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Microsatellite DNA markers and their correlation with growth traits in mandarin fish (*Siniperca chuatsi*)

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ABSTRACT. The mandarin fish (*Siniperca chuatsi*) is a traditionally cultured freshwater fish with high commercial value in China. To facilitate marker-assisted selection in genetic improvement of this species, 120 microsatellite markers from the literature were characterized in the 25 largest and 25 smallest individuals. Eighteen polymorphic loci were then used to genotype 200 individuals, and the associations between their genotypes and growth traits were examined. We found that eight genotypes of six loci (*AP 37-06*, *AP 37-11*, *AP 37-16*, *AP 37-48*, *AP 38-32,* and *AP 39-05*) were positively correlated with growth traits (body weight, length, and height) in the mandarin fish population. The average observed and expected heterozygosities were 0.68 and 0.59, respectively, and the average PIC value was 0.50, indicating a population with high genetic diversity. Therefore, these markers could be useful for assisted selection in genetic breeding of this species and its related species.

Key words: Mandarin fish; Microsatellite; Growth traits; Genetic diversity

INTRODUCTION

The mandarin fish, *Siniperca chuatsi* (Basilewsky), mainly distributed in the Yangtze and Pearl Rivers, is an economically important species in China and has a relatively high market value, and it is widely cultured throughout the country (Liang, 1996; Liu et al., 1998). It has a fast growth rate but is susceptible to diseases and viral infections (Sun and Nie, 2004). Artificial reproduction and selective breeding programs have been undertaken to meet market demand for *S. chuatsi* (Yang et al., 2007; Mi et al., 2010). An assessment of the population genetics of this species is urgently needed to reveal its available germplasm resources as a basis for breeding programs and to determine the potential genetic risk of translocation.

Microsatellites, also known as simple sequence repeats (SSRs), are rapidly developing molecular markers in recent years. Microsatellites are characterized as being highly polymorphic, plenty and well-distributed in the genome (Gpta and Rustgi, 2004). Therefore, they have become a useful tool in population genetics analysis, genetic mapping and marker-assisted selection (MAS) to assess genetic diversity and develop molecular-breeding techniques in fish (Walter and Epperson, 2001; Saha et al., 2004).

The aim of this study was to identify and confirm molecular markers that are associated with growth traits in the mandarin fish. They could provide a valuable theoretical basis for MAS to breed a disease-resistant and faster growing strain and to preserve the fish germplasm.

MATERIAL AND METHODS

Fish and DNA samples

A hatchery population was produced by mass spawning of *S. chuatsi* from Qingyuan Yushun Farming & Fishery Science and Technology Service Co. Ltd. (Qingyuan, Guangdong Province, China) and 200 individuals were randomly sampled half a year post-fertilization. Genomic DNA of these 200 individuals was extracted from the caudal fin using the TIANamp Genomic DNA kit (Tiangen Biotechnology (Beijing) Co. Ltd., China) following the manufacturer directions.

Polymorphic analysis and genotyping

Library preparation for transcriptome analysis and sequence assembly was as previously described (Wang et al., 2010). This unigene set was used for mining EST-SSR markers using the default parameters of BatchPrimer3 v1.0 software (You et al., 2008). In this study, a subset of 120 SSR markers were collected from the literature (Cnaani et al., 2003) and initially screened in the 25 largest and 25 smallest individuals from 200 samples according to total weight and length for marker association analysis in an isolated population. Subsequently, the initially selected microsatellites were further screened in another 200 individuals for marker association analysis in a randomly selected population (Cnaani et al., 2003). Polymerase chain reaction (PCR) conditions were optimized for each of the primers (Table 1). PCR was performed in 25-μL reaction volumes containing 2.5 μL 10X PCR buffer, 1.0-3.0 mM $MgCl_2$, 50 μM dNTPs, 0.4 μM each primer, 1 U *Taq* polymerase (Takara Biotechnology (Dalian) Co. Ltd., China) and 50 ng genomic DNA. PCR conditions were as follows: initial denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 30 s, the optimized annealing temperature for 30 s, 72°C for 30 s, and then a final extension

step at 72°C for 10 min. PCR products were separated by 8% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining. A denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen Biotechnology (Beijing) Co. Ltd., China) was used as a size standard to identify alleles.

