Characterization of microsatellite markers and their correlations with growth traits in Mandarin fish (*Siniperca chuatsi*)

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ABSTRACT. Mandarin fish (*Siniperca chuatsi*) is a traditionally cultured freshwater fish with high commercial value in China. To facilitate marker-assisted selection for genetic improvement of this species, 100 microsatellite markers identified in previous studies were characterized in the 25 largest and 25 smallest individuals. Twenty polymorphic loci were used to genotype 200 individuals, and the associations between their genotypes and growth traits were examined. We found that 9 genotypes at 8 loci (*SC-10*, *Sin 135*, *Sin 166*, *AP 34-23*, *AP 38-11*, *AP 37-22*, *AP 37-08*, and *AP 37-37*) were positively correlated with growth traits (body weight, body length, body height) in the mandarin fish population. The average of observed and expected heterozygosities were 0.71 and 0.59, respectively, and the average

polymorphism information content value was 0.54, indicating that the population had high genetic diversity. The markers developed in this study are useful for selection of genetic breeding in this species and its related species.

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

INTRODUCTION

Mandarin fish, *Siniperca chuatsi* (Basilewsky), is a peculiar freshwater fish species in China (Liang and Cui, 1982; Liu et al., 1998) that has a relatively high market value and is widely cultured throughout the country. However, because of river damming, water pollution, and over-fishing, natural resources of *S. chuatsi* have been exhausted and genetic diversity is declining. In addition, numerous disease outbreaks caused by parasites, bacteria, and viruses have caused huge economic losses to the *S. chuatsi* industry (He et al., 2002). Artificial reproduction and selective breeding programs have been implemented to meet the market demand for *S. chuatsi* (Yang et al., 2007; Mi et al., 2010). Therefore, breeding a disease-resistant and faster growing strain as well as preserving fish germplasm are becoming urgent goals in China.

Selective breeding is a proven, powerful approach for enhancing domesticated species for production. Currently, 10 of 19 major aquacultures species are being produced using selective breeding to identify improved brood stocks, and new tools such as molecular markers have been developed to increase the precision of this process, allowing the identification of quantitative trait loci and the application of marker-assisted selection to speed the selection process (Hershberger, 2006). Genetic selection is effective for improving growth rate (Wada, 1986).

Microsatellites or simple sequence repeats (SSRs) are useful tools for population genetic analysis, genetic mapping, and marker-assisted selection in fish because of their codominant nature, high allelic, ubiquitous distribution within the genome polymorphism, and high reproducibility (Gupta and Rustgi, 2004; Saha et al., 2004). In this study, we identified and confirmed microsatellites affecting growth in mandarin fish. We studied the genetic diversity and distribution of microsatellite marker alleles and analyzed the association between these makers and growth. Our results provide a valuable theoretical basis for marker-assisted selection for improving the growth of mandarin fish.

MATERIAL AND METHODS

Fish and DNA samples

A hatchery population was produced by mass spawning *S. chuatsi* from the Qingyuan Yushun Farming & Fishery Science and Technology Service Limited Corporation (Qingyuan, Guangdong Province, China), and 250 individuals were randomly sampled for 6 months post-fertilization. Genomic DNA of the 250 individuals was extracted from the caudal fin ray using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer instructions.

Polymorphic analysis

The library for transcriptome analysis was prepared, and the sequence was assembled as described previously (Wang et al., 2010). This unigene set was used to mine expressed sequence tag-SSR markers using the default parameters of the BatchPrimer3 v1.0 software (You et al., 2008). In this study, a subset of 100 SSR markers were collected from previously published studies (Cnaani et al., 2003) and initially screened in the 25 largest and 25 smallest individuals from 250 samples based on total weight and length for the isolated population marked for association analysis (Cnaani et al., 2003). The initially selected microsatellites were further screened in the other 200 individuals for a randomly selected population for associated analysis (Cnaani et al., 2003). Polymerase chain reaction (PCR) conditions were optimized for each primer set (Table 1). PCRs were performed in a 25-μL reaction volume containing 2.5 μL 10X PCR buffer, 1.0-3.0 mM MgCl$_2$, 50 μM dNTPs, 0.4 μM of each primer, 1 U *Taq* polymerase (Takara, Shiga, Japan), 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 on an 8% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining. A denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen) was used as a size standard to identify alleles.

