorphisms in *Pangasianodon hypophthalmus*. The primers characteristics used to amplify the five microsatellite loci are presented in Table 1.

Amplification of Microsatellite Locations and Scoring

A total of 2 µL (≤ 200 ng) of the extracted genomic DNA was amplified using type-it microsatellite PCR (Qiagen, Germany). The PCR process was carried out using the following settings: 95°C for 5 minutes; (95°C for 30 seconds, 6°C-62°C for 90 seconds according to the degree of attaching temperature (Tm) per primer (Table 1); 72°C for 30 seconds) of 28 cycles; and 72°C for 30 minutes. The PCR amplification results were loaded on 2% (w/v) agarose gel by an electrophoresis method. The electrophoresis results observed under UV transilluminator and photographed using a Canon EOS 1100D digital camera.

Microsatellite locus polymorphisms were screened using the QIAxcel (Qiagen, Germany) fragment analyzer and using QIAxcel DNA High resolution (Qiagen) kit, and the size of the alleles was determined based on the PCR product size relative to the size of the DNA fragment on QX size marker 50-800 bp (Qiagen, Germany) and alignment marker 50-1,000 bp (Qiagen, Germany). The patterns of DNA band and electrophoregram data were analyzed using QIAxcel screen Gel software 1.5 (Qiagen, Germany) to scan alleles that emerged. The allele score data were then used for the analysis of the relevant genetic parameters.

Statistical Analysis

Allelic frequencies, genotype frequencies, Hardy-Weinberg equilibrium, and observed (*HO*) and expected (*HE*) heterozygosities were statistically analyzed using POPGENE software version 1.32, Fstat's statistical genetic software version 2.9.3. (Goudet, 2001) and Arlequin (Excoffier *et al*., 2006). The associations between genotypes and growth traits were considered significant if two-way P values were less than 0.05. The statistical analyses were carried out using the SPSS software (Version 19.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

A total of five simple sequence repeat (SSR) markers were screened from the 160 samples of the isolated fish population. Three of these loci were polymorphic in striped catfish and significantly associated with the body weight and standard length (Table 2).

A random population was amplified using these five microsatellite loci (Table 2), and 27 alleles were detected in striped catfish. The number of alleles per locus (N_A) ranged from 4 to 7, with an average of 5.4 alleles per locus. The effective number of alleles per locus (N_E) ranged from 3.940 to 6.939, with an average of 5.32 alleles per locus. H_O and H_E ranged from 0.125 to 0.944 (average of 0.472) and from 0.564 to 0.775 (average of 0.697), respectively. Heterozygosity is one of the indicators of the degree of genetic variation (Allendorf & Luikart, 2007). PIC ranged from

Table 1. Primer sequences and characteristics of five polymorphic microsatellites loci of striped catfish populations

Loci	Repeat motif	Primer sequence (5'-3')	Range size (bp)	Temperature (°C)
Pg-1	$(CA)_{18}$	F : GGCCTGTCACAATGTGTATTGC R : GTCTGAGGTAGGCCTGTGAGGAG	231-239	64
Pg-2	$(GA)_{14}N_{116}(GT)_{11}$	F : TGTGTCTAATCTTGTCCGTGCTG R : TACTGTTGGACCAGACGTTCCTC	258-276	60
Pg-3	$(GT)_{16}$	F : CCAGCCCACATTAGGTAGCATC R : ACTAAAAGGCCTGACCCTTAGC	204-220	60
Pg-13	$(CA)_{25}$	F : GTTTTCCATCCAGGTTGTTTTCC R : TAAGTCCATGTGGGTTTCCTCTG	232-262	58
Pg-14	$(GT)_5AT(GT)_{11}$ AT$(GT)_{13}$	F : ACCGTGCATGTGCATTATCATAG R : AGAATGTGACCTGGAAATGAGCA	289-293	60

0.573 to 0.799 (average of 0.706), indicating that they were highly polymorphic loci.

These novel markers will facilitate further studies on genetic diversity evaluation, conservation genetics, construction of high-density linkage map, and molecular marker-assisted breeding of striped catfish and its related species. *PIC* is the change of function of allele frequency and the allele's number, and it is a good indicator of genetic information capacity of a genetic marker (Zhu *et al*., 2008). Moreover, PIC is also an indicator of the degree of genetic variation (Li *et al*., 2017). The five loci of this study showed a high polymorphism (PIC > 0.5).