Statistical analysis

Allelic frequencies, genotype frequencies, Hardy-Weinberg equilibrium, and observed (H_O) and expected (H_E) heterozygosities were statistically analyzed using the POPGENE software (Version 1.31; University of Alberta, Alberta, Canada). Polymorphism information content (PIC) was determined according to the formula:

$$PIC = 1 - (\sum_{i=1}^{n} q_i^2) - (\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2q_i^2 q_j^2)$$

where q_i and q_j are the frequencies of the *i*th and *j*th alleles at one locus; *n* is the number of alleles at one locus. Associations between genotypes and growth traits were evaluated using the chi-square test. Results were considered to be statistically significant if two-way P values were less than 0.05. Statistical analyses were carried out using the SPSS software (Version 19.0; SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 120 SSR markers were initially screened in the 25 largest and 25 smallest individuals from 200 samples based on total weight and length for marker association analysis in an isolated population. Eighteen of these loci were polymorphic in *S. chuatsi* and were selected for further random population tests (Table 1).

A random population was amplified by using these 18 microsatellite loci (Table 2), and 70 alleles were detected in *S. chuatsi*. The number of alleles per locus (N_A) ranged from 2 to 6, with an average of 3.61 alleles per locus. The effective number of alleles per locus (N_E) ranged from 1.74 to 4.83, with an average of 2.87 alleles per locus. H_O and H_E ranged from 0.44 to 1.00 (average of 0.68) and from 0.35 to 0.78 (average of 0.59), respectively. PIC ranged from 0.31 to 0.72 (average of 0.50).

In examining the associations between microsatellite loci and growth traits (body weight, body length, body height) (Table 3), microsatellite loci of *AP 37-16* were significantly associated with weight, length, and height (P < 0.01), while *AP 37-06*, *AP 37-11*, *AP 37-48* and *AP 38-32* were significantly associated with length and height (P < 0.05 or P < 0.01) and *AP 39-05* significantly associated with weight and body (P < 0.05). *AP 37-40* was only significantly associated with length (P < 0.05), and *AP 38-28* only significantly associated weight (P < 0.05).

The eight microsatellites that showed significant differences (P < 0.05) were used to analyze the associations between their genotypes and growth traits (Table 4). The results showed that five genotypes were discovered at *AP 37-16,* and growth traits of genotype AB were significantly greater than those of other genotypes (P < 0.01). For *AP 37-06*, length and height of genotype AB were significantly greater compared to genotypes BB and AC (P < 0.05). For *AP 37-11*, length and height of genotype AA were significantly greater compared to AC and BB (P < 0.01). For *AP 37-40*, length of AB was significantly greater than that of AC and AB (P < 0.05). For *AP 37-48*, length

Table 1. Primer sequences and characteristics of 18 polymorphic microsatellites loci of *Siniperca chuatsi*.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	Ta (°C)	Accession No.
AP37-02	CATGATACAATTTCCCAGC CACACTAACTATGCAGGTCAC	$(TTAG)_7$	211-309	58.0	JX503494
AP37-06	TATCTGAAGACGGTGGGAA TCTGGATGAGTTGGATGGT	$(ATGCC)_6$	199-247	58.0	JX503490
AP37-10	GTGAGGCAAAGTGGCTATT TTACAACTAGGACCCTTTAGACC	$(CT)_{15}$	222-277	58.0	JX503486
AP37-11	TCAGGAGGAGACAAGGGACA TGAGATGTTCTGTGACGCATTA	$(ATCC)_6$	202-284	57.0	JX503485
AP37-16	GGAAGCACCTTGTCGTGTA GACTTTCCACTCCTTCTCCA	$(TCG)_{11}$	165-211	57.5	JX503480
AP37-40	GTAGTCAGGGCTCAGTTCAG AGCCTACGAAGAGAGACAGATA	$(CCGATG)_5$	157-192	57.5	JX503456
AP37-48	TACAGAATCAGGAAGTGGCT CTCCTGTTGCTGTGTCAAA	$(ACT)_{15}$	273-302	59.0	JX503448
AP38-05	ACAGGCCACTGTGGAAATG TCAAGCACTTGATGATGTACAAT	$(CAG)_9$	204-231	53.0	JX503440
AP38-06	AAGCCTTGCAGTGACATAT TTTGGCTTGATTGATGGTG	$(CA)_{10}$	247-306	54.0	JX503439
AP38-31	GCGCTTGCATGTGAGGAC GTCAGACTGGAGCTGGATGTG	$(AC)_{18}$	270-320	55.0	JX503419
AP38-19	CACATATTAACAAGTCAGCGTGAG ATGGCTTTGAGTTCTGAGACGA	$(AATA)_5$	210-270	56.0	JX503426
AP38-25	GCAGCAGGGCAACAACCA ACACGGGAACCAGGCAGA	$(GT)_{23}$	205-236	55.0	JX503422
AP38-32	CGAAGGCAAGAAGCAAGG TGCCCTCAGGAAGGAATCTAC	$(ACC)_7$	156-231	59.0	JX503418
AP38-28	AACTGTTGGTGGTGATGAGGG GATCGTGTTGGAAAGAATGTCG	$(TGA)_8$	160-220	57.0	JX503420
AP38-39	CCCGCCCTTCTTTCTTTAGC CCACAGTGACGTATAAATATTCAGC	$(CCT)_{10}$	142-157	55.0	JX503411
AP39-02	AGGCCAGAGCTGCTACCAAG TCAAAGGGGTGATGGAGAAA	$(CA)_{20}$	257-279	57.0	JX503401
AP39-03	TTGGTTTGCTGCTTTCCCTT CACGGCGACATCCAATCACT	$(AC)_{22}$	128-165	57.0	JX503400
AP39-05	CTGACAGGCAGAAGGTAGCA ATTTAGCAGAGCTTTGACCC	$(CTG)_7$	180-280	57.0	JX503398