Statistical analysis

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

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

where q_i and q_j are the frequencies of the ith and jth alleles at 1 locus and n is the number of alleles at 1 locus. The associations between genotypes and growth traits were performed using the chi-squared test. The results were considered to be statistically significant if bilateral P values were less than 0.05. Statistical analyses were carried out using the SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA).

RESULTS

A subset of 100 SSR markers were initially screened in the 25 largest and 25 smallest individuals of 250 samples based on total weight and length for an isolated population used for association analysis. Twenty of these loci were polymorphic in *S. chuatsi* and were selected for further random population tests (Table 1).

A random population was amplified using these 20 microsatellite loci (Table 2), and 77 alleles were detected in *S. chuatsi*. The number of alleles per locus ranged from 2-6, with an average of 3.85 alleles per locus. The effective number of alleles per locus ranged from

1.14-4.79, with an average of 2.84 alleles per locus. The H_O and H_E ranged from 0.14-1.00 (average of 0.71) and from 0.13-0.80 (average of 0.59), respectively. PIC ranged from 0.12-0.76 (average of 0.54).

Table 1. Primer sequences and characteristics of 20 polymorphic microsatellites loci of *Siniperca chuatsi*.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	Ta (℃)	Accession number
SC-1	TTTTAAAGACGGGGCAGCGG ACCAACGTTTGGCGTAAAGC	$(TCA)_{12}$	211-309	55.5	JQ686834
SC-9	CACGAACTACTCGCCCTCAC TCTGTGGGAACCGTGGTGAT	$(GCCTGA)_7$	199-247	58.5	JQ686842
SC-10	CAGGGAACGGGGAAAAAGCA AGAATTCCTCCGTACCTGTCTG	$(TTTC)_{10}$	222-277	53.5	JQ686843
SC-24	ACGGAGAGCAGAGTCTTACGTC ACTCTCCAGCTGCCGTCACA	$(CA)_{42}$	202-284	54.5	JQ686857
SC-52	GAATGCCATGTGTCGTTGCG CCGGCCTGACATCCCTAAGA	$(TG)_{19}$	165-211	55.5	JQ686883
Sin 118	AGGCCACACTTTAGTCACATC ACCACACTCCAGCATTTCC	$(GT)_{14}$	157-192	54.5	JQ804773
Sin 135	GTGATATCTCCTCCTGACGGC ACATTCTGAATTGCAAAGGCTCA	$(TG)_{14}$	273-302	54.5	JQ804790
Sin 166	GAAATTGAAGAAGACAAGGTGATG CTGCTTTTGGCAGGAGCTAA	$(GA)_{13}$	204-231	53.5	JQ804819
AP 34-23	CAGCAGGAATTGGGATGAAA CAGATGCGGCCAATACAAGA	$(GT)_{25}..(GA)_7..(GAT)_4$	247-306	55.0	JX503177
AP 35-43	GTAATACTGTTGCACTTCGT GTAGGCATCAAGTGAAGC	$(GT)_{15}$	270-320	55.0	JX503331
AP 40-16	ATCTGACACGATAAACCCTC GCTGATGCTGAGGAGGAAAT	$(CCT)_{10}$	205-236	58.0	JX449077
AP 38-11	TCACTAAGATGATTGCTGC TGACTACTGTTGTGATGGG	$(CA)_{20}$	232-260	54.0	JX503434
AP 37-22	TAACAGACAAAACCTGGACT ATGGCTCCTGGTATTTAGTG	$(AC)_{22}$	301-336	58.0	JX503474
AP 41-25	CCCTCTCTGTTGTCTTGGCT GTCTACTCCCTAACCCCAGT	$(TG)_{20}$	141-181	60.0	JX449130
AP 41-23	CTCTGTCTGGCACAATAACA GTCCACATACACTGCTGCTC	$(TG)_{20}$	247-309	60.0	JX449128
AP 37-08	TCTGTACCACCTGAGCGTTC TCCTGTTTATGCTCCACGAC	$(GT)_{17}$	142-157	60.0	JX503488
AP 37-09	CTTGGACCTGAACCTGGA CCACCAACACCTACTCCCTAT	$(CAG)_9$	257-279	58.0	JX503487
AP 37-32	ATTTGCATTCGAGGTCATC ATTTGCATTCGAGGTCATC	$(CA)_{10}$	191-233	55.0	JX503464
AP 37-37	AGTCTCAGCATCTCCTCCAG CGACCTGAGGAGACTGTGTT	$(AT)_{11}$	110-125	60.0	JX503459
AP 41-03	GTAGAAGGACAGAGTGCCAG CTCTGATGATGTTGGTGGTT	$(GAG)_{11}$	265-306	56.0	JX449105

Examination of the associations between microsatellite loci and growth traits (mandarin fish body weight, body length, body height) (Table 3) revealed that the microsatellite loci of *SC-10*, *Sin 135*, *Sin 166*, *AP 34-23*, *AP 38-11*, *AP 37-22*, *AP 37-08*, and *AP 37-37* were significantly associated with body weight, body length, and body height (P < 0.05 or P < 0.01), while *SC-52* and *AP 37-09* were only significantly associated with body length (P < 0.05) and *Sin 118* was only significantly associated with body height (P < 0.05).