In this study, one microsatellites loci (Pg13) showed a significant difference ($P < 0.01$) in the associations between their genotypes and growth traits, while Pg3 and Pg14 were significantly associated with the standard length ($P < 0.01$) and body weight ($P < 0.05$). However, Pg1 and Pg2 were not significantly associated with the body weight and standard length ($P > 0.05$). Four genotypes of three loci were positively correlated with growth traits (body weight and standard length), i.e. genotypes 194/194 for Pg3, 227/227 and 229/229 for Pg13, 279/279 for Pg14 (Table 4). These significantly correlated loci carry an important function in the evolution because they control the viability of individuals bearing different genotypes of the locus (Xu, 2008). Therefore, these kinds of genotypes could indirectly be used to select preferred growth traits in striped catfish. The study about correlated marker to growth traits also has been reported by Sun *et al*. (2015) for Mandarin fish. The result of the study showed that the eight genotypes of six loci were positively correlated with growth traits (body weight, length, and height) in the mandarin fish population.

CONCLUSION

This study had confirmed that the identified microsatellite DNA markers are closely associated with growth traits in striped catfish. There are four genotypes of three loci positively correlated with growth traits (body weight and standard length) in striped catfish. It could be useful for marker-assisted selection in breeding programs of striped catfish. It also

Table 2. Genetic diversity in *Pangasianodon hypophthalmus* population

Locus	N_A	N_E	H_o	H_E	PIC
Pg-1	7	6.939	0.944	0.775	0.799
Pg-2	5	5.000	0.563	0.742	0.755
Pg-3	4	3.940	0.213	0.564	0.573
Pg-13	5	4.985	0.125	0.696	0.702
Pg-14	6	5.785	0.513	0.709	0.703

Table 3. Association between five microsatellite loci to body weight and standard length of *Pangasianodon hypophthalmus*

Locus	Body weight (g)	Standard length (cm)
Pg-1	0.49	0.5
Pg-2	0.5	0.14
Pg-3	0.012*	0.001**
Pg-13	0.003**	0.0006**
Pg-14	0.0422*	0.001**

Note: * significantly correlated with markers at $P < 0.05$
** significantly correlated with markers at $P < 0.01$

Table 4. Association between three microsatellite loci with body weight and standard length of *Pangasianodon hypophthalmus*

Locus	Genotype	N	Body weight (g)	Standard length (cm)	Number of individual	
					Smallest fish	Largest fish
Pg-3	198/206	1	1,283[a]	42[a]	1	0
	192/198	4	2,775.10 ± 1,939.40[a]	49.85 ± 12.07[a]	2	2
	194/196	10	2,534.9 ± 2,099.41[a]	48.22 ± 10.37[a]	5	5
	194/194	14	3,023.727 ± 1,507.33[b]	55.33 ± 8.76[b]	2	12
	192/192	8	4,144.667 ± 1,730.96[a]	59.59 ± 7.34[a]	4	4
	192/206	1	6,800[a]	69.5[a]	1	1
	198/198	1	7,400[a]	70.5[a]	0	1
Pg-13	231/231	12	3,364 ± 1,536.21[a]	55.94 ± 9.15[a]	2	10
	229/229	31	3,147.323 ± 1,890.74[b]	53.01 ± 10.22[b]	5	26
	233/233	11	3,774.455 ± 945.04[a]	58.79 ± 4.79[a]	5	6
	227/227	17	2,492.471 ± 1,624.29[b]	49.51 ± 8.16[b]	2	15
	229/233	3	3,872 ± 1,197.14[a]	59.00 ± 4.58[a]	0	3
	227/231	2	7,200 ± 565.69[a]	69.25 ± 0.35[a]	0	2
	229/233	1	7,400[a]	69.33[a]	0	1
	231/233	1	7,600[a]	70.01[a]	0	1
	227/233	2	3,671 ± 1,030.96[a]	60.25 ± 7.42[a]	0	2
Pg-14	285/291	1	2,028[a]	47.5[a]	1	0
	289/289	1	4,600[a]	64[a]	1	0
	279/291	12	5,364 ± 746.12[a]	45.90 ± 6.58[a]	6	6
	279/285	1	6,000[a]	67.5[a]	0	1
	285/285	10	6,337 ± 1,175.92[a]	54.75 ± 8.13[a]	5	5
	279/279	11	3,182.70 ± 2,162.71[b]	59.95 ± 7.81[b]	0	11
	279/291	2	25,438 ± 1,859.3[a]	53.65 ± 10.36[a]	1	1
	279/289	4	30,949 ± 2,132.96[a]	57.32 ± 9.88[a]	2	2
	281/281	5	58,221 ± 1512.24[a]	55.50 ± 8.18[a]	3	2
	281/291	4	75,321 ± 1,940.78[a]	55.90 ± 10.31[a]	2	2

Data labeled with b superscript letters in the same column by individual locus mean significant difference. In contrast, data labeled with a superscript letters in the same column by individual locus mean insignificant difference.

provides new information on striped catfish growth traits to guide future breeding programs of this species. The prospective microsatellites highlighted in this study could be used in the validation of MAS in fish species belong to the striped catfish family.

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