Table 2. Genetic diversity in panmictic population of *Siniperca chuatsi*.

Locus	N_A	N_E	H_O	H_E	PIC
AP37-02	5	4.8272	0.7656	0.5548	0.5351
AP37-06	3	2.8056	0.7998	0.6483	0.5697
AP37-10	3	1.7388	0.5345	0.4694	0.4199
AP37-11	3	1.946	0.5432	0.4967	0.4039
AP37-16	2	1.9429	0.8219	0.7897	0.5687
AP37-40	3	2.8381	0.6719	0.6529	0.5727
AP37-48	3	2.9554	1	0.6667	0.5889
AP38-05	3	2.5173	0.5045	0.6055	0.5192
AP38-06	6	4.0443	0.4362	0.3561	0.3145
AP38-31	6	3.7146	0.6864	0.6487	0.4169
AP38-19	3	1.9788	0.6577	0.4969	0.4438
AP38-25	5	3.5596	0.5329	0.5106	0.4842
AP38-32	3	2.4943	0.6435	0.6038	0.5138
AP38-28	3	2.5135	0.5634	0.6069	0.5186
AP38-39	5	3.7138	0.7321	0.7498	0.7235
AP39-02	4	3.7662	0.7712	0.6289	0.5449
AP39-03	2	1.7841	0.5942	0.4427	0.3429
AP39-05	3	2.6602	1	0.6298	0.5532

Table 3. Associations between 18 microsatellite loci and body weight, body length, and body height of *Siniperca chuatsi*.

Locus	Body weight	Body length	Body height
AP37-02	0.181	0.868	0.326
AP37-06	0.222	0.004**	0.018*
AP37-10	0.328	0.174	0.384
AP37-11	0.073	0.002**	0.001**
AP37-16	0.001**	0.000**	0.000**
AP37-40	0.085	0.014*	0.07
AP37-48	0.096	0.032*	0.008**
AP38-05	0.366	0.123	0.254
AP38-06	0.068	0.096	0.222
AP38-31	0.318	0.02	0.064
AP38-19	0.703	0.773	0.847
AP38-25	0.569	0.069	0.071
AP38-32	0.476	0.002**	0.005**
AP38-28	0.031*	0.217	0.683
AP38-39	0.318	0.256	0.098
AP39-02	0.195	0.064	0.061
AP39-03	0.318	0.203	0.064
AP39-05	0.046*	0.017*	0.107

*Significantly correlated with markers ($P < 0.05$). **Significantly correlated with markers ($P < 0.01$).

Table 4. Multiple comparisons of body weight, body length, and body height in 8 microsatellite loci.