Eleven microsatellites showing significant differences (P < 0.05) were used to analyze the associations between genotypes and growth traits (Table 4). The results showed that 3 genotypes were present at *SC-10* and the growth of fish with genotype AB was significantly higher than those with other genotypes (P < 0.01). At *SC-52*, the body length of genotypes BC and CE was significantly (P < 0.05) superior to that of genotype AD.

Table 2. Genetic diversity in panmictic population of *Siniperca chuatsi*.					
Locus	N_A	N_E	H_O	H_E	PIC
SC-1	4	1.8272	0.5856	0.4548	0.4051
SC-9	4	2.4595	0.5856	0.5961	0.5282
SC-10	3	1.6880	0.5045	0.4094	0.3699
SC-24	4	3.4948	1.0000	0.7171	0.6618
SC-52	5	4.6521	1.0000	0.7886	0.7512
Sin 118	2	1.1442	0.1351	0.1266	0.1181
Sin 135	3	2.4959	0.6306	0.6021	0.5260
Sin 166	3	2.5173	0.5045	0.6055	0.5192
AP 34-23	6	4.0443	1.0000	0.7561	0.7145
AP 35-43	6	1.7146	0.3964	0.4187	0.3969
AP 40-16	3	1.9788	0.6577	0.4969	0.4438
AP 38-11	5	3.5596	1.0000	0.7243	0.6742
AP 37-22	6	4.7873	1.0000	0.7968	0.7582
AP 41-25	4	3.6254	0.6892	0.6705	0.6359
AP 41-23	3	2.7138	0.7321	0.7498	0.7235
AP 37-08	3	2.6620	1.0000	0.6289	0.5449
AP 37-09	2	1.7841	0.5942	0.4427	0.3429
AP 37-32	5	4.5873	0.5637	0.5368	0.5167
AP 37-37	3	2.4100	1.0000	0.5893	0.4956
AP 41-03	3	2.6324	0.6983	0.6837	0.6234

Table 3. Associations between 20 microsatellite loci and body weight, body length, and body height of *Siniperca chuatsi*.			
Locus	Body weight	Body length	Body height
SC-1	0.181	0.868	0.326
SC-9	0.523	0.926	0.843
SC-10	0.000**	0.000**	0.000**
SC-24	0.185	0.371	0.071
SC-52	0.164	0.027*	0.230
Sin 118	0.698	0.124	0.048*
Sin 135	0.000**	0.045*	0.003**
Sin 166	0.000**	0.003**	0.000**
AP 34-23	0.000**	0.000**	0.000**
AP 35-43	0.318	0.020	0.064
AP 40-16	0.703	0.773	0.847
AP 38-11	0.015*	0.014*	0.001**
AP 37-22	0.003**	0.005**	0.016*
AP 41-25	0.765	0.684	0.533
AP 41-23	0.318	0.256	0.098
AP 37-08	0.195	0.046*	0.031*
AP 37-09	0.318	0.02*	0.064
AP 37-32	0.711	0.753	0.867
AP 37-37	0.014*	0.033*	0.095
AP 41-03	0.687	0.279	0.413

*Trait was significantly correlated with markers ($P < 0.05$). **Trait was significantly correlated with markers ($P < 0.01$).

Therefore, genotype AD was negatively correlated with body length. At *Sin 135*, all growth traits of BC were significantly superior to AA; the body weight of AB was significantly superior to AA. For *Sin 166*, all growth traits of AC and CC were significantly superior to those of genotype AB, while those of AC and CC showed no significant difference. At *AP 34-23*, genotype DE was negatively correlated with all growth traits. At *AP 38-11*, all growth traits of BC were significantly superior to those of genotype AB and DE and for *AP 37-22*, all growth traits of AB and CD were significantly superior to those of genotype EF. Growth traits for genotypes AB and CD showed no significant difference. At *AP37-37*, all growth traits of AB and BC were significantly superior to those of genotype AC, while those of AB

and BC showed no significant difference. At *AP37-09*, the body length of BB was significantly superior to that of AA. Therefore, genotype AA was negatively correlated with body length. For *AP37-08*, all growth traits of AC were significantly superior to those of AB. Therefore, genotype AB was negatively correlated with growth traits.