Locus	Genotype*	N	Body weight (g)	Body length (cm)	Body height (cm)
AP37-06	AB	18	663.04 ± 22.83	30.20 ± 0.50^{A}	11.39 ± 0.21^{a}
	BC	12	651.94 ± 22.88	28.93 ± 0.34^{B}	10.96 ± 0.14^{ab}
	AA	12	610.40 ± 19.57	29.15 ± 0.35^{AB}	10.88 ± 0.12^{ab}
AP37-11	BB	9	606.32 ± 25.51	28.19 ± 0.35^{B}	10.84 ± 0.16^{b}
	AC	19	604.59 ± 24.04	28.40 ± 0.28^{B}	10.70 ± 0.11^{b}
	AA	26	658.38 ± 16.03^{a}	29.93 ± 0.32^{A}	11.33 ± 0.13^{A}
AP37-16	AB	32	634.34 ± 17.37^{ab}	28.69 ± 0.25^{AB}	10.83 ± 0.09^{AB}
	AC	6	579.08 ± 24.65^{ab}	28.45 ± 0.50^{B}	10.69 ± 0.15^{B}
	BB	6	572.62 ± 28.14^{b}	27.79 ± 0.61^{B}	10.39 ± 0.24^{B}
	AA	28	678.92 ± 14.56^{A}	30.08 ± 0.30^{A}	11.32 ± 0.13^{A}
	BB	16	612.57 ± 26.50^{B}	28.19 ± 0.39^{B}	10.56 ± 0.15^{B}
AP37-40	AB	26	596.78 ± 14.45^{B}	28.48 ± 0.21^{B}	10.84 ± 0.08^{B}
	BB	15	664.59 ± 18.03^{b}	29.29 ± 0.25^{ab}	11.08 ± 0.11^{ab}
	AB	16	648.71 ± 25.22^{b}	30.11 ± 0.59^{b}	11.31 ± 0.24^{b}
AP37-48	AA	8	637.67 ± 29.71^{ab}	28.89 ± 0.47^{ab}	10.70 ± 0.16^{a}
	AC	21	613.62 ± 20.28^{ab}	28.56 ± 0.29^{a}	10.87 ± 0.13^{ab}
	BC	10	569.14 ± 26.06^{a}	28.18 ± 0.30^{a}	10.69 ± 0.12^{a}
	AB	12	662.89 ± 16.33^{b}	29.19 ± 0.32^{ab}	10.90 ± 0.14^{AB}
	BB	7	655.03 ± 34.33^{ab}	29.87 ± 0.95^{b}	11.48 ± 0.31^{C}
	AC	18	646.38 ± 23.61^{ab}	29.86 ± 0.41^{b}	11.26 ± 0.15^{BC}
	CC	7	640.61 ± 47.38^{ab}	28.17 ± 0.44^{a}	10.43 ± 0.20^{A}
AP38-28	AA	12	615.81 ± 24.84^{ab}	28.51 ± 0.25^{ab}	10.79 ± 0.11^{AB}
	BC	14	569.88 ± 18.72^{a}	28.41 ± 0.43^{ab}	10.81 ± 0.19^{AB}
	CC	4	657.58 ± 88.28^{a}	26.95 ± 0.13^{b}	10.31 ± 0.12^{b}
	AA	15	653.85 ± 26.52^{a}	29.80 ± 0.58^{a}	11.18 ± 0.23^{a}
	BB	16	623.88 ± 20.70^{ab}	29.04 ± 0.26^{a}	10.99 ± 0.13^{a}
	AB	25	621.81 ± 16.97^{ab}	29.10 ± 0.31^{a}	10.93 ± 0.12^{a}
AP38-32	AC	10	606.17 ± 19.55^{b}	28.71 ± 0.35^{a}	10.97 ± 0.16^{a}
	AA	13	670.10 ± 19.68	30.27 ± 0.50^{A}	11.42 ± 0.20^{A}
	AB	28	625.26 ± 17.72	29.10 ± 0.29^{AB}	10.95 ± 0.12^{AB}
	BC	8	621.75 ± 44.86	27.39 ± 0.44^{C}	10.36 ± 0.17^{C}
	BB	12	613.25 ± 19.44	28.85 ± 0.34^{B}	10.83 ± 0.13^{BC}
	AC	9	608.24 ± 33.51	28.92 ± 0.42^{B}	11.09 ± 0.20^{AB}
AP39-05	AC	19	656.58 ± 19.37^{a}	28.96 ± 0.29^{ab}	10.85 ± 0.13^{ab}
	AA	13	639.57 ± 23.33^{b}	29.40 ± 0.42^{a}	11.05 ± 0.16^{ab}
	AB	25	636.39 ± 19.77^{b}	29.56 ± 0.39^{a}	11.17 ± 0.16^{a}