Table 4. Multiple comparisons of body weight, body length, and body height in 11 microsatellite loci.

Locus	Genotype*	N	Body weight (g)	Body length (cm)	Body height (cm)
SC-10	AB	34	705.165 ± 12.248^A	30.703 ± 2.484^A	11.338 ± 0.729^A
	AA	55	606.939 ± 9.630^a	28.760 ± 2.103^a	10.675 ± 0.849^a
	AC	22	637.425 ± 15.226^a	28.849 ± 1.439^a	10.644 ± 0.722^a
SC-52	BC	29	655.647 ± 85.243^a	29.759 ± 2.222^a	10.959 ± 0.838^a
	CE	36	645.856 ± 75.940^a	29.636 ± 2.538^a	10.766 ± 0.916^a
	AD	42	624.646 ± 85.887^a	28.669 ± 1.932^b	10.818 ± 0.780^a
Sin 118	AE	4	667.906 ± 122.746^a	27.970 ± 2.950^a	10.937 ± 0.636^{ab}
	BB	65	651.145 ± 81.074^a	29.834 ± 2.043^{Aa}	11.041 ± 0.837^a
	BE	15	644.458 ± 95.391^a	29.743 ± 2.895^{ab}	10.724 ± 0.962^{ab}
	BD	15	624.952 ± 76.640^a	27.984 ± 2.252^{Bb}	10.368 ± 0.713^b
	BC	7	596.292 ± 70.744^a	28.720 ± 1.581^{ab}	10.472 ± 0.741^{ab}
Sin135	BC	36	681.197 ± 77.006^a	29.885 ± 2.714^b	11.237 ± 0.757^{Ab}
	AB	34	650.628 ± 71.422^a	29.573 ± 1.736^{ab}	10.784 ± 0.729^{Aa}
	AA	41	593.581 ± 78.796^A	28.590 ± 2.219^a	10.591 ± 0.941^a
Sin166	CC	31	684.288 ± 58.772^a	30.371 ± 1.805^c	11.263 ± 0.616^A
	AC	27	656.727 ± 88.072^a	29.704 ± 2.415^{bc}	11.110 ± 0.795^A
	AA	24	652.358 ± 70.571^a	28.984 ± 1.831^{ab}	10.679 ± 0.608^a
	AB	29	578.601 ± 74.228^A	28.320 ± 2.511^a	10.392 ± 0.999^a
AP 34-23	AC	30	734.732 ± 40.656^a	30.515 ± 2.620^a	11.331 ± 0.739^a
	BD	57	638.877 ± 50.369^a	29.463 ± 1.739^b	10.873 ± 0.714^A
	DE	24	538.443 ± 45.984^A	27.731 ± 2.085^A	10.295 ± 0.918^B
AP 38-11	BC	40	670.42 ± 73.21^b	29.43 ± 1.91^b	11.23 ± 0.68^B
	DE	15	613.26 ± 69.47^a	28.62 ± 1.72^a	10.79 ± 0.88^A
	AB	14	602.19 ± 78.36^a	28.63 ± 1.86^a	10.63 ± 0.76^A
AP 37-22	AB	28	675.42 ± 76.44^B	29.63 ± 1.94^B	11.18 ± 0.73^b
	CD	34	637.73 ± 73.45^B	29.19 ± 1.86^B	10.99 ± 0.76^b
	EF	7	551.72 ± 43.22^A	27.69 ± 1.88^A	10.45 ± 0.55^a
AP 37-37	BC	54	659.86 ± 69.34^b	29.43 ± 1.97^b	11.10 ± 0.74^a
	AB	5	617.91 ± 73.33^b	29.00 ± 1.91^b	10.82 ± 0.68^a
	AC	10	572.48 ± 72.31^a	28.15 ± 1.72^a	10.67 ± 0.72^a
AP 37-09	BB	26	666.39 ± 71.23^a	29.87 ± 1.99^b	11.23 ± 0.77^a
	AB	41	688.36 ± 69.32^a	29.43 ± 1.35^{ab}	11.36 ± 0.55^a
	AA	4	672.42 ± 73.28^a	28.35 ± 1.72^a	10.79 ± 0.68^a
AP 37-08	AC	13	682.13 ± 89.53^b	29.87 ± 1.16^b	11.36 ± 0.79^b
	BC	42	641.84 ± 73.32^{ab}	29.19 ± 1.35^{ab}	10.98 ± 0.82^{ab}
	AB	14	615.86 ± 66.35^a	28.69 ± 1.23^a	10.81 ± 0.71^a