Data labeled with different superscript letters in the same column by individual locus mean significant difference. Different lowercase letters indicate significant differences ($P < 0.05$); different uppercase letters indicate significant differences ($P < 0.01$). Each genotype was considered when individual numbers were more than 4.

of BB and AC was significantly greater compared to CC ($P < 0.05$), height of BB was significantly greater than that of CC ($P < 0.01$). For *AP 38-28*, weight of genotypes AA and CC was significantly greater than that of AC ($P < 0.05$). For *AP 38-32*, length and height of AA were significantly greater compared to BC ($P < 0.01$). Therefore, BC showed a negative correlation with length and height. For *AP 39-05*, weight of AC was significantly greater compared to BC ($P < 0.05$), while length of AA and AB was significantly greater compared to BC ($P < 0.05$).

DISSCUSSION

For aquatic animals, molecular markers are not only used for genetic monitoring in selective breeding lines (Hao et al., 2010; Yu et al., 2011) but also association analysis of target traits (Kang et al., 2002). Currently, there are two methods for using molecular markers: one is marker association analysis in a randomly selected population (Cnaani et al., 2003), and the other is marker association analysis in an isolated population (Gross and Nilsson, 1999). In this study, these two methods were used together. First, polymorphic analyses of these microsatellite loci were analyzed between the maximal weight group and minimal weight group. Second, these polymorphic markers were used for genotyping and association analysis in a random group. As a result, 18 microsatellite loci were screened between the maximal weight group and minimal weight group, and 8 loci showed significant differences in subsequent association analysis with random population testing and growth traits. The results indicated that screening markers in extreme groups could improve the efficiency of screening.

Many authors have reported that the different genotypes of microsatellite loci are positively correlated with growth traits (Fan et al., 2009; Liu et al., 2012). In this study, the results showed that microsatellite loci of *AP 37-16* were significantly correlated with body weight, length and height of *S. chuatsi*, while *AP 37-06*, *AP 37-11*, *AP 37-48* and *AP 38-32* were significantly associated with length and height. *AP 39-05* was significantly associated with weight and length ($P < 0.05$), *AP 37-40* was only significantly associated with length ($P < 0.05$), and *AP 38-28* was only significantly associated with weight ($P < 0.05$). The finding that these loci were significantly correlated with economic traits could be useful for marker-assisted selection in breeding programs of this important aquatic species.

Among the eight loci showing significant correlations in the *S. chuatsi* population, eight genotypes of six loci were positively correlated with growth traits (body weight, length, and height). Genotypes AB for *AP 37-06*, AA for *AP 37-11,* AA for *AP 37-16*, BB and AC for *AP 37-48*, AA for *AP 38-32*, and AC and AA for *AP 39-05* exhibited significantly larger traits compare with other genotypes of the same marker. These significantly correlated loci carry an important function in evolution because they control the viability of individuals bearing different genotypes of the locus (Xu, 2008). Therefore, these kinds of genotypes could indirectly help select growth traits for *S. chuatsi*.

Heterozygosity is one of the indicators of the degree of genetic variation (Bin et al., 1999). In this study, average H_O and H_E were 0.68 and 0.59, respectively, showing a population with a high genetic diversity. *PIC* is the change of function allele frequency and the alleles number, and it is also a good indicator of genetic information capacity of a genetic marker (Zhu et al., 2008). According to Botstein et al. (1980), PIC is an indicator of the degree of genetic variation. In the eighteen loci of this study, eleven loci showed high polymorphism ($PIC > 0.5$), and seven loci medium polymorphism ($0.25 < PIC < 0.5$). The average PIC value for the 18 microsatellite loci detected in *S. chuatsi* was

0.50, showing 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 *S. chuatsi* and its related species.

In conclusion, 120 SSR markers were initially screened in the 25 largest and 25 smallest individuals from 200 samples on the basis of total weight and length for marker association analysis in an isolated population. Eighteen of these loci were polymorphic in *S. chuatsi* and were selected for further random population tests. We found that eight genotypes of six loci were positively correlated with growth traits (body weight, length and height) in the *S. chuatsi* population. Average H_O and H_E were 0.68 and 0.59, respectively, and the average PIC value was 0.50, indicating a population with high genetic diversity. In addition, these loci were significantly correlated with growth traits, indicating that they could be useful for marker-assisted selection in breeding programs of this important aquatic species and its related species.

Conflicts of Interest

The authors declare no conflict of interest.

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