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

DISCUSSION

Molecular markers for association analysis of target traits in aquatic animals can be used with 2 methods, including isolating a population marked for association analysis (Gross and Nilsson, 1999; Kang et al., 2002) and random selection of a population marked for associated analysis (Cnaani et al., 2003). In this study, these 2 methods were used together. First, polymorphic analyses of microsatellite loci were analyzed in a maximal weight group and a minimal weight group. Second, the polymorphic markers were used for genotyping and association analysis in a random group. Twenty microsatellite loci were screened in the maximal and minimal weight groups, 11 loci showed significant differences in subsequent association

analysis of a random population testing and growth traits. The results indicated that screening for markers in extreme groups may improve screening efficiency.

Fan et al. (2009) reported that the different genotypes of 7 microsatellite loci were positively correlated with growth traits (body weight, body length, body height). In this study, the microsatellite loci *SC-10*, *Sin 135*, *Sin 166*, *AP 34-23*, *AP 38-11*, *AP 37-22*, *AP 37-08*, and *AP 37-37* were significantly correlated with body weight, body length, and body height of *S. chuatsi*, while *SC-52* and *AP 37-09* were only significantly associated with body length and *Sin 118* was only significantly associated with body length and body height. Loci that were significantly correlated with economic traits will be useful for marker-assisted selection during breeding of this important aquacultural species.

Among the 11 significantly correlated loci in the *S. chuatsi* population, 9 genotypes of 8 loci were positively correlated with growth traits (body weight, body length, body height), including genotype AB for *SC-10*, genotype BC for *Sin 135*, genotype CC for *Sin 166*, genotype AC and BD for *AP 34-23*, genotype BC for *AP 38-11*, genotype AB for *AP 37-22*, genotype AC for *AP 37-08*, and genotype AB for *AP 37-37*, which were significantly higher than other genotypes for the same marker. These significantly correlated loci have an important function in evolution because they control the viability of individuals with different genotypes of the locus (Xu, 2008). Therefore, these genotypes may indirectly assist in the selection of growth traits for *S. chuatsi*.

The average gene heterozygosities of a population are ideal for measuring the variation in a population (Bin et al., 1999). Average gene heterozygosities can reflect the variation of the genetic diversity. In this study, the average H_O and H_E values were 0.71 and 0.59, respectively, indicating that the population was highly diverse. The average H_O and H_E values for *S. chuatsi* were higher than those of *S. obscura* (Huang et al., 2013). The reason may be a combination of the 2 test methods that was used to isolate both a specific and a random population for analysis. PIC is a value that is commonly used in genetics to measure the usefulness of a polymorphism as a marker (Shete et al., 2000). Bostein et al. (1980) described that a locus exhibits low polymorphism when the PIC < 0.25, medium polymorphism when the value is 0.25 < PIC < 0.5, and high polymorphism when the PIC > 0.5. PIC is related to the availability and efficiency of the locus. If PIC is high, the proportion of heterozygous individuals is greater and more genetic information can be obtained. In this study, the average PIC value for the 20 microsatellite loci detected in *S. chuatsi* was 0.54, indicating highly polymorphic loci. These novel markers will facilitate further studies on genetic diversity, conservation genetics, the construction of high-density linkage map, and molecular marker-assisted breeding of *S. chuatsi* and its related species.

In conclusion, a subset of 100 SSR markers were initially screened in the 25 largest and 25 smallest individuals from 250 samples based on total weight and length for an isolated population marked for association analysis. Among them, 20 loci were polymorphic in *S. chuatsi* and were selected for further random population tests. A combination of an isolated population marked for association analysis and a randomly selected population marked for association analysis screening markers may improve screening efficiency. Based on random population tests, 9 genotypes of 8 loci were positively correlated with growth traits (body weight, body length, body height) in the *S. chuatsi* population. The average H_O and H_E values were 0.71 and 0.59, respectively, and the average PIC value was 0.54, indicating that the population was highly genetically diverse. These loci were significantly correlated with

growth traits and will be useful for marker-assisted selection in the breeding programs of this important aquacultural species and its related species.